KMJ
KUWAIT MEDICAL JOURNAL

The Official Journal of The Kuwait Medical Association

Continued inside

KUWAIT MEDICAL JOURNAL

C O N T E N T S

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Kuwait Medical Journal

Guidelines for Authors

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Review Article

The potential of gene therapy and future approaches in the management of pancreatic cancer

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ABSTRACT

The pancreas is indeed an essential organ that plays a role in digestion and hormone production. Common pancreatic problems include inflammation (pancreatitis), pain (pancreatic discomfort), tumours (pancreatic neuroendocrine, cystic, pancreatic ductal, endocrine), and diabetes. Although a variety of therapies have been developed and used, there is a pressing need to enhance the effectiveness of both tried-and-true approaches and emerging ones. Although we are aware of positive trends over the past few years, we also know that the mortality rate has not decreased, and this particular form of cancer is projected to be etiologically the second leading type of cancer due to fatalities. Thus, efforts to discover and implement fresh treatment strategies continue. This review article focuses on the most current preclinical research and clinical studies using gene addition for pancreatic ailments,

notably pancreatic cancer. Finding effective and safe gene treatments for all pancreatic illnesses, including pancreatic tumours, is our primary focus. New medicines to improve prognosis of pancreatic cancer, especially pancreatic ductal adenocarcinoma, is urgently needed. There is an immediate need to discover novel treatment approaches that can be used in tandem with standard chemotherapy. Gene therapy is an exciting new treatment option. Significant research has been focused on increasing the specificity and sensitivity of diagnostic techniques for pancreatic cancer to acclimatize pancreatic cancer patients to diagnosis and therapy accurately. Gene therapy has been used to treat various diseases, including pancreatic cancer. This review article describes gene therapy's applicability, therapeutic procedures, and research status in pancreatic cancer diagnosis, treatment and survival.

KEY WORDS: gene therapy, inoculation, interventional studies, oncogenic virus, pancreatic cancer

INTRODUCTION

The pancreas is an essential organ of the human body system, with an important role in digestive and endocrine functions. It's located in the retroperitoneal space of the abdominal cavity and its size is about 15 to 20 centimeters long. The pancreas and the pancreatic duct run together and connect the pancreas to the 2nd part of the duodenum. The pancreas is a vital organ in our body, and its primary purpose is to digest fat, carbohydrates and protein with the help of enzymes released through its ducts. The second key role is the endocrine activity, mediated by several hormones of the pancreas, for example, insulin. These hormones are secreted from the pancreas into the blood circulation

system, which plays a vital role in maintaining homeostasis. Pancreatitis, cystic disorders, diabetes mellitus, tumors of islet cells and endocrine tumors persuaded by these disorders are the most frequent pancreas diseases[1].

HISTORY

Cancer of the pancreas is one of the main essential causes of cancer-related mortality globally and also the fourth major etiology of cancer-related fatalities in the United States of America[2]. Pancreatic ductal adenocarcinoma (PDAC) as we all know is the most prevalent histological subtype of pancreatic cancer, characterized by rapid development and a poor

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Table 1: Clinical trial summaries published.

Conditions	Registration	Interventions	Phases	Carrier	Title	Reference
Pancreatic carcinoma	-7	AdV/Interleukin 2	Phase I/ Phase II	Adenovirus	Adv-IL-2 gene therapy incurable patients with gastrointestinal cancer: an interim report of a Phase I-II study	[132]
Pancreatic carcinoma	-23	AdV/ONYX-015	Phase I	Adenovirus	Phase I experiments assessed the safety and feasibility of injecting a replicating selective adenovirus (ONYX-015) with the deletion of the E1B-55kDa gene into primary pancreatic cancer	[133]
Pancreatic carcinoma	-14	Lipofectamine/ Cyto. P450	Phase I/ Phase II	Lipofectamine [Plasmid DNA]	In phase I/II clinical studies, cell-based local chemotherapy can be used to treat inoperable pancreatic cancer	[134]
Pancreatic carcinoma	-10	plasmid DNA– cationic liposome	Phase I/ Phase II	plasmid Deoxyribonucleic acid - lipoplexes	Phase 1/2 clinical study of (MUC1-DC) transfected as immunization	[135]
Pancreatic carcinoma	-21	Adenovirus	Phase I/ Phase II	Adenovirus	EUS-guided fine needle injection of E1B 55kDa mutant adenovirus dl1520 and Gemzar for stage I/II pancreatic cancer	[116]
Pancreatic carcinoma	-3	Rv/Rexin-G	Phase I	Retrovirus	Injectable retroviral vectors [Rexin-G] can be used as the first clinical experience of stage IV pancreatic cancer intervention	[136]
Pancreatic carcinoma	-7	AdV/Interleukin 12	Phase I	Adenovirus	Intratumoral injection of interleukin-12 adenovirus as a phase I study of advanced gastrointestinal cancer	[137]
Pancreatic carcinoma	-30	TNFerade	Phase I	Adenovirus	TNFerade biologic is an adenoviral vector containing the human TNF- gene with radiation-inducible promoters	[138]
Pancreatic carcinoma	-11	Adv. encoding interleukin-12 gene	Phase I	Adenovirus	In metastatic gastrointestinal cancer, recombinant adenovirus modified dendritic cells to release interleukin-12	[139]
Pancreatic carcinoma	-10	variolae vaccinae / Poxvirus expressing CEA-MUC-1-TRICOM vaccine and Co-stimulation	Phase I	Poxviruses/ Vaccinia	Pox virus-based vaccination management helps advanced pancreatic cancer patients	[140]
Pancreatic carcinoma	-50	Plasmid/GVAX	Phase I	Plasmid DNA	Preliminary investigation on the safety, effectiveness, and immunological activity of allogeneic granulocyte macrophage colony stimulating factor released by chemotherapy for pancreatic cancer	[141]
Pancreatic carcinoma	-9	Mx-dnG1 at 2 Dose	Phase I / Phase II	Retrovirus	Phase 1/2 research. Rexin-G for Infugem resistant pancreatic cancer	[21]
Pancreatic carcinoma	-6	HF10 oncolytic herpes virus	Phase I	Herpes virus	Intratumoral canerpaturev oncolytic virus immunotherapy for advanced pancreatic cancer: a phase I trial	[142] [145]
Pancreatic carcinoma	-7	EBV-Ras mutant-modified lymphocytes	Phase I	Immunotherapy	One preliminary study was based on pancreatic cancer vaccination of lymphoblastic-like cell lines transfected with Ras and transformed by EBV	[143] [146]
Pancreatic carcinoma	-9	Diphtheria plasmid/gene expression	Phase I	Plasmid DNA	In the phase I / 2A of dosage escalation, pharmacokinetics, safety and preliminary effectiveness trial, bBC-819 intratumoral injection is being examined in patients with unresectable pancreatic cancer	[144]
Pancreatic carcinoma	-50	Adenovirus	Phase I	Adenovirus	Percutaneous guided intratumoral TNFerade biologic or EUS coupled with radiotherapy: a phase I/II research	[145]
Pancreatic carcinoma	-26	attenuated listeria immunization	Phase I	Cancer vaccine	First, researchers tested the safety and immunological induction of live attenuated Listeria vaccines (ANZ-100 and CRS-207) in advanced malignant tumours	[146]
Pancreatic carcinoma	-70	Algenpantucel-L + gemcitabine + 5FU	Phase II	Immuno-therapy	In phase 2, algenpantucel-L immunotherapy was added to normal pancreatic cancer adjuvant treatment	[147]

Pancreatic carcinoma	-304	AdV/TNFerade+ chemotherapy and chemotherapy	Phase III	Adenovirus	Final randomised phase III multi-institutional research findings on TNFerade coupled radiotherapy and fluorouracil for locally advanced pancreatic cancer	[129]
Pancreatic carcinoma	-13	Lipofectamine/ Cyto. P450	Phase II	Lipofectamine (Plasmid DNA)	Encapsulated cells expressing chemotherapy-activated enzymes enable safe and efficient subtoxic chemotherapy: results of two clinical trials of pancreatic cancer	[148]
Pancreatic carcinoma	-24	AdV/HSV thymidine kinase	Phase I	Adenovirus	Gene-mediated cytotoxic immunotherapy as an adjunctive therapy for pancreatic cancer after surgery or chemo-radiotherapy	[149]
Pancreatic carcinoma	-15	SiG12-LODER® + gemcitabine	Phase I/ Phase II	RNAi	RNAi treatment of KRAS is combined with chemotherapy for locally advanced pancreatic cancer	[23]
Pancreatic carcinoma	-90	GVAX + CRS 2017	Phase II	Cancer vaccine	Vaccination of metastatic pancreatic cancer is enhanced using mesothelin-expressing monocytogenes proliferative factor (CRS-207)	[127]
Pancreatic carcinoma	-73	Reolysin + paclitaxel + carboplatin	Phase II	Reovirus	Oncolytic virus Pelareorep [Reolysin] in phase II pancreatic cancer study	[150]
Pancreatic carcinoma	-9	AdV/Thera gene + Radiotherapy	Phase I	Adenovirus	AdV/Theragene combined chemotherapy	[151]

prognosis[3]. Even though poor glycemic control, decreased patient weight, and long-term diabetes are considered valuable indicators of pancreatic tumors, there is no early detection of tumor markers or strategies currently[4]. Although early fat (adipose) and skeletal muscle tissue deficiencies are common in cancer, a recent study shows that early pancreatic cancer is associated with loss of peripheral tissue but may not affect survival[5], which is why the development of a better understanding involved in the mechanism of this invasive cancer progression is crucial. Current epidemiological evidence shows that the pancreatic cancer mortality rate has not decreased. It is also determined that by 2030, pancreatic cancer will be the second major cause of death related to cancer[6]. As a result, significant efforts are being made to develop new treatments for this devastating disease.

Even though some new chemotherapeutic agents, including liposomal irinotecan, FOLFIRINOX and nab-paclitaxel have been tested, these drugs are effective in clinical trials as routine management[7], particularly considering the poor prognosis of pancreatic cancer[8]. Because of its prognosis, research and the number of clinical trials are rising daily, intending to improve pancreatic cancer treatment and survival rate shortly[7]. Furthermore, the need to provide comprehensive precision medical tools for implementing treatment programs in the community and academic fields, is clearly essential[9]. The combinations of new tools with established tools are intended to contribute to the improvement of patients undergoing appropriate treatments for pancreatic cancer in terms of progression-free survival. In a disease like pancreatic cancer, the urgent need for new treatment innovations, and the combination of new therapies for cancer, including gene therapy and cell therapy with remaining cytotoxic chemotherapy and radiotherapy, provide hope for patients to achieve better results in the future with progressive pancreatic cancer. Gene therapy is emerging as a promising strategy among all these potential new therapies, as evidenced by multiple completed and ongoing research projects and clinical trials. A synopsis of all these investigations (research and clinical trials) is provided in Tables 1 and 2 below.

LITERATURE REVIEW
Preclinical studies are enabling us to better understand pancreatic cancer well and learn about the most important therapeutic target genes, which are very potential, including suicide genes, tumor suppressor genes, promoting-apoptosis and anti-angiogenic genes[10]. In 2018, a prospective study conducted by Hu et al showed that germline MLH1, tumor protein p53, ATM, BRCA1, BRCA2 and cyclin-dependent kinase inhibitor 2A mutations were significantly connected to an elevated probability of pancreatic cancer in an analysis of 3,030 individuals[11]. The role of Kras gene studies shows similar results in tumor growth[12], in DNA inactivation studies; maintenance genes, including PALB2, BRCA1 and BRCA2 indicated that pancreatic cancer with DNA which involve defective DNA maintenance genes are more and more responsive to platinum chemotherapy as well[13]. As a result, there are some promising therapeutic targets in preclinical trials. For pancreatic cancer patients, MiR-98-5p is a potential therapeutic target because it promotes pancreatic cancer by downregulating MAP4K4 and blocking the downstream MAPK/ERK pathway[14].

Table 2: Summary of ongoing clinical trials

NCT	Carrier	Title	Phases	Conditions	Reg.	Study Started	Study Finished	Ref.
NCT00415454	Adenovirus	PDAC gene therapy prodrug transforming genes with Chemoradiation for non-metastatic pancreatic cancer	Phase I	Pancreatic Carcinoma	8	11-2006	N/A	
NCT01274455	Plasmid DNA	PDAC gene therapy	Phase I	Pancreatic Carcinoma	22	12-2010	03-2013	[22]
NCT02806687	Plasmid DNA	Intratumoral gene therapy for pancreatic cancer	Phase II	Pancreatic Carcinoma	100	01-2017	06-2019	
NCT02894944	Adenovirus	Theragene phase I trial Pancreatic cancer chemotherapy	Phase I	Pancreatic Carcinoma	9	08-2016	07-2018	
NCT00121745	Retrovirus	Mx-dnG1 Gene confer for altered Pancreatic Cancer	Phase I	Pancreatic Carcinoma	12	07-2005	07-2007	[152]
NCT03165188	Immunotherapy	Long-term follow-up of algenpantucel-L immunotherapy patients	N/A	Pancreatic Carcinoma	500	09-2017	06-2031	
NCT01583686	CAR-T	CAR-T targeting mesothelin in metastatic cancer patients	Phase I/ Phase II	Pancreatic Carcinoma	136	04-2012	12-2028	
NCT02830724	CAR-T	The transfected peripheral blood lymphocytes were given human chimeric antigen receptor expressing CD70 cancer	Phase I/ Phase II	Pancreatic Carcinoma	113	04-2017	01-2028	
NCT00638612	Adenovirus	Surgical and chemotherapy for Adv. TK Pancreatic cancer (PaTK02)	Phase I	Pancreatic Carcinoma	27	08-2008	06-2015	
NCT02465983	CAR-T	Artificial T cell receptors in metastasis pancreatic cancer	Phase I	Pancreatic Carcinoma	4	05-2015	11-2017	
NCT03190941	Immunotherapy	Transgenic peripheral blood cells recognise G12V and Ras mutations in HLA-A * 1102 individuals	Phase I/ Phase II	Pancreatic Carcinoma	110	09-2017	06-2028	
NCT03225989	Adenovirus	Experimental study on tumor oncolytic by immune stimulation adenovirus for cancer	Phase I/ Phase II	Pancreatic Carcinoma	50	03-2018	12-2022	
NCT03192462	Immunotherapy	Pancreatic cancer patients' TAA-specific cytotoxic T cells	Phase I/ Phase II	Pancreatic Carcinoma	45	01-2018	11-2025	
NCT00004178	Immunotherapy	Gene addiction for cases with pancreatic cancer	Phase I	Pancreatic Carcinoma	Null	04-1998	12-2001	
NCT00084383	Cancer vaccine	Adjuvant chemoradiotherapy and vaccination treatment Grade 1 and 2 pancreatic cancers	Phase II	Pancreatic Carcinoma	60	01-2002	07-2006	[131]
NCT00836407	Cancer vaccine	Patients treated with ipilimumab + / - vaccine. locally advanced, non-resectable or metastatic pancreatic cancer	Phase I	Pancreatic Carcinoma	30	02-2009	07-2012	[130]
NCT02750657	Genetic Profiling	Study of patient genetic changes and characteristics better treatment options with pancreatic cancer	N/A	Pancreatic Carcinoma	180	12-2015	12-2021	
NCT00303927	Genetic Profiling	Capecitabine as a second-line treatment with TSG for stage IV pancreatic cancer	Phase II	Pancreatic Carcinoma	65	12-2005	N/A	
NCT01188109	Genetic Profiling	Pancreas cancer resection Gemcitabine/Cisplatin establishes ERCC2's function in therapy decision-making	Phase II	Pancreatic Carcinoma	25	07-2010	07-2015	
NCT00389610	Cancer vaccine	Vaccines are used in surgically removed patients with pancreatic cancer	Phase II	Pancreatic Carcinoma	56	09-2006	12-2018	
NCT01394120	For Targeted and Tailored Treatment	Chemotherapy scheme based on treatment target. advanced pancreatic cancer	Phase II	Pancreatic Carcinoma	60	08-2011	12-2013	
NCT00066404	Recombinant adenovirus	Intrapleural BG00002 treat malignant pleural effusions or malignant pleural mesothelioma in patients	Phase I	Pancreatic Carcinoma	N/A	04-2003	N/A	

NCT01474564	Genetic Profiling	Collect and analyse circulating tumorigenic cells from pancreatic cancer patients	N/A	Pancreatic Carcinoma	60	11-2011	11-2019	
NCT02405585	Immunotherapy	Immunotherapy and SBRT studies of pancreatic cancer with borderline resectable	Phase II	Pancreatic Carcinoma	10	04-2016	Not Applicable	[22]
NCT02705196	Adenovirus	Treatment of pancreatic cancer with LOAd704 oncolytic virus	Phase I/ Phase II	Pancreatic Carcinoma	26	11-2016	08-2019	
NCT00669734	Cancer vaccine	Vaccine and Sargramostim cure unresectable pancreatic cancer	Phase I	Pancreatic Carcinoma	18	02-2011	Not Applicable	
NCT00727441	Vaccine for Cancer	Patients with stage I pancreatic cancer are treated with or without cyclophosphamide, vaccination, chemotherapy, and radiation	N/A	Pancreatic Carcinoma	87	07-2008	03-2018	[152]
NCT00947102	Observation	Gemcitabine and pancreatic cancer immunity and serology	Not Applicable	Pancreatic Carcinoma	N/A	02-2009	12-2011	
NCT00051467	Adenovirus	TNFerade biological study of 5-FU and radiation local therapy that cannot be resected with first-line therapy advanced pancreatic cancer	Phase III	Pancreatic Carcinoma	N/A	N/A	N/A	
NCT03531125	Endoscopic Ultrasound Procedure	Easy-to-resect pancreatic cancer genes	Not Applicable	Pancreatic Carcinoma	10	06-2018	12-2019	
NCT00429858	Chemotherapy	Gemcitabine / S-2 in pancreatic cancer	Phase II	Pancreatic Carcinoma	21	01-2007	10-2010	[131]
NCT02568267	Genetic Profiling	Management of solid tumours with NTRK 1/2/3/ROS2 or ALK gene rearrangement with Entrectinib (RXDX-101)	Phase II	Phase II	300	11-2015	10-2020	[130]
NCT00159471	Genetic Profiling	Genes as on docetaxel, capecitabine (GTX) and gemcitabine, are used in metastatic or non-resectable pancreatic cancer	N/A	Pancreatic Carcinoma	1	02-2005	07-2006	
NCT00386399	Genetic Profiling	BRCA3 mutation's effect on Mitomycin C in pancreatic cancer	Phase II	Pancreatic Carcinoma	0	10-2006	02-2008	
NCT01836432	Immunotherapy	Immunotherapy for localised pancreatic cancer	Phase III	Pancreatic Carcinoma	302	05-2013	06-2017	
NCT00255827	Cancer vaccine	Vaccine therapy for pancreatic cancer which is surgically resectable	Phase I/ Phase II	Pancreatic Carcinoma	7	11-2005	11-2007	
NCT01938716	Chemotherapy	Preoperative Gemcitabine pharmacokinetics chemo-radiation therapy	N/A	Pancreatic Carcinoma	40	03-2012	03-2019	
NCT03193190	Immunotherapy	Therapeutic studies sased on multiplex immunotherapy combine with participants in metastatic pancreatic cancer. ductal adenocarcinoma	Phase I/ Phase II	Pancreatic Carcinoma	185	07-2017	09-2020	
NCT00089024	Chemotherapy	Radiotherapy combined with CTX for LAPC	Phase II	Pancreatic Carcinoma	50	02-2003	Not Applicable	
NCT02465060	Genetic Profiling	Gene detection oriented targeted therapy for multiple myeloma, lymphoma or advanced refractory solid tumor	Phase II	Pancreatic Carcinoma	6452	08-2014	Not Applicable	
NCT01191684	Vaccine for cancer	Pancreatic, Colorectal, and Stomach Cancer Vaccine	Phase I	Pancreatic Carcinoma	12	10-2011	08-2013	
NCT01088789	Plasmid DNA	Experimental study on enhancing immunity with pancreatic tumor cell vaccine	Phase II	Pancreatic Carcinoma	72	04-2010	04-2023	
NCT02514421	Device of Electroporation	Safety and efficacy evaluation of electrochemical therapy Treatment of pancreatic cancer	N/A	Pancreatic Carcinoma	24	07-2015	07-2017	
NCT02414100	Genetic Profiling	The molecular changes of patients with primary pancreatic cancer were identified by gemcitabine hydrochloride-based chemotherapy	N/A	Pancreatic Carcinoma	0	12-2013	12-2016	
NCT00936104	Genetic Profiling	Side Group in Pancreatic Ductal Adenocarcinoma	N/A	Pancreatic Carcinoma	20	08-2008	07-2012	

NCT Number	Category	Description	Phase	Condition	N	Start	End	Ref
NCT03302637	Genetic Profiling	Pancreatic cancer and oral microbiome	N/A	Pancreatic Carcinoma	732	12-1992	12-2010	
NCT03602079	Genetic Profiling	A166 study in patients with reperfusion / refractory cancer Expression of HER2 antigen or amplified HER3 gene	Phase I/ Phase II	Pancreatic Carcinoma	82	07-2018	05-2021	[22]
NCT03337087	Chemotherapy	Liposomal irinotecan, fluorouracil, Rucaparib, and Leucovorin Calcium in the treatment of GERD, Biliary, colorectal or metastatic pancreatic Cancer	Phase I/ Phase II	Pancreatic Carcinoma	110	08-2018	12-2022	[152]
NCT00128622	Cancer vaccine	Denileukin Diftitox was subsequently treated with the vaccine therapy	Phase I	Pancreatic Carcinoma	24	09-2005	05-2009	
NCT02592395	Device of Electroporation	Patients with metastatic cancer study on FOLFIRINOX electro chemotherapy treatment of pancreatic cancer	Phase I	Pancreatic Carcinoma	0	10-2015	10-2017	
NCT02432963	Cancer vaccine	Pembrolizumab and vaccine treatment of patients with solid tumor which previous treatment failure	Phase I	Pancreatic Carcinoma	19	11-2015	02-2019	
NCT00959946	Chemotherapy	Study on bosutinib combined with capecitabine in the treatment of solid tumors. and metastatic or advancement in local breast tumor	Phase I/ Phase II	Pancreatic Carcinoma	32	09-2009	03-2011	[131]
NCT01643499	Genetic Profiling	Folfirinox genotype guided chemotherapy dose in patients with advanced gastrointestinal malignancies	Phase I	Pancreatic Carcinoma	79	03-2012	08-2020	[130]
NCT02576665	Retrovirus	The use of Toca 511 and Toca FC, two retroviral duplicating vectors, in the management of solid tumours such as lymphomas is being investigated(Toca seven)	Phase I	Pancreatic Carcinoma	30	07-2016	11-2019	
NCT00711997	Plasmid DNA	Non-resectable pancreatic cancer patients with stage 1/2a DTA-H19	Phase I/ Phase II	Pancreatic Carcinoma	9	08-2009	12-2010	
NCT02239861	Immunotherapy	TAA solid tumor specific CTL (TACTASOM)	Phase I	Pancreatic Carcinoma	16	04-2015	12-2018	
NCT03281382	Adenovirus	Interleukin 13 Gene Therapy phase 1 clinical trial for metastatic pancreatic cancer	Phase I	Pancreatic Carcinoma	9	07-2017	06-2021	
NCT02340117	Immunotherapy	gemcitabine / paclitaxel Combined SGT-54 in the treatment metastatic pancreatic adenocarcinoma	Phase II	Pancreatic Carcinoma	28	01-2015	12-2020	
NCT00868114	Cell	Application of direct injection of KLH-Pulsed dendritic cells in non-resectable pancreatic carcinoma	Phase II	Pancreatic Carcinoma	35	07-2006	12-2015	
NCT01437007	RNAi	Primary or secondary liver cancer, TKM 080302 Used	Phase I	Pancreatic Carcinoma	1	08-2011	06-2012	
NCT02416466	CAR-T	For liver metastases, chimeric immunoreceptors, Hepatic arterial infusion (HAI) and SIR-Spheres microspheres are used	Phase I	Pancreatic Carcinoma	8	04-2015	01-2019	
NCT01116791	The procedure of debulking surgery Plus	Intraoperative peritoneal chemotherapy (HIPC) in Cytoreductive surgery (CRS) plus hyperthermia combined with Cisplatin for peritoneal cancer of upper gastrointestinal cancer	Phase II	Pancreatic Carcinoma	34	07-2010	12-2015	
NCT02315625	Hyperthermia and HIPEC Genetic Profiling	Sunitinib or everolimus mutations are targets for the treatment of advanced gastrointestinal and pancreatic low-grade neuroendocrine tumors, with or without Cytoreduction	Phase II	Pancreatic Carcinoma	120	04-2015	12-2025	
NCT00444444	N/A	Genetic analysis (AIP) is used to predict recurrence during steroid therapy for autoimmune Pancreatitis	N/A	Pancreatic Carcinoma	40	02-2002	06-2007	

In the same way, splat like protein 4 induces endothelial mesenchymal transition in the cells of PDAC to promote metastasis potential, suggesting that splat like protein 4 somehow may be a marker for the management of pancreatic ductal adenocarcinoma; targeting this type of protein may provide anti-proliferation and anti-metastasis therapy[15]. Contemporary studies have shown that the short palindrome repeat / CRISPR-associated protein nine (Cas9) genome-edit tools can reduce the migration and proliferation propensity of MIA PaCa-2 pancreatic cancer cell line by knocking out epithelial cell transformation two genes[16]. In addition, in the three tumor-infiltrating immune cell markers (CD206, CD117, CD15), there was a smad4 mutation discovered to be associated with patient survival and recurrence following PDAC surgical treatment[17]. The mechanism of carcinogenesis driven by G protein alpha, SIKs, showed Kras mutation in pancreatic tumors, and there is evidence that it is a potential tumor inhibitor[18].

However, other preclinical studies have shown a relationship between gene expression and chemotherapy[19]. P53 dependent gene expression and gemcitabine-mediated tumor suppression increases the prospect of focusing the Bax-dependent cell death route instead of the p53 upregulated modulator of the apoptosis pathway, which may expressively improve the patients' outcome and prognosis with pancreatic tumors[20]. In phases I and II trial of gemcitabine (GEM)-resistant pancreatic cancer, intravenous dosing of the Rexin G™ showed excellent efficacy and safety[21]. Endoscopic ultrasonography was found to be safe for intratumoral injection of a non-viral gene therapy product in the phase I experiment involving 22 patients receiving gemcitabine concurrently (CYL-02)[22]. On the other hand, to assess the efficacy of RNAi targeting Kras in treating locally advanced pancreatic cancer, a combination of RNAi targeting Kras and chemotherapy can be used[23]. Among these, G protein plays a critical function in coupling the family C, member five and group A genes in MIA PaCa-2 cells, resulting in an increase in drug resistance in all of those MIA PaCa-2 cells[24].

All of the mentioned data can help implement personalized treatment of pancreatic cancer patients to improve the prognosis. Ongoing research aims to create actual gene therapy for pain and infection of the pancreas, in addition to pancreatic cancer. These conclusions are based on numerous completed clinical studies (Table 1) and several continuing clinical studies at present (Table 2). So, gene therapy as a safe and effective method provides a promising way[25].

For gene therapy, several technologies including molecular tools, viral and synthetic vectors, and genome editing procedures, have shown outstanding outcomes in bridging gaps between clinical trials (cancer-related) and experimental models of cancer[10,26]. Hence, we also summarize gene transfer procedures to the pancreas in this review of pancreatic cancer (Table 3).

Gene therapy for pancreatic cancer: diagnostic methods and target specificity

There are several unique gene therapy strategies and experimental models of pancreatic cancer. First of all, it needs an effective vector, and the ability to transfer a large number of genes expressed may not be extended. It should be remembered that an inflammatory response is associated with the use of many vectors, which is less critical than other adaptations, as we know that the drug-gene tests are not planned to be used regularly and continuously. The anatomical positioning of the pancreas is important because the deep positioning of the organ is closely related to the abundance of blood vessel formation and nearby structures. According to Buscail et al, from a scientific point of view, imaging technology is essential for diagnosing and executing stages of PDAC. For diagnosis and executing stages of pancreatic cancer therapies, computed tomodensitometry is to be done along with particularly vital endoscopic ultrasound (EUS) procedures. EUS combines endoscopes with high-resolution echography which is placed with high frequency ultrasound devices at tip of the endoscope. Under ultrasound imaging for pancreatic lesions, fine needle aspiration can be done, and EUS is the primary technique to confirm the pathology of PDAC. This gene therapy product can be quickly and safely completed by injecting therapeutic compounds into the same tumor[27].

According to Rouanet et al and Klemm et al, pancreatic cancer cells are known to be invasive, with high proliferation rates and solid local invasion and metastasis properties (peritoneum, liver and lymph nodes). Growth factors and their receptors, proangiogenic factors and increased invasive factors are engaged in this proliferation and aggression. Also, regardless of tumor size, this invasiveness occurs and has a remarkable effect on the tumor micro-environment. This micro-environment also shows a crucial role in the invasion and metastasis of pancreatic cancer. We are shown that there is a close connection between cancer cells, pancreatic stellate cells and extracellular substitutes causing tumor hypoxia, insufficient vascular formation and fibrosis, resulting in demanding access to the drug[10,28]. The genetic basis of PDAC is well established; in the majority of pancreatic cancers, the tumor protein P53, the SMAD family member 4/deleted in pancreatic

Table 3: Clinical and pre-clinical stages need the utilization of gene delivery techniques.

Methods of Gene Transfer	Functional Components	Targeted Genes	Characteristic	Characteristic
Vector Virus				
Foamy virus	Ribonucleic acid (RNA)	Pro-cell death genes, prodrug transforming genes, anti-oncogenes genes, siRNA, and microRNA	Extremely effective	Random integration and low titer
Oncoretro-virus	Ribonucleic acid (RNA)		Sustained and efficient gene expression	Random integration and low titer
Lentivirus	Ribonucleic acid (RNA)		Sustained and efficient gene expression	Random integration and low titer
HSV (Herpes simplex virus)	Double Helix DNA		Extremely effective, long-lasting gene expression, infect non-dividing cells	Host innate immune response
AAV (deno-associated virus)	Single-stranded DNA		No harmful, persistent gene expression infects cells that are not dividing	Integration may occur, the transgene's capability is limited, and the titer is low
Adenovirus	Double Helix DNA		Without integration, gene expression is not sustained	Inefficient transduction
(Chemicals) Non-viral vectors				
Proteins	Cationic lipids Natural or artificially modified		In vitro efficacy, simplicity of preparation	Low effectiveness in vivo, acute immune response
Polymers	Cationic polymers		In vitro, highly effective, and simple to prepare	Toxic to cells elicits an inflammatory response
Lipids	Cationic proteins		It is highly effective in vitro and has low toxicity, and can be directed at specific targets	low activity in vivo,
Peptides	Peptide lysine or arginine residues		It is highly effective in vitro and has low toxicity, and can be directed at specific targets	low activity in vivo,
(Physical Methods) Non-viral vectors				
Injection via needle	Mechanic force		Easy	Efficacy is low, and expression is restricted to the needle track.
Helios gene gun	Pressure		Adequate efficiency	Restricted to the target location, internal organ surgery required
Electropermeabilization	Electric pulse		High-level efficiency	Damage to the tissue, a small target area, and the requirement for an internal organ surgical procedure
Cellular sonication	Ultrasonography		Site-specific	Ineffectiveness, tissue damage
Magnetic and transfection	Magnetic field		Site-specific	Inefficient, limited target region necessitates internal organ surgery
Hydrodynamic gene delivery (HD)	Hydrodynamic pressure		Easy, increased efficiency, site-specific	Large animals need catheter insertion technology.
Cancer Immuno-therapy	Cytokine		Ex vivo cell culture required	
Adoptive Immuno-therapy	Chimeric immunoreceptors		Ex vivo cell culture required	
Immunization	Pulsing dendritic cells (DCs)		Administration through IV, subcutaneous, or local route	

cancer 4, and the INK4a/ARF tumor suppression routes are hereditarily inhibited (associated with hemodynamic deficiencies of 9p21, 17p or 18q, respectively). At the same time, the carcinogenic (KRAS) pathway is also stimulated[29-31]. Telomerase activity increases as tumors progress through the late stages of development. However, the main event remains the activation site mutation at codon twelve (exon two) of the Kras oncogene[12,31,32].

Most of the animal models employed in preclinical gene therapy investigations have been performed on pancreatic cancerous cell lines because they are amenable to ectopic and in situ fixation into athymic nude mouse. These models are less immune active. However, the mice themselves produce subunit reactions. Other in vivo models are genetically modified, including mouse models showing G12D Kras mutations[33]. Furthermore, crossed models can be used in animals carrying TP53 or other tumor suppressor genes knocked out[34,35]. It usually takes some time for transgenic mice to obtain primary PDAC[33-35].

In conclusion, humanized models, patient-derived xenografts can be applied in theory but need initial characterization in variation, somatic cell mutation, chemotherapy sensitivity, or drug resistance. That is why a large number of immune modes whose phenotypic and genotype characteristics were closely related to the model of humans are observed according to pancreatic cancer or orthotopic allotransplantation cells in hamsters. This is a rapidly breeding hamster model, but there is a shortage of molecular tools for hamster research[36-38].

On the other side, it has been proved many years ago that it is difficult for cancer cell of human pancreas to transfect with numerous synthetic vectors as well as adenoviruses[36,37]. While using viral vectors often results in higher DNA transfection and expression rates, not all models can be employed well. Some models require specialized receptors, and human viruses do not always operate in organs or rodents' cells. Finally, two things are essential when considering PDAC's gene therapy strategies:

1) 80% of tumors progress rapidly, with a medium survival of 8-11 months[38-40]. It should be noted that chemotherapy usually has to be incorporated into the treatment strategy (before it is used as first-line treatment or combined with gene therapy). Chemotherapy should be integrated into the treatment strategy for other invasive and metastatic diseases. The time required to establish second line (ideal) treatment is concise.

2) Select the best gene: As soon as possible, we should review and study the carrier and future route of administration (frequency) in the clinical stage to "integrate it into all stages of clinical development" proof of concept, preclinical trials (expand the production of gene therapy, toxicology, biodistribution) and the first phase.

Gene-therapy administration strategies

There are three main gene delivery methods: in vitro, in vivo and situ. We collect cells from patients in vitro gene therapy, further modify those cells with vectors carrying therapeutic genes, and back all those modified gene cells in the same patient[10]. This gene delivery process is very suitable for blood stem cells because this method is clinically used in gene therapy of hemoglobinopathy or immune deficiency syndrome[41,42]. This strategy requires in vitro culture, and adult stem cell samples that are used in cell therapy. This was done several times and took at least 4-8 weeks. For PDAC and other tumors with rapid growth and invasion processes, in vitro approach isn't appropriate, and theoretically, "ready to use" gene therapy products are the best. Another type of these therapies is gene in vivo therapy. It involves the direct injection of vectors carrying therapeutic genes into the bloodstream. So, in this type of gene therapy, the injection is simple and easy both ways (intravenous or intra-arterial) but will add additional obstacles, such as blood and blood vessels. Several factors in circulating blood in the human body remove foreign body (DNA) and its carriers, endonucleases and macrophages. If the transgene must reach the organ, it has to cross the capillary and endothelial cell barriers. In situ is the third kind of gene therapy, which directly transfers therapeutic genes to the targeted cells or tumor. This kind of method is particularly suitable for cystic fibrosis, myopathy (e.g., injection of vectors carrying Duchenne myopathy gene dystrophy into muscles), and cancer, to inject vectors carrying therapeutic genes into tumors. This method needs two things: one is a good targeting of organs, and the other is an effective carrier. In other words, this can be performed by doctors or surgeons through simple needles or catheters, which are mainly physical methods. In PDAC patients, the injection of therapeutic genes is guided by the surgical procedure, performed by radiologists under ultrasound or CT scanning, and finally by gastroenterologists under EUS. This is the most common accurate technology, regardless of the location of PDAC in the pancreas, which can target primary PDAC. This seems to be the most active approach for PDAC gene therapy. Still, it also requires the particular and selective direction of PDAC cell gene therapy products. This presupposes that one specific promoter represents a "molecular target" or a specific viral direction. Since molecular targeting is ideally potential, it must also use PDAC cell-specific promoters. Unfortunately, although MUC-1 and mesothelin appear to be the most significant candidates for highly particular transgene expression in PDAC cells, no distinct promoters exist. In situ gene therapy seems to be the most effective treatment option for PDAC.

Vector-based gene therapy

In the 2014 study by Yin et al, we note that there is no apparent advantage in the cancer therapy of naked DNA; synthetic vectors also have an advantage in the chemical industry for easy production. The products themselves are relatively harmless and trigger gene therapy properties, combining all nucleic acids. There are also two major synthetic carriers: which are lipid and multi-cation[43]. Completely pure lipid carriers can encapsulate DNA and promote DNA to pass through a composed cell membrane, a lipid bilayer. For transferring genes into human PDAC cells, these vectors do not seem to have succeeded because they have speedily changed into mixed vectors associated with lipid and cationic fractions. The role of the cationic

portion is straight: the molecules are a series of positive charges that are encapsulated and recombined with negatively charged DNA. Due to the cationic part of the carrier, the carrier encapsulates DNA to promote the membrane pathway of the lipid part and is protected when it "passes" through the cytoplasm into the nucleus.

Finally, according to Yin *et al*, if the mixed carrier performs better than the pure lipid carrier in PDAC cells, it can induce marker DNA transfection. The second kind of composite vector seems to be more attractive. Cationic polymers or polycation are the vectors derived from polylysine and polyethyleneimine (PEI). Therefore, a polylysine carrier, a vital amino acid polymer lysine, can concentrate DNA and protect DNA from lysosomal digestion. Some of the chemical groups, like polyethylene glycol, are used to improve the stability of blood and the internal environment or reduce cytotoxicity.

We also found that PEI is a perfect polycation with a linear structure, which can promote the nuclear and cellular diffusion of DNA by providing decent opposition for digestion action of the lysosome. Some carriers are formed from PEI, and some of the carriers are also added by chemists, which are polyethylene glycol, mannose, cholesterol, glucose, and many other chemical compounds[44-49]. In patients, some of these DNA complex vectors have been tested with no significant adverse reactions of administration at any site of the body (ovarian cancer, lung cancer, lymphocyte, bladder cancer and subcutaneous vaccination). Experimental studies of PEI and safety have been directed in patients with PDAC (including our primary experience) and may be effective until the ongoing phase II trial results are available. Lastly, it must be well-known that DNA polymerized complex has specific structures, particularly close to the size of the manometer. Therefore, these manometers are known as "nano-complexes" or "DNA nanoparticles."

In addition, viral and synthetic vectors have been developed to infect human cells due to their natural characteristics[43,50,51] (Table 4). Adenovirus infection is the leading cause of head, neck, lung and digestive tract infections of children and adults. They infect many cells; therefore, adenovirus-derived vectors are widely used in clinical and preclinical strategic gene therapy for PDAC. They also prompt the process of transient expression and produce a solid immune response. Many clinical trials of adenovirus vectors have been carried out, and no significant events have occurred. To overcome some toxicity problems, the 3rd generation adenovirus vector was used lacking viral sequence and has been developed[50-52].

Adeno-associated virus (AAV) is a nonpathogenic and naturally defective virus that widely exists in humans. AAV comes from the parvo-virus family and is used as single-stranded DNA carriers, without packaging and small in size, to divide cells and transfer quiescent cells. They are randomly integrated and rare, so they have a low mutation risk. That's why the term "associated adenovirus vectors" is used since the parent strain, adeno-associated satellite (AAS), depends on some other viruses such as the existence of adenoviruses, human papillomavirus, or herpes simplex virus (HSV) for replication. These viruses can successfully transfer cells from the liver, brain and other body parts, especially blood cells, but are hardly detected in human PDAC cells. Retroviruses and lentiviruses are from the Retroviridae family. Some tiny, encapsulated RNA vectors can effectively transduce many cells of their interest, are not more immunogenic, and are also easy to produce.

However, the disadvantage of these vectors is that they integrate into the chromatin open region of the

Table 4: The primary features and viral vectors employed in the preclinical and clinical techniques of gene addiction in cases with pancreatic cancer.

Virus	Target Cells	Insert Capacity	Delivery	Transgene Expression	Level of Expression	Pre-existing Immunity	Biosafety
Adenovirus (Vector)	Divided / Not Divided	36 KB	Ex Vivo	Transient	High	Yes	Immunogenicity, inflammation, unconformity
Adeno-Associated (Vector)	Divided / Not Divided	4.7 KB	Ex Vivo	Steady	Medium	Yes	Mutational integration
Retroviral (Vector)	Divided / Not Divided	7 KB	Ex Vivo	Steady	Medium	Negative	Mutational integration
Lentiviral (Vector)	Divided / Not Divided	11 KB	Ex Vivo	Steady	High	No	Mutation integration and WT HIV recombination
Herpes Simplex Virus (Vector)	Divided / Not Divided	29 KB	Ex Vivo	Transient	High	Yes	Mutational integration, neurotoxicity
Pox and vaccinia virus (Vector)	Divided / Not Divided	24 KB	In Vivo	Steady	Peak	Negative	Immunologic adjuvant to immunization
Simian virus 40	Divided / Not Divided	6 KB	Ex Vivo	Steady	Medium	Negative	Integrating mutational

target cell nucleus and have the risk of mutation. They are primarily used in vitro, but they can transduce proliferating cells, for example, in cancer cells that allow in situ administration[41,42,51,52]. A new group of lentiviral and retroviral vectors have been improved to prevent oncogene transactivation upon integration into host cells, hence, the term self-activating vector, which avoids the trans-activation of oncogenes (such as LMO oncogenes). Successfully tested, the improved lentivirus is becoming a part of the PDAC model using an integral defect lentivirus vector[53].

It has been hypothesised by some researchers that HSV (HSV-1 in particular) are the key instrument for gene therapy since their benefits and disadvantages are so unlike to those of the AAV virus. The herpes simplex virus type 1 (HSV-1) is capable of transferring a substantial quantity of Deoxyribonucleic acid and is highly immunogenic. Compared with most other carotid artery resections, the virus has a solid predisposition to nerve cells. Other viruses generated from more hazardous viruses, including bovine pox, acne or murine parainfluenza virus type 1, have been used to transfer genes in vivo and in vitro studies. Despite their promise in vaccination approaches and in vivo dosage, their utility in gene therapy remains restricted. Numerous viruses formed from other pathogenic viruses, which are cowpox, Sendai or acne virus, have been used in vivo and vitro to transfer genes. Although they are only used infrequently in gene therapy, they have demonstrated potential in vaccination or in vivo approaches. The virus vectors and main virus characteristics are summarized in Table 4. Several writers have added their notion of "biological vectors" to the list in Table 4, recognizing that decreased bacterial strains can be easily used for vaccination and gene therapy via the introduction of therapeutic genetically modified organisms[52,54]. Finally, it is worth emphasizing that the adaptation of nanotechnology and nanomaterials to DNA transport has significantly expanded the endpoint of gene transfer[55].

The carrier type dictates the administration route (vectors). Ex vivo drug delivery is typically done with lentiviral and retroviral vectors, while in situ drug delivery is done with AAV, adenovirus and synthetic vectors. Intravenously or subcutaneously, an oncolytic virus can be injected with plasmid DNA and transfected cells or bacteria, whether a gene delivery system or a PDAC solid tumor; reaching all tumor cells is challenging throughout chemotherapy. One can benefit from the situ method from radiologic targeting, but it is not known how many cancer cells have been metastasized. Gene transfers in vivo can be blocked by natural barriers such as nuclease, capillary, blood flow and tumor matrix. Once again, targeting specific "robust" molecules (promoter-driven genes of interest or molecular model genes) in pancreatic cancer cells is the best way.

Expression of a therapeutic gene and preclinical development of a therapeutic approach

The most often employed strategies and instruments in cancer gene therapy are copying defective genes, introducing therapeutic genes that are absent or under-expressed, such as cytokines or suicide genes, or inhibiting non-coding gene expression. On the other side, no matter what drug delivery strategy (in vivo or situ) is used, most tumors should not be expected to achieve gene transfer and gene expression. Most persistent barriers need to be got over, such as entry into cancerous cells, nuclear invasion and intracellular, protein, mature mRNA, and expression. In the experiment, the researcher injected Pei or adenovirus complex in situ with a complex plasmid vector and obtained up to 15% primary tumor cells[36-38]. This percentage means obtaining the bypass effect, which is expected to produce an apparent anti-tumor impact. This prerequisite must be studied well to prove the concept of any epithelial cancer gene therapy program. This negative outcome was first reported following in vitro and in vivo transfers, particularly when prodrug transforming genes were used. As pro-apoptotic signals spread, it often activates pro-apoptotic medicines and anti-angiogenic paracrine signals. Methods for PDAC gene therapy in preclinical settings are detailed in (Table 5).

Oncogenes that inhibit tumor growth

This study aims to better understand the transduction of an oncogene lost in the carcinogenesis process. The gene encoding TP53 is the most famous tumor suppressor gene, a protein controlling

Table 5: Gene therapy for pancreatic cancer: main approaches and experimental investigation

Strategies	Gene's Exhaustive
Adoptive immunotherapy of cells	CAR-T cells (focusing mesothelin)
Immunization	Dendritic cells that have been stimulated DNA, peptides, artificial cells, and bacteria are all examples
Active immunotherapy	Expression of interleukin (IL) and expression of cytokine
Genetic invalidation	Kirsten rat sarcoma virus, ERBB2, MDR1, hsa-mir-21
Transfer Gene	Anti-oncogenes (protein16, Tumor protein 53, Smad4 (DPC4), Antiangiogenesis-related genes and involved in apoptosis (Trail, TNF)

apoptosis and mutation of 50% of tumor cells. The direct inhibition of TP53 in vitro on tumor growth and neoangiogenesis in vivo complement each other. Treatment of liver, stomach, lung, colon, ovarian, and head and neck cancers have all been achieved using the TP53 gene transfer technique. The overall results are still poor, which usually manifest very low efficacy of gene transfer in vivo and different cell type sensitivity. Even the TP53 mutation in tumors is inconsistent. However, it is worth noting that the 1st licensed gene therapy drug for treatment of cancer includes the transfer of the p53 gene. Along with TP53, additional tumor suppressor genes such as DPC, p21WAF1 or p16INK4A have been identified[20,52,56-61].

Genes that inhibit angiogenesis and promote cell death

Numerous techniques, including gene transfer of anti-angiogenic molecules, have been probed in all models (in vivo and vitro), including thrombospondin-1, endostatin, soluble vascular endothelial growth factor receptor, angiostatin and vasostatin[26,52,62-68]. Restoring the somatostatin receptor two subtype gene (which is deleted in PDAC) in animal models of cancer has been shown to have both local and systemic anti-tumor effects. The side effects are facilitated by apoptosis and antiangiogenesis, mediated by a negative secretory loop persuaded by natural ligand growth inhibitor (somatostatin) gene transfer[36,37].

Prodrug transforming genes

One of the most significant issues is that the gene responsible for suicide transmission has a proximal solid anti-tumor effect that can compensate for the tumor's lack of gene expression. The most well-known sample of this suicide gene strategy is thymidine kinase gene, which is herpes virus. Typically, this kind of gene encoding the thymidine kinase enzyme responsible for the metabolization of ganciclovir into a dangerous substance by an antiviral drug, which inhibits DNA replication and predominantly induces death, has no antitumor effect. The approach supplies sensitive genes and their substrates based on conditional toxicity. On the other hand, the gene is toxic to the cells in which it is produced, when the substrate (ganciclovir) is added, and its toxicity increases further. Thus, the latter is dangerous in its untreated state but becomes toxic to cells when phosphorylated with thymidine kinase. Following subsequent ganciclovir medication, just 10% of the cell population expresses enough. All cells die in culture increased ganciclovir phosphorylation expressing the HSV-TK enzyme results in remote toxicity.

Additionally, cells carrying the HSV-TK gene communicate information and enzymes related to apoptosis. In vivo, the system against tumor cells also induces a robust immune response. Tumor cells may release antigens, eliciting the body's response to other tumor cells as well, a phenomenon known as the distant bystander effect[69-71]. Several other instances of the suicide gene/prodrug gene system that were successfully tested in the PDAC model include the following: 5-fluorocytosine is converted to 5-fluorouracil by the cytosine deaminase gene[52,72,73]; nitroreductase gene transformed CB1954 into 4-hydroxy amine[74,75]; ifosfamide was converted to acrolein by the cytochrome P450 gene. Clinically, the cytochrome P450 / isophosphoramide system was cross developed in vitro and in vivo during the phase 1 follow-up of PDAC patients[76,77].

Gene therapy utilizing small interfering RNAs (miRNAs), antisense mRNAs and mRNA interference

In addition to the traditional use of DNA vectors (synthetic or viral vectors) for gene transfer, another strategy shown directly affects the differentiation and transcription of RNA and DNA. Since the late 1990s, small non-coding RNAs and the other two kinds of small RNAs, interfering RNAs and microRNAs, such as short hairpin RNA (shRNAs) and small interfering RNA (siRNAs), have been discovered.

Gene expression can be repressed at the transcriptional level thanks to these non-coding RNAs. Single-stranded RNAs, or microRNAs, have a nucleotide sequence between 21 and 24 bases long. These natural molecule post-transcriptional regulators can also silence genes. Target mRNA supplementation and sequence-specific pairing may prevent translational degradation of mRNA. Their primary functions are to control cell proliferation, death and differentiation. It's been found that in cancer patients, in particular, their failure to express might cause serious malfunction.

Large-scale expression studies, known as miRNA tumors, have made it possible to regulate the primary function of certain miRNAs in the growth of numerous tumors and the process leading to all these dysfunctions. Most of these types of miRNAs, known as cancer cells, can be reduced by gene therapy. It has been successfully proved that targeting oncogene miRNA-21 can significantly inhibit the full development of pancreatic cancer in vitro and in vivo in all aspects[78]. The translation of messenger RNA into protein and its subsequent destruction are both slowed when interference RNA (which can be single- or double-stranded and has 21 RNA nucleotides) is present. Similar to micro-immune cytochemistry (ICT), interfering ICT can "silence" a gene to inhibit

translation. Also, stems and rings made out of microscopic hairs or small RNAs (short hairy RNA) are required; they play a role in RNA interference. Protons or viral vectors are often used to drive the expression of shRNAs in cells. Eventually, RNA tools will steer clear of the nucleus altogether, skipping through the transcription and post-transcription phases of a cell's life cycle.

There have been several successful trials in vitro and in vivo, and numerous preclinical as well as clinical trials that are presently utilizing RNA intervention methodologies. In the same type of tools, there are other strategies to deal with gene expression directly. Additionally, when the target gene is expressed, a strand of nucleic acid can be synthesized to bind to it. This results in the imprisonment of genes (and sometimes even destruction). Additionally, it may impact the linking of pre-RNA and alter the messenger RNA (the exon content). The sequence of synthetic nucleic acid employed is referred to as the right sequence since it complements the messenger RNA sequence of the gene. As a result, oligonucleotides can be produced to inhibit the expression of specific genes via overdevelopment or expression[79].

Antisense, siRNA and ribosomal molecular methods target the Kras oncogene expression. The clinical results do not match the positive in vivo results[32,52,80-83]. One of the most challenging approaches developed recently is genome editing, which entails comprehensive group of procedures to change the genome by "recomposition genetic information," a time-consuming process that may be applied to bacteria, fungi, plants and animals. It is applied to the genome of human genome used as a tool of gene therapy, which some laboratories suggest. The three new systems mentioned below have biomedical research potential and the possibility of individualized medicine. The first two methods relied on nuclease cleavage via zinc-finger nucleases and transcription activator-like effector nucleases. The CRISPR-Cas9 system, our third most important tool, constitutes a genuine technical revolution up to this point. In 2012, it was recognized as a bacterial defense mechanism. Bacteria incorporate "memories" of previous infections into their DNA via CRISPR. When external DNA is detected, RNA can direct the Cas9 enzyme (also called CRISPR-related protein nine or endonuclease) into the exogenous DNA to remove it. Based on this idea, CRISPR-Cas9 artificial compound selectivity for cleavage and modification of specific DNA sequences (typically between 10 and 24 nucleotides) can be generated. Naturally, this is a unique tool for molecular biology and gene therapy: eliminate a particular damaging segment, replace it, and introduce a critical sequence to rapidly remodel the genome[84-86]. This strategy has been applied to gene therapy for HIV infection, malaria or lung cancer[84]. In various cancer models, including PDAC, gene transfer to the system is possible[85]. A question that needs to be solved is, due to the target, which key bases should be used to decrease cell proliferation or invasion? In addition to gene specificity (many candidate genes can be selected in the case of PDAC), we must pass the milestones of CRISPR-Cas9 gene therapy strategy technology, such as accuracy, effectiveness and safety.

Immuno-oncology and immunizations

The identification and clearance of tumor cells by the failure immune system is closely related to the occurrence and development of malignant tumors. PDAC is described by the connective tissue growth substations rich in inflammatory cells in the filtered fluid[86]. Although there is a vast number of immune cells in the substitute, they are primarily sub-groups of immunosuppression, such as regulatory T-cells, T-cells (Th) 17 cells, numerous myeloid cell subsets, myeloid-derived suppressor cells and tumor-associated macrophages[17,87,88]. Compared to many other solid tumors, in-tumor effector T-cells are rare and, when extant, they show very high levels of immune checkpoints, which is PD-1, indicating depletion. As detected in many cancers, PDAC survives the effects of micro-environmental remodeling. PDAC specifically promotes immunosuppressive micro-environments and tumor response. We can emphasize the unique role of PDAC astrocytes, whose side secretion signals are arbitrated by cytokines, which is IL-6 and granulocyte-macrophage collection stimulation factor (GM-CSF)[89]. Furthermore, cancer cells themselves express checkpoint molecules[90]. PDAC researched these immune checkpoint inhibitors. CTLA-4 and programmed death-ligand one inhibitor were explored in two clinical trials in metastatic PDAC patients. Inappropriately, we know that PDAC is not melanoma, and clinical results are unsatisfactory[87,91]. The PDAC micro-end environment generally comprises complex signaling networks between stellate cells, immune cells and PDAC cells, leading to immune suppression environment resistance to single-dose immunotherapy, including gene therapy[87,90].

As we know, there are so many immunotherapy strategies used in cancer treatment. More classic is the non-specific immunotherapy, the purpose of which is to dispose of cytokines (interleukins, interferon, etc.) with anti-tumor effects. To decrease the ratio of the side effects of systemic drug action, for induction of local interleukin or cytokines, gene therapy strategies have been developed through tumor cells themselves or by ex-vivo[52,92-97].

Preclinical studies on the production of interleukin and cytokines were carried out in vivo and in vitro. Most of the trials are positive as evidence of the concept of future clinical trials. There are several ways at the end of particular immunotherapy. The first is monoclonal antibodies which are placed against immunity, tumor growth, vascularization-related receptors or molecules. The second is adoptive immunotherapy, which involves the introduction of tumor antigen into lymphocytes, entails activating the tumor-immune system of patients and encouraging cancer cell detection and clearance. The patient's antigen-presenting cells or T lymphocytes are stimulated with antigens (known as "dendritic cells"). Gene therapy introduces a gene that encodes a protein associated with cancer detection and eradication via patients' several arteries. Mucin-1 (MUC-1) and mesothelin are two frequently utilized antigens in PDAC. The antigen-pulsed smart cell vaccine experiment was carried out on the PDAC model. New active immunotherapy for cancer has been produced based on embedding antigen receptors. Specifically, CD8+ T cells are isolated from a patient's body, then engineered to recognize and eliminate tumour cells that express tumour antigens. Lymphovirus (or retrovirus) vectors are used to deliver monoclonal antibody fragments (really many pieces, so-called "chimeric") into the patient's cells, which are subsequently transfused back into the patient. As well as the ongoing preliminary test for PDAC, many more preliminary tests for haematological malignancies include acute B-lymphocytic, B-lymphocytic lymphoma, chronic lymphocytic leukaemia, and leukaemia are planned or underway[98-102].

The last method of immunotherapy is to vaccinate tumors in peptides, proteins, tumor cell lysates, DNA protons, or recombinant viral vectors through systemic administration, local administration or subcutaneous. In addition to antigen-pulsed dendrites, various strategies have been developed: immunization using DNA encoding the VEGF receptor, peptide vaccination, CRS-207, TELOVAC, and GVAX [52,103-109].

Viral oncolysis

PDAC progression is followed by a series of variations which confer selective growth advantages to cancerous cells. Though, it is precisely because of these modifications (absence of interferon response, loss of cell cycle control and increased metabolic activity) that cancerous cells become particularly susceptible to viral infection. Therefore, selective replication virus (oncolytic) is an intriguing novel therapeutic approach. These viruses can naturally occur "cancer-specific" replication or require genetic alteration. Clinical trial outcomes from the last few years describe the oncolytics application in cancer treatment[110]. Priority replication when tumor cells are dividing until the death of the cell occurs, specificity tumor cells targeted dysfunction pathways (e.g., TP53), activation of precise and non-specific anti-tumor immune responses as well as the ultimate destruction of tumor stem cells are some of the mechanisms by which oncolytic viruses exert their anticancer activity[111]. The oncolytic virus is a naturally occurring pathogen that has been specifically selected or engineered to infect and put an end to cancerous cells.

All of these tumor viruses have been created in pharmaceutical laboratories and academic institutes worldwide in the last two decades. Cytokines, antigens or suicide genes can all be programmed into them. The adenovirus named oncorine H101 (made by Shanghai Sunway Biotech) is the first cancer virus approved for clinical usage. It has been designed and adjusted to multiply and destroy tumor cells with the p53 mutation preferentially. Virus Onyx-15, which it closely resembles, is close to this one. Onyx-15 has been tested to treat PDAC, a cancer of the neck and head. The second is OncoVex GM-CSF, which has been endorsed by the USFDA and the exponential moving average for the management of melanoma that has spread to other parts of the body due to the HSV-1.

Jenerex's GM-CSF-expressing poxvirus, JX-594, is undergoing clinical trials for hepatoma; Reolysin® is being studied for use against brain tumours; and Toca 511 (a retrovirus replication vector encoding yeast cytosine deaminase and cytosine adenine dinucleotide phosphate) is being studied for use against pancreatic cancer[112]. Using PDAC as an example, researchers examined the ability of different viruses in an experimental model to increase and prevent tumor growth[51,113,114]. Preclinical investigations have confirmed that conditional replication adenovirus can be utilized with gemcitabine to treat PDAC[115]. Onyx-15 intratumorally injected in patients in phase one and two clinical studies with PDAC has been demonstrated to be exceptionally well-tolerated compared to gemcitabine[116]. Recent studies from the German-French partnership Conditional Replication Adenovirus include changes to the hexon protein that enhance viral replication in stromal cells and tumor cells[117]. Since PDAC exhibits a less pronounced substring response, it impairs the dissemination of treatment medicines within the tumor.

Interestingly, there is mounting evidence that the PDAC microenvironment is not an impenetrable barrier but encourages the oncolytic virus' reproduction and therapeutic effectiveness[118]. Recently we confirmed that engineered HSV is highly effective at inhibiting the growth of experimental tumors, specially HSV-1 when used alone or in combination with gemcitabine[119]. Since HSV-1 does not unify into the

genomes of infected cells, it is secure to administer to a sick person who has been exposed to several infections of the tumour cell type. There are a variety of options for dealing with any adverse effects of anti-HSV-1 medication. In addition, even tiny doses of viruses can quickly eradicate an entire population of cancer cells. In order to limit HSV-1 reproduction to cancerous cells, it is common practice to delete viral replication genes and so prevent the development of protective immune responses at birth. The elimination of the 34.5 viral protein that was used to treat OncoVex and HF10, the first generation of tumor-soluble HSV-1 viruses, reduced the efficacy of treatment[114]. We found that the herpes simplex (HSV-1) based virus Myb34.5, in which the viral protein 34.5 was produced under the control of the tumor-specific B-Myb promoter, could competently proliferate and kill PDAC cells. Naked mice with human tumours were able to successfully reproduce Myb34.5, which dramatically slowed the growth of the tumour and prevented it from spreading. On a cellular level, gemcitabine enhances Myb34.5's anticancer action when it is administered externally[119].

The H-1 (H-1PV) oncolytic parvovirus is a member of the small virus family. It has a small, membrane-free twenty-sided body shell that contains a linear single-stranded deoxyribonucleic acid of approximately 5kb in length. The fundamental process by which viruses replicate selectively and are specifically toxic to cancer cells in rats and humans is multifactorial and complex. If H-1PV binds to usual cells, transforms them, and infects them, in cancer cells the molecular abnormalities observed, and it's thought to be beneficial for viral gene expression, amplification of viral DNA, shell assembly, cell lesions and viral particle maturation. Variable activation ranks of specific metabolic/signaling pathways responsible for all these cell events maybe account for differences in H-1PV pots, as various types of permitted, semi-permissible and drug-resistant cancer cells have been described[120]. In rats with PDAC, H-1PV was found to be productive and safe in all those rats, including cells from the same gene human or rat cancer cells in the immunodeficient model[121-123]. Other studies have reached the same conclusions using PDAC tumors of human, implanted in immunocompromised mice that could not be infected with Parvovirus H-1.

Additionally, the effectiveness of Parvovirus H-1 in combined therapy for PDAC was investigated. Given the several mechanisms by which H-1PV kills cancer cells, oncolytic H-1PV may kill gemcitabine-resistant cells. Concerning the therapeutic use of Parvovirus H1 in patients with tumors, study and research work in French found that injecting the same dose of H-1PV into 12 patients of skin metastases with melanoma, breast cancer, lung cell cancer, pancreatic cancer, or renal smoothness muscle tumor caused relatively mild damage. The presence of protein and viral DNA in the lesion and distal tumor site of the injected tumor indicates that the virus has been transmitted systemically. Shown no visible toxicity in investigations of early clinical setup, the solution H-1PV test (ParvOryx01) was performed in malignant brain tumor patients, and it is recommended that the H-1PV dose be raised[122]. It is determined to be safe following injection or intravenous injection at the primary location. Clinical trials involving patients with cancer who received H-1PV have confirmed that it's safe and does not cause any clinically significant adverse effects.

Additionally, the ParvOryx01 study, which was just finished and is currently being evaluated clinically, presents intriguing indications of virus multiplication within cells, generation of cytotoxic and viral effects, and particular T-cell responses activation[123]. This could eventually result in a treatment for pancreatic cancer. In conjunction with immunotherapy, a novel treatment concept has evolved. Indeed, virus-induced tumor disintegration results in the release of mutant proteins or tumor-associated antigens developed during tumor progression[124]. Imlygic® demonstrates the first ever solid data of human supporting a systematic and long-lasting anti-cancer immune response with advanced cancer patients by multiplying viruses that directly and locally destroy tumors[125]. The oncolytic virus has been demonstrated to advance the penetration of T-cells into the cancer and elicit a systematic immune response to tumor-related antigens[126]. As a result, the combination of oncolytic viruses with immuno-checkpoint inhibitors has appeared as one of the most capable anti-cancer therapies until now and it is logical to predict that resistance to immune checkpoint inhibitors (ICI) treatment could be overcome to the patient's benefit with the oncolytic virus by heating immune-cold pancreatic tumors.

Experimental and clinical evidence indicates that spontaneous induction and antitumor immunity mediated purely by soluble tumors are "hit or miss" strategies. Rather than inducing an immune response, the oncolytic virus facilitates the encoding of genetically modified foreign transgenes, and developed a more effective PDAC oncolytic viral vaccine[26]. The "boosted" immune response to specific antigens creates an ideal immunological storm within the tumor on the patient's cells, resulting in widespread healing effects on the primary site as well as metastatic sites. An oncolytic virus can be engineered to produce a variety of PDAC antigens (mesothelin, MUC-1 and others), which have been revealed that combined with GVAX, induce robust anti-tumor immune responses[127]. It is used in chimeric T cell receptors approach[124]. The
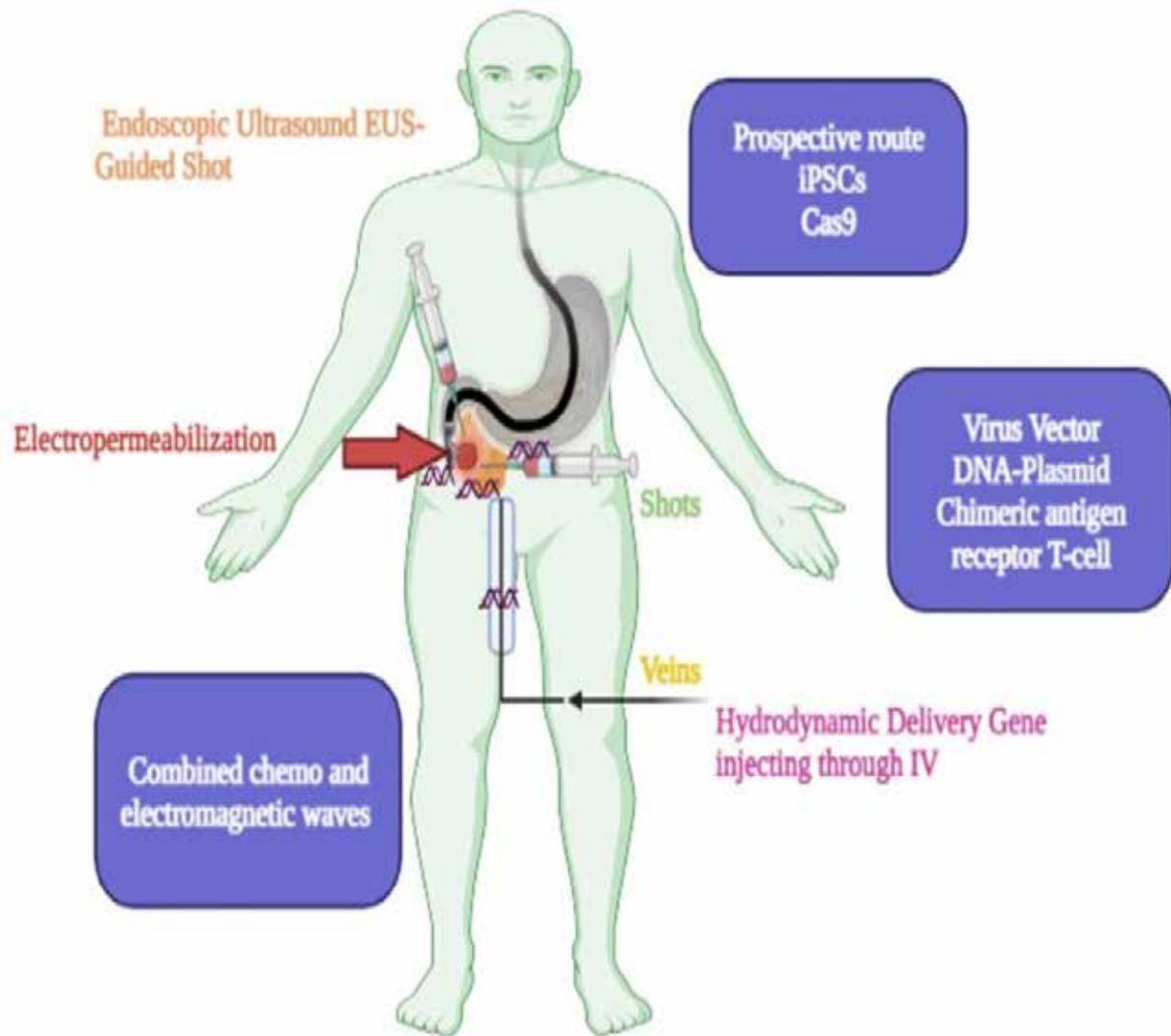
Figure 1: CRISPR/Cas9 and CRISPR-associated protein 9, CAR-T cells, DNA, endoscopic ultrasound (EUS), and induced pluripotent stem cells (iPSCs) cells are all short palindromic repeats. The following graphic summarizes gene therapy for the pancreas.

pressurized oncolytic viruses can be used with immunosuppressive agents to measure tumor damage and immune response to tumors in conjunction.

Gene therapy for pancreatic disease

In pancreatic clinical and preclinical studies, two gene transfer methods are used: in vitro and in vivo models[128]. In vitro gene transfection is genetically modifying cells isolated from experimental animals or patients and then transfecting them into patients or animals. Delivery methods of gene can be classified into in situ, in vivo and systemic. The pancreas gene therapy strategy is depicted in Figure 1.

Thus far, the study has used tumor virus, HSV, lentivirus, foam virus, adenovirus and AAV as virus vectors (Tables 1 and 2). Additionally, table 3 contains information about the characteristics of various viral vectors. Clinical trials frequently employ adenovirus and AAV (Tables 1 and 2). To give just one example, a phase three trial (NCT00051467) is currently testing whether the adenovirus vector TNFerade, which delivers TNF-α to cancer cells and is controlled by a chemically induced promoter, can improve the efficacy of radiotherapy and 5-fluorouracil for the management of locally advanced, unresectable pancreatic cancer. Non-viral vector establish approach, such as plasmid DNA injections, used in clinical trials to direct in vivo gene transfer to the pancreas. As previously indicated, some other non-viral vector establishes approach, including physical gene transfer and chemical compound vectors, have been investigated as preclinical setting. Chemical methods make extensive use of chemically modified proteins, polymers, and cation lipids; physical approaches make extensive use

of acupuncture, gene guns, electro-perforation, ultrasonic polarization, magnetic induction, and fluid-power gene transfer. Additionally, table 3 discusses the characteristics of several genes transfer methods. Along with ongoing clinical trials evaluating prosthetic gene injections and viral vectors, another study (NCT02514421) evaluates the safety and efficacy of electro-perforated electrochemical therapy for pancreatic cancer.

This technique has been successfully used to treat chronic pancreatic pain in the clinical setting by blocking sympathetic innervation, injecting neurolytic solutions into the celiac plexus for neurolysis, and using endoscopic ultrasound; this technique can also be used for gene transfer. The method has been combined with the plasmids, immune-modified cells and injection of virus vectors between tumors in ongoing clinical trials (Table 3).

Interventional studies

Gene addiction for pancreatic illnesses, and notably pancreatic cancer, has been the subject of a large number of clinical studies. The results of all trials are included in Table 1. Plasmids, adenoviruses and synthetic vectors are used for therapeutic delivery of suicide genes and interleukin. These genes are injected intradermally or subcutaneously using endoscopic ultrasonography in situ or an oncolytic virus.

Furthermore, radiation or chemotherapy is typically incorporated into the treatment regimen of these trials. Only one type of experiment advanced to phase III, while the others were done at the phase I and phase II levels. To determine whether or not TNFerade, a novel gene transfer strategy for tumour necrosis factor-alpha delivery, improves viability in cases with locally advanced pancreatic cancer when used in conjunction with radiation and 5-fluorouracil, a randomized, multi-institution phase III trial is underway. Intratumoral injections of TNFerade are administered through ultrasound-guided percutaneous transabdominal or endoscopic tumour injection prior to irradiation[129]. While the anti-tumor efficacy of TNFerade is small, the method has been demonstrated to be feasible and safe when combined with clinically proven procedures.

Current trials are summarized in Table 2[26]. These contain gene treatment with suicide genes and interleukin-13, oncolytic virus-based gene therapy, and plasmid DNA-based gene vaccination for pancreatitis, and pancreatic cancer. Additionally, clinical trials evaluating cell treatment, notably CAR T-cell therapy for adoptive immunotherapy, have been done. The technique is designed to be an adoptive active immunotherapy approach in which CD8+ T lymphocytes from patients are extracted and in vitro modified to recognize and destroy cancer cells

expressing tumor antigens. Over twenty clinical trials enrolled participants, and future trials will focus on developing effective and safe gene therapy for pancreatic diseases, especially pancreatic cancer treatment.

Several different types of chemotherapy, including fludarabine, gemcitabine, capecitabine, cisplatin and cyclophosphamide are used in clinical studies for pancreatic disorders. Everolimus, docetaxel, sunitinib, fluorouracil and paclitaxel are all part of the nanoparticle albumin-bound (NAB). Trials evaluating the safety of CYL-02, a non-viral gene product that imparts gemcitabine sensitivity on tumour cells by generating a fusion gene expressing the mouse somatostatin receptor two subtypes as deoxycytidine and monophosphate kinases, may be found at (NCT01274455)[22]. NCT00836407 is a clinical trial using Yervoy to evaluate the transfection of GM-CSF gene into pancreatic cancerous cells[130]. This colony stimulating factor 1 (CSF1) is also used in cancer therapeutic vaccines[131]. Many of these studies are still in their infancy or mid-stages, and they all need to be registered and evaluated for safety and effectiveness. Nevertheless, advances in delivery methods, vectors and clinical technologies will allow targeting of genes unique to the pancreas. In addition, a trial is now recruiting participants for its second phase to test the effectiveness of precision medication and mutation targeting treatment for intermediate and advanced pancreatic low-grade neuroendocrine tumours (NCT02315625).

CONCLUSIONS

The pancreas is an essential organ with roles in both the digestive and hormonal systems. Pancreatic cancer is the main cause of mortality from cancer globally. As gene therapy research improves, a number of promising clinical studies are now ongoing. Moreover, it is the primary preclinical investigation of gene delivery. It is essential to simultaneously develop delivery technologies, vectors and procedures alongside CRISPR-associated protein 9 induced genome engineering, and pluripotent stem cells. The outcomes of these studies and the novel gene therapy approaches may also be used for pancreatic diseases that are presently incurable. Additional study that combines these strategies will stimulate the current development of novel treatment choices. Based on CRISPR/Cas9 technology, in vivo gene editing may prefer short-term transgenic expression to avoid targeting. Thus, non-viral vector-based delivery approaches may be more suitable for transitory gene expression than gene editing. The life expectancy and prognosis of patients with pancreatic illnesses are enhanced by the customization of these procedures based on specific biomarkers, even if further study is necessary to optimize their efficiency.

Clinical trials have begun, and this lengthy and rigorous procedure must be completed before medication development can proceed: proof of concept, early phase, preclinical safety trials and phase II. They are innovative pharmaceuticals that have been genetically modified, which adds another layer of intricacy. Due to the severity of this condition, innovative treatments must be used in conjunction with conventional chemotherapy, which has only a minor effect. Almost certainly, solutions will be uncovered through these treatment combinations. Gene therapy has also widened and strengthened the field of tumor virus therapy and adoptive immunotherapy.

ACKNOWLEDGMENT

REFERENCES

1. Standring S. Gray's anatomy: the anatomical basis of clinical practice. 41st ed. Elsevier; 2015.
2. Saad AM, Turk T, Al-Husseini MJ, Abdel-Rahman O. Trends in pancreatic adenocarcinoma incidence and mortality in the United States in the last four decades; a SEER-based study. BMC Cancer 2018; 18(1):688.
3. Outani H, Akita H, Nakai T, Takada R, Imura Y, Tanaka T, et al. Clinical features and prognosis of patients with the bone metastasis of pancreatic cancer: A single-institutional cohort study. Pancreas 2018; 47(7):e43-e46.
4. Mueller AM, Meier CR, Jick SS, Schneider C, et al. The potential of glycemic control and body weight change as early markers for pancreatic cancer in patients with long-standing diabetes mellitus: a case-control study. Pancreas 2018; 47(7):807-15.
5. Danai LV, Babic A, Rosenthal MH, Dennstedt EA, Muir A, Lien EC, et al. Altered exocrine function can drive adipose wasting in early pancreatic cancer. Nature 2018; 558:600-604.
6. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014; 74(11):2913-21.
7. Rossi ML, Rehman AA, Gondi CS. Therapeutic options for the management of pancreatic cancer. World J Gastroenterol 2014; 20(32):11142-59.
8. Hegewisch-Becker S, Aldaoud A, Wolf T, Krammer-Steiner B, Linde H, Scheiner-Sparna R, et al. Results from the prospective German TPK clinical cohort study: treatment algorithms and survival of 1,174 patients with locally advanced, inoperable, or metastatic pancreatic ductal adenocarcinoma. Int J Cancer 2019; 144(5):981-90.
9. Pishvaian MJ, Bender RJ, Halverson D, Rahib L, Hendifar AE, Mikhail S, et al. Molecular profiling of patients with pancreatic cancer: initial results from the Know Your Tumor initiative. Clin Cancer Res 2018; 24(20):5018-27.
10. Rouanet M, Lebrin M, Gross F, Bournet B, Cordelier P, Buscail L. Gene therapy for pancreatic cancer: specificity, issues and hopes. Int J Mol Sci 2017; 18(6):1231.
11. Hu C, Hart SN, Polley EC, Gnanaolivu R, Shimelis H, Lee KY, et al. Association between inherited germline mutations in cancer predisposition genes and risk of pancreatic cancer. JAMA 2018; 319(23):2401-9.
12. Di Magliano MP, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. Gastroenterology 2013; 144(6):1220-9.
13. Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015; 518(7540):495-501.
14. Fu Y, Liu X, Chen Q, Liu T, Lu C, Yu J, et al. Downregulated miR-98-5p promotes PDAC proliferation and metastasis by reversely regulating MAP4K4. J Exp Clin Cancer Res 2018; 37(1):130.
15. Huynh DL, Zhang JJ, Chandimali N, Ghosh M, Gera M, Kim N, et al. SALL4 suppresses reactive oxygen species in pancreatic ductal adenocarcinoma phenotype via FoxM1/Prx III axis. Biochem Biophys Res Commun 2018; 503(4):2248-54.
16. Liu B, Yang H, Taher L, Denz A, Grutzmann R, Pilarsky C, et al. Identification of prognostic biomarkers by combined mRNA and miRNA expression microarray analysis in pancreatic cancer. Transl Oncol 2018; 11(3):700-14.
17. Wang WQ, Liu L, Xu HX, Wu CT, Xiang JF, Xu J, et al. Infiltrating immune cells and gene mutations in pancreatic ductal adenocarcinoma. Br J Surg 2016; 103(9):1189-99.
18. Patra KC, Kato Y, Mizukami Y, Widholz S, Boukhali M, Revenco I, et al. Mutant GNAS drives pancreatic tumourigenesis by inducing PKA-mediated SIK suppression and reprogramming lipid metabolism. Nat Cell Biol 2018; 20(7):811-22.
19. Thakar M, Hu Y, Morreale M, Lerner L, Lin WY, Sen R, et al. A novel epigenetic modulating agent sensitizes pancreatic cells to a chemotherapy agent. PloS One 2018; 13(6):e0199130.
20. Hill R, Rabb M, Madureira Pa, Clements D, Gujar SA, Waisman DM, et al. Gemcitabine-mediated tumour regression and p53-dependent gene expression: implications for colon and pancreatic cancer therapy. Cell Death Dis 2013; 4(9):e791.
21. Chawla SP, Chua VS, Fernandez L, Quon D, Blackwelder WC, Gordon EM, et al. Advanced phase I/II studies of targeted gene delivery in vivo: intravenous Rexin-G for gemcitabine-resistant metastatic pancreatic cancer. Mol Ther 2010; 18(2):435-41.
22. Buscail L, Bournet B, Vernejoul F, Cambois G, Lulka H, Hanoun N, et al. First-in-man phase 1 clinical trial of gene therapy for advanced pancreatic cancer: safety, biodistribution, and preliminary clinical findings. Mol Ther 2015; 23(4):779-89.

23. Golan T, Khvalevsky EZ, Hubert A, Gabai RM, Hen N, Segal A, et al. RNAi therapy targeting KRAS in combination with chemotherapy for locally advanced pancreatic cancer patients. Oncotarget 2015; 6(27):24560-70.

24. Liu B, Yang H, Pilarsky C, Weber GF. The effect of GPRC5a on the proliferation, migration ability, chemotherapy resistance, and phosphorylation of GSK-3β in pancreatic cancer. Int J Mol Sci 2018; 19(7):1870.

25. Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR. Gene therapy clinical trials worldwide to 2017: An update. J Gene Med 2018; 20(5):e3015.

26. Vassaux G, Angelova A, Baril P, Midoux P, Rommelaere J, Cordelier P. The promise of gene therapy for pancreatic cancer. Hum Gene Ther 2016; 27(2):127-33.

27. Buscail L, Faure P, Bournet B, Selves J, Escourrou J. Interventional endoscopic ultrasound in pancreatic diseases. Pancreatology 2006; 6(1-2):7-16.

28. Klemm F, Joyce JA. Microenvironmental regulation of therapeutic response in cancer. Trends Cell Biol 2015; 25(4):198-213.

29. Hahn SA, Schmiegel WH. Recent discoveries in cancer genetics of exocrine pancreatic neoplasia. Digestion 1998; 59(5):493-501.

30. Delpu Y, Hanoun N, Lulka H, Sicard F, Selves J, Buscail L, et al. Genetic and epigenetic alterations in pancreatic carcinogenesis. Curr Genomics 2011; 12(1):15-24.

31. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 2011; 39(Suppl_1):D945-50.

32. Bournet B, Buscail C, Muscari F, Cordelier P, Buscail L. Targeting KRAS for diagnosis, prognosis, and treatment of pancreatic cancer: Hopes and realities. Eur J Cancer 2016; 54:75-83.

33. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003; 4(6):437-50.

34. Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, et al. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 2003; 17(24):3112-26.

35. Bardeesy N, Aguirre AJ, Chu GC, Cheng KH, Lopez LV, Hezel AF, et al. Both p16 (Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. Proc Natl Acad Sci USA 2006; 103(15):5947-52.

36. Rochaix P, Delesque N, Esteve JP, Saint-Laurent N, Voight JJ, Vaysse N, et al. Gene therapy for pancreatic carcinoma: local and distant antitumor effects after somatostatin receptor sst2 gene transfer. Hum Gene Ther 1999; 10(6):995-1008.

37. Vernejoul F, Faure P, Benali N, Calise D, Tiraby G, Pradayrol L, et al. Antitumor effect of in vivo somatostatin receptor subtype 2 gene transfer in primary and metastatic pancreatic cancer models. Cancer Res 2002; 62(21):6124-31.

38. Ryan DP, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. N Engl J Med 2014; 371(11):1039-49.

39. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 1997; 15(6):2403-13.

40. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011; 364(19):1817-25.

41. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 2010; 363(4):355-64.

42. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature 2010; 467(7313):318-22.

43. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nature Reviews Genetics 2014; 15(8):541-55.

44. Doody AM, Korley JN, Dang KP, Zawaneh PN, Putnam D. Characterizing the structure/function parameter space of hydrocarbon-conjugated branched polyethylenimine for DNA delivery in vitro. J Control Release 2006; 116(2):227-37.

45. Forrest ML, Meister GE, Koerber JT, Pack DW. Partial acetylation of polyethylenimine enhances in vitro gene delivery. Pharm Res 2004; 21(2):365-71.

46. Thomas M, Klibanov AM. Enhancing polyethylenimine's delivery of plasmid DNA into mammalian cells. Proc Natl Acad Sci USA 2002; 99(23):14640-5.

47. Oskuee RK, Dehshahri A, Shier WT, Ramezani M. Alkylcarboxylate grafting to polyethylenimine: a simple approach to producing a DNA nanocarrier with low toxicity. J Gene Med 2009; 11(10):921-32.

48. Midoux P, Pichon C, Yaouanc JJ, Jaffres PA. Chemical vectors for gene delivery: a current review on polymers, peptides and lipids containing histidine or imidazole as nucleic acids carriers. Br J Pharmacol 2009; 157(2):166-78.

49. Bertrand E, Goncalves C, Billiet L, Gomez JP, Pichon C, Cheradame H, et al. Histidinylated linear PEI: a new efficient non-toxic polymer for gene transfer. Chem Commun (Camb) 2011; 47(46):12547-9.

50. Nayerossadat NT, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. Adv Biomed Res 2012; 1:27.

51. Touchefeu Y, Harrington KJ, Galmiche JP, Vassaux G. Review article: gene therapy, recent developments and future prospects in gastrointestinal oncology. Aliment Pharmacol Ther 2010; 32(8):953-68.

52. Liu SX, Xia ZS, Zhong YQ. Gene therapy in pancreatic cancer. World J Gastroenterol 2014; 20(37):13343-68.

53. Hanoun N, Gayral M, Pointreau A, Buscail L, Cordelier P. Initial characterization of integrase-defective lentiviral vectors for pancreatic cancer gene therapy. Hum Gene Ther 2016; 27(2):184-92.

54. Vassaux G, Nitcheu J, Jezzard S, Lemoine NR. Bacterial gene therapy strategies. J Pathol A 2006; 208(2):290-8.

55. Riley MK, Vermerris W. Recent advances in nanomaterials for gene delivery—a review. Nanomaterials (Basel) 2017; 7(5):94.

56. Kobayashi S, Shirasawa H, Sashiyama H, Kawahira H, Kaneko K, Asano T, et al. P16INK4a expression adenovirus vector to suppress pancreas cancer cell proliferation. Clin Cancer Res 1999; 5(12):4182-5.

57. Joshi US, Dergham ST, Chen YQ, Dugan MC, Crissman JD, Vaitkevicius VK, et al. Inhibition of pancreatic tumor cell growth in culture by p21WAF1 recombinant adenovirus. Pancreas 1998; 16(2):107-13.

58. Craig C, Kim M, Ohri E, Wersto R, Katayose D, Li Z, et al. Effects of adenovirus-mediated p16^{INK4A} expression on cell cycle arrest are determined by endogenous p16 and Rb status in human cancer cells. Oncogene 1998; 16(2):265-72.

59. Gupta S, Sathishkumar S, Ahmed MM. Influence of cell cycle checkpoints and p53 function on the toxicity of temozolomide in human pancreatic cancer cells. Pancreatology 2010; 10(5):565-79.

60. Azmi AS, Philip PA, Wang Z, Banerjee S, Zafar SF, Goustin AS, et al. Reactivation of p53 by novel MDM2 inhibitors: implications for pancreatic cancer therapy. Curr Cancer Drug Targets 2010; 10(3):319-31.

61. Shen W, Tao GQ, Li DC, Zhu XG, Bai X, Cai B. Inhibition of pancreatic carcinoma cell growth in vitro by DPC4 gene transfection. World J Gastroenterol 2008; 14(40):6254-60.

62. Kuo CJ, Farnebo F, Yu EY, Christofferson R, Swearingen RA, Carter R, et al. Comparative evaluation of the antitumor activity of antiangiogenic proteins delivered by gene transfer. Proc Natl Acad Sci 2001; 98(8):4605-10.

63. Ogawa T, Takayama K, Takakura N, Kitano S, Ueno H. Anti-tumor angiogenesis therapy using soluble receptors: enhanced inhibition of tumor growth when soluble fibroblast growth factor receptor-1 is used with soluble vascular endothelial growth factor receptor. Cancer Gene Ther 2002; 9(8):633-40.

64. Zhang X, Xu J, Lawler J, Terwilliger E, Parangi S. Adeno-associated virus–mediated antiangiogenic gene therapy with thrombospondin-1 type 1 repeats and endostatin. Clin Cancer Res 2007; 13(13):3968-76.

65. Shan YF, Fang YF, Wang XQ, Jin R, Zhang QY, Andersson R. Experimental studies on treatment of pancreatic cancer with double-regulated duplicative adenovirus AdTPHre-hEndo carrying human endostatin gene. Pancreatology 2013; 13(4):393-400.

66. Tysome JR, Briat A, Alusi G, Cao F, Gao D, Yu J, et al. Lister strain of vaccinia virus armed with endostatin–angiostatin fusion gene as a novel therapeutic agent for human pancreatic cancer. Gene Ther 2009; 16(10):1223-33.

67. Rigg AS, Lemoine NR. Adenoviral delivery of TIMP1 or TIMP2 can modify the invasive behavior of pancreatic cancer and can have a significant antitumor effect in vivo. Cancer Gene Ther 2001; 8(11):869-78.

68. Katz MH, Spivack DE, Takimoto S, Fang B, Burton DW, Moossa AR, et al. Gene therapy of pancreatic cancer with green fluorescent protein and tumor necrosis factor–related apoptosis-inducing ligand fusion gene expression driven by a human telomerase reverse transcriptase promoter. Ann Surg Oncol 2003; 10(7):762-72.

69. Wang J, Lu XX, Chen DZ, Li SF, Zhang LS. Herpes simplex virus thymidine kinase and ganciclovir suicide gene therapy for human pancreatic cancer. World J Gastroenterol 2004; 10(3):400-3.

70. Fogar P, Greco E, Basso D, Habeler W, Navaglia F, Zambon CF, et al. Suicide gene therapy with HSV-TK in pancreatic cancer has no effect in vivo in a mouse model. Eur J Surg Oncol 2003; 29(9):721-30.

71. Greco E, Fogar P, Basso D, Stefani AL, Navaglia F, Zambon CF, et al. Retrovirus-mediated herpes simplex virus thymidine kinase gene transfer in pancreatic cancer cell lines: an incomplete antitumor effect. Pancreas 2002; 25(2):e21-9.

72. Erbs P, Regulier E, Kintz J, Leroy P, Poitevin Y, Exinger F, et al. In vivo cancer gene therapy by adenovirus-mediated transfer of a bifunctional yeast cytosine deaminase/uracil phosphoribosyltransferase fusion gene. Cancer Res 2000; 60(14):3813-22.

73. Fogar P, Navaglia F, Basso D, Greco E, Zambon CF, Fadi E, et al. Suicide gene therapy with the yeast fusion gene cytosine deaminase/uracil phosphoribosyltransferase is not enough for pancreatic cancer. Pancreas 2007; 35(3):224-31.

74. Grove JI, Searle PF, Weedon SJ, Green NK, McNeish IA, Kerr DJ. Virus-detected enzyme prodrug therapy using CB1954. Anticancer Drug Des 1999; 14(6):461-72.

75. Green NK, Youngs DJ, Neoptolemos JP, Friedlos F, Knox RJ, Springer CJ, et al. Sensitization of colorectal and pancreatic cancer cell lines to the prodrug 5-(aziridin-1-yl)-2, 4-dinitrobenzamide (CB1954) by retroviral transduction and expression of the E. coli nitroreductase gene. Cancer Gene Ther 1997; 4(4):229-38.

76. Carrió M, Visa J, Cascante A, Estivill X, Fillat C. Intratumoral activation of cyclophosphamide by retroviral transfer of the cytochrome P450 2B1 in a pancreatic tumor model. Combination with the HSVtk/GCV system. J Gene Med 2002; 4(2):141-9.

77. Löhr M, Muller P, Karle P, Stange J, Mitzner S, Jesnowski R, et al. Targeted chemotherapy by intratumour injection of encapsulated cells engineered to produce CYP2B1, an ifosfamide activating cytochrome P450. Gene Ther 1998; 5(8):1070-8.

78. Sicard F, Gayral M, Lulka H, Buscail L, Cordelier P. Targeting miR-21 for the therapy of pancreatic cancer. Mol Ther 2013; 21(5):986-94.

79. Brummelkamp TR, Bernards R, Agami R. Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer Cell 2002; 2(3):243-7.

80. Réjiba S, Wack S, Aprahamian M, Hajri A. K-ras oncogene silencing strategy reduces tumor growth and enhances gemcitabine chemotherapy efficacy for pancreatic cancer treatment. Cancer Sci 2007; 98(7):1128-36.

81. Morioka CY, Machado MCC, Saito S, Nakada Y, Matheus AS, Jukemura J, et al. Suppression of invasion of a hamster pancreatic cancer cell line by antisense oligonucleotides mutation-matched to K-ras gene. In Vivo 2005; 19(3):535-8.

82. Shen YM, Yang XC, Yang C, Shen JK. Enhanced therapeutic effects for human pancreatic cancer by application K-ras and IGF-IR antisense oligodeoxynucleotides. World J Gastroenterol 2008; 14(33):5176-85.

83. Alberts SR, Schroeder M, Erlichman C, Steen PD, Foster NR, Moore Jr DF, et al. Gemcitabine and ISIS-2503 for patients with locally advanced or metastatic pancreatic adenocarcinoma: a North Central Cancer Treatment Group phase II trial. J Clin Oncol 2004; 22(24):4944-50.

84. Huang Z, Tomitaka A, Raymond A, Nair M. Current application of CRISPR/Cas9 gene-editing technique to eradication of HIV/AIDS. Gene Ther 2017; 24(7):377-84.

85. Chiou SH, Winters IP, Wang J, Naranjo S, Dudgeon C, Tamburini FB, et al. Pancreatic cancer modeling using retrograde viral vector delivery and in vivo CRISPR/Cas9-mediated somatic genome editing. Genes Dev 2015; 29(14):1576-85.

86. Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. Cancer Res 2007; 67(19):9518-27.

87. Sideras K, Braat H, Kwekkeboom J, van Eijck CH, Peppelenbosch MP, Sleijfer S, et al. Role of the immune system in pancreatic cancer progression and immune modulating treatment strategies. Cancer Treat Rev 2014; 40(4):513-22.

88. Seo YD, Pillarisetty VG. T-cell programming in pancreatic adenocarcinoma: a review. Cancer Gene Ther 2017; 24(3):106-13.

89. von Ahrens D, Bhagat TD, Nagrath D, Maitra A, Verma A. The role of stromal cancer-associated fibroblasts in pancreatic cancer. J Hematol Oncol 2017; 10(1):76.

90. Johnson III BA, Yarchoan M, Lee V, Laheru DA, Jaffee EM. Strategies for increasing pancreatic tumor immunogenicity. Clin Cancer Res 2017; 23(7):1656-69.

91. Johansson H, Andersson R, Bauden M, Hammes S, Holdenrieder S, Ansari D. Immune checkpoint therapy for pancreatic cancer. World J Gastroenterol 2016; 22(43):9457-76.

92. Kimura M, Tagawa M, Takenaga K, Kondo F, Yamaguchi T, Saisho H, et al. Loss of tumorigenicity of human pancreatic carcinoma cells engineered to produce interleukin-2 or interleukin-4 in nude mice: a potentiality for cancer gene therapy. Cancer Lett 1998; 128(1):47-53.

93. Liu L, Meng J, Zhang C, Duan Y, Zhao L, Wang S, et al. Effects on apoptosis and cell cycle arrest contribute to the antitumor responses of interleukin-27 mediated by retrovirus in human pancreatic carcinoma cells. Oncol Rep 2012; 27(5):1497-503.

94. Ravet E, Lulka H, Gross F, Casteilla L, Buscail L, Cordelier P. Using lentiviral vectors for efficient pancreatic cancer gene therapy. Cancer Gene Ther 2010; 17(5):315-24.

95. Armstrong L, Davydova J, Brown E, Han J, Yamamoto M, Vickers SM. Delivery of interferon alpha using a novel Cox2-controlled adenovirus for pancreatic cancer therapy. Surgery 2012; 152(1):114-22.

96. Murugesan SR, King CR, Osborn R, Fairweather WR, O'Reilly EM, Thornton MO, et al. Combination of human tumor necrosis factor-alpha (hTNF-α) gene delivery with gemcitabine is effective in models of pancreatic cancer. Cancer Gene Ther 2009; 16(11):841-7.

97. Lortal B, Gross F, Peron JM, Penary M, Berg D, Hennebelle I, et al. Preclinical study of an ex vivo gene therapy protocol for hepatocarcinoma. Cancer Gene Ther 2009; 16(4):329-37.

98. Mohammed S, Sukumaran S, Bajgain P, Watanabe N, Heslop HE, Rooney CM, et al. Improving chimeric antigen receptor-modified T cell function by reversing the immunosuppressive tumor microenvironment of pancreatic cancer. Mol Ther 2017; 25(1):249-58.

99. Posey Jr AD, Schwab RD, Boesteanu AC, Steentoft C, Mandel U, Engels B, et al. Engineered CAR T cells targeting the cancer-associated Tn-glycoform of the membrane mucin MUC1 control adenocarcinoma. Immunity 2016; 44(6):1444-54.

100. O'Hara M, Stashwick C, Haas AR, Tanyi JL. Mesothelin as a target for chimeric antigen receptor-modified T cells as anticancer therapy. Immunotherapy 2016; 8(4):449-60.

101. DeSelm CJ, Tano ZE, Varghese AM, Adusumilli PS. CAR T-cell therapy for pancreatic cancer. J Surg Oncol 2017; 116(1):63-74.

102. Ren J, Liu X, Fang C, Jiang S, June CH, Zhao Y. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. Clin Cancer Res 2017; 23(9):2255-66.

103. Kudrin A. Overview of cancer vaccines: considerations for development. Hum Vaccin Immunother 2012; 8(9):1335-53.

104. Plate JM.D Advances in therapeutic vaccines for pancreatic cancer. Discov Med 2012; 14(75):89-94.

105. Maki RG, Livingston PO, Lewis JJ, Janetzki S, Klimstra D, Desantis D, et al. A phase I pilot study of autologous heat shock protein vaccine HSPPC-96 in patients with resected pancreatic adenocarcinoma. Dig Dis Sci 2007; 52(8):1964-72.

106. Rong Y, Qin X, Jin D, Lou W, Wu L, Wang D, et al. A phase I pilot trial of MUC1-peptide-pulsed dendritic cells in the treatment of advanced pancreatic cancer. Clin Exp Med 2012; 12(3):173-180.

107. Gjertsen MK, Buanes T, Rosseland AR, Bakka A, Gladhaug I, Soreide O, et al. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: Clinical and immunological responses in patients with pancreatic adenocarcinoma. Int J Cancer 2001; 92(3):441-50.

108. Bernhardt SL, Gjertsen MK, Trachsel S, Moller M, Eriksen JA, Meo M, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: a dose escalating phase I/II study. Br J Cancer 2006; 95(11):1474-82.

109. Senovilla L, Vacchelli E, Garcia P, Eggermont A, Fridman WH, Galon J, et al., Trial watch: DNA vaccines for cancer therapy. Oncoimmunology 2013; 2(4):e23803.

110. Pol J, Buque A, Aranda F, Bloy N, Cremer I, Eggermont A, et al. Trial watch: oncolytic viruses and cancer therapy. Oncoimmunology 2016; 5(2):e1117740.

111. Huang F, Wang BR, Wu YQ, Wang FC, Zhang J, Wang YG. Oncolytic viruses against cancer stem cells: A promising approach for gastrointestinal cancer. World J Gastroenterol 2016; 22(35):7999-8009.

112. Husseini F, Delord JP, Federico CF, Guitton J, Erbs P, Homerin M, *et al*. Vectorized gene therapy of liver tumors: proof-of-concept of TG4023 (MVA-FCU1) in combination with flucytosine. Ann Oncol 2017; 28(1):169-74.

113. Wennier S, Li S, McFadden G. Oncolytic virotherapy for pancreatic cancer. Expert Rev Mol Med 2011; 13:e18.

114. Xu C, Li H, Su C, Li Z. Viral therapy for pancreatic cancer: tackle the bad guys with poison. Cancer Lett 2013; 333(1):1-8.

115. Bhattacharyya M, Francis J, Eddouadi A, Lemoine NR, Hallden G. An oncolytic adenovirus defective in pRb-binding (dl 922–947) can efficiently eliminate pancreatic cancer cells and tumors in vivo in combination with 5-FU or gemcitabine. Cancer Gene Ther 2011; 18(10):734-43.

116. Hecht JR, Bedford R, Abbruzzese JL, Lahoti S, Reid TR, Soetikno RM, *et al*. A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma. Clin Cancer Res 2003; 9(2):555-61.

117. Lucas T, Benihoud K, Vigant F, Schmidt CA, Bachem MG, Simmet T, *et al*. Hexon modification to improve the activity of oncolytic adenovirus vectors against neoplastic and stromal cells in pancreatic cancer. PLoS One 2015; 10(2):e0117254.

118. Ilkow CS, Marguerie M, Batenchuk C, Mayer J, Neriah DB, Cousineau S, *et al*. Reciprocal cellular cross-talk within the tumor microenvironment promotes oncolytic virus activity. Nat Med 2015; 21(5):530-6.

119. Gayral M, Lulka H, Hanoun N, Biollay C, Selves J, Vignolle-Vidoni A, *et al*. Targeted oncolytic herpes simplex virus type 1 eradicates experimental pancreatic tumors. Hum Gene Ther 2015; 26(2):104-13.

120. Angelova AL, Aprahamian M, Grekova SP, Hajri A, Leuchs B, Giese NA, *et al*. Improvement of gemcitabine-based therapy of pancreatic carcinoma by means of oncolytic parvovirus H-1PV. Clin Cancer Res 2009; 15(2):511-519.

121. Rejiba S, Bigand C, Parmentier C, Masmoudi A, Hajri A. Oncosuppressive suicide gene virotherapy "PVH1-yCD/5-FC" for pancreatic peritoneal carcinomatosis treatment: NFκB and Akt/PI3K involvement. PLoS One 2013; 8(8):e70594.

122. Geletneky K, Huesing J, Rommelaere J, Schlehofer JR, Leuchs B, Dahm M, *et al*. Phase I/IIa study of intratumoral/intracerebral or intravenous/intracerebral administration of Parvovirus H-1 (ParvOryx) in patients with progressive primary or recurrent glioblastoma multiforme: ParvOryx01 protocol. BMC Cancer 2012; 12:99.

123. Geletneky K, Leoni AL, Pohlmeyer-Esch G, Loebhard S, Leuchs B, Hoefer C, *et al*. Bioavailability, biodistribution, and CNS toxicity of clinical-grade parvovirus H1 after intravenous and intracerebral injection in rats. Comp Med 2015; 65(1):36-45.

124. Breitbach CJ, Lichty BD, Bell JC. Oncolytic viruses: therapeutics with an identity crisis. EBioMedicine 2016; 9:31-36.

125. Kaufman HL, Kim DW, De Faffele G, Mitcham J, Coffin RS, Kim-schulze S. Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. Ann Surg Oncol 2010; 17(3):718-30.

126. Andtbacka RHI, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, *et al*. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol 2015; 33(25):2780-8.

127. Le DT, Wamg-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, *et al*. Safety and survival with GVAX pancreas prime and Listeria monocytogenes–expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. J Clin Oncol 2015; 33(12):1325-33.

128. Kamimura K, Suda T, Zhang G, Liu D. Advances in gene delivery systems. Pharmaceut Med 2011; 25(5):293-306.

129. Herman JM, Wild AT, Wang H, Tran PT, Chang KJ, Taylor GE, *et al*. Randomized phase III multi-institutional study of TNFerade biologic with fluorouracil and radiotherapy for locally advanced pancreatic cancer: final results. J Clin Oncol 2013; 31(7):886-94.

130. Le DT, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, *et al*. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother 2013; 36(7):382-9.

131. Lutz E, Yeo CJ, Lillemoe KD, Biedrzycki B, Kobrin B, Herman J, *et al*. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma: a phase II trial of safety, efficacy, and immune activation. Ann Surg 2011; 253(2):328-35.

132. Gilly FN, Beaujard A, Bienvenu J, Lenoir VT, Glehen O, Thouvenot D, *et al*. Gene therapy with Adv-IL-2 in unresectable digestive cancer: phase I-II study, intermediate report. Hepatogastroenterology 1999; 46 Suppl 1:1268-73.

133. Mulvihill S, Warren R, Venook A, Adler A, Randlev B, Heise C, *et al*. Safety and feasibility of injection with an E1B-55 kDa gene-deleted, replication-selective adenovirus (ONYX-015) into primary carcinomas of the pancreas: a phase I trial. Gene Ther 2001; 8(4):308-15.

134. Salmons B, Lohr M, Gunzburg WH. Treatment of inoperable pancreatic carcinoma using a cell-based local chemotherapy: results of a phase I/II clinical trial. J Gastroenterol 2003; 38 Suppl 15:78-84.

135. Pecher G, Haring A, Kaiser L, Thiel E. Mucin gene (MUC1) transfected dendritic cells as vaccine: results of a phase I/II clinical trial. Cancer Immunol Immunother 2002; 51(11-12):669-73.

136. Gordon EM, Cornelio GH, Lorenzo 3rd CC, Levy JP, Reed RA, Liu L, *et al*. First clinical experience using a 'pathotropic' injectable retroviral vector (Rexin-G) as intervention for stage IV pancreatic cancer. Int J Oncol 2004; 24(1):177-85.

137. Sangro B, Mazzolini G, Ruiz J, Herraiz M, Quiroga J, Herrero I, *et al*. Phase I trial of intratumoral injection of an adenovirus encoding interleukin-12 for advanced digestive tumors. J Clin Oncol 2004; 22(8):1389-97.

138. Senzer N, Mani S, Rosemurgy A, Nemunaitis J, Cunningham C, Guha C, et al. TNFerade biologic, an adenovector with a radiation-inducible promoter, carrying the human tumor necrosis factor alpha gene: a phase I study in patients with solid tumors. J Clin Oncol 2004; 22(4):592-601.

139. Mazzolini G, Alfaro C, Sangro B, Feijoo E, Ruiz J, Benito A, et al. Intratumoral injection of dendritic cells engineered to secrete interleukin-12 by recombinant adenovirus in patients with metastatic gastrointestinal carcinomas. J Clin Oncol 2005; 23(5):999-1010.

140. Kaufman HL, Kim-Schulze S, Manson K, DeRaffele G, Mitcham J, Seo KS, et al. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. J Transl Med 2007; 5:60.

141. Laheru D, Lutz E, Burke J, Biedrzycki B, Solt S, Onners B, et al. Allogeneic granulocyte macrophage colony-stimulating factor–secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: a pilot study of safety, feasibility, and immune activation. Clin Cancer Res 2008; 14(5):1455-63.

142. Nakao A, Kasuya H, Sahin TT, Nomura N, Kanzaki A, Misawa M, et al. A phase I dose-escalation clinical trial of intraoperative direct intratumoral injection of HF10 oncolytic virus in non-resectable patients with advanced pancreatic cancer. Cancer Gene Ther 2011; 18(3):167-75.

143. Kubuschok B, Pfreundschuh M, Breit R, Hartmann F, Sester M, Gartner B, et al. Mutated Ras-transfected, EBV-transformed lymphoblastoid cell lines as a model tumor vaccine for boosting T-cell responses against pancreatic cancer: a pilot trial. Hum Gene Ther 2012; 23(12):1224-36.

144. Hanna N, Ohana P, Konikoff FM, Leichtmann G, Hubert A, Appelbaum L, et al. Phase 1/2a, dose-escalation, safety, pharmacokinetic and preliminary efficacy study of intratumoral administration of BC-819 in patients with unresectable pancreatic cancer. Cancer Gene Ther 2012; 19(6):374-81.

145. Hecht JR, Farrell JJ, Senzer N, Nemunaitis J, Rosemurgy A, Chung T, et al. EUS or percutaneously guided intratumoral TNFerade biologic with 5-fluorouracil and radiotherapy for first-line treatment of locally advanced pancreatic cancer: a phase I/II study. Gastrointest Endosc 2012; 75(2):332-8.

146. Le DT, Brockstedt DG, Nir-Paz R, Hampl J, Mathur S, Nemunaitis J, et al. A live-attenuated Listeria vaccine (ANZ-100) and a live-attenuated Listeria vaccine expressing mesothelin (CRS-207) for advanced cancers: phase I studies of safety and immune induction. Clin Cancer Res 2012; 18(3):858-68.

147. Hardacre JM, Mulcahy M, Small W, Talamonti M, Obel J, Krishnamurthi S, et al. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. J Gastrointest Surg 2013; 17(1):94-101.

148. Löhr JM, Haas SL, Kroger JC, Friess HM, Hoft R, Goretzki PE, et al. Encapsulated cells expressing a chemotherapeutic activating enzyme allow the targeting of subtoxic chemotherapy and are safe and efficacious: Data from two clinical trials in pancreatic cancer. Pharmaceutics 2014; 6(3):447-66.

149. Aguilar LK, Shirley LA, Chung VM, Marsh CL, Walker J, Coyle W, et al. Gene-mediated cytotoxic immunotherapy as adjuvant to surgery or chemoradiation for pancreatic adenocarcinoma. Cancer Immunol Immunother 2015; 64(6):727-36.

150. Noonan AM, Farren MR, Geyer SM, Huang Y, Tahiri S, Ahn D, et al. Randomized phase 2 trial of the oncolytic virus pelareorep (Reolysin) in upfront treatment of metastatic pancreatic adenocarcinoma. Mol Ther 2016; 24(6):1150-8.

151. Inoko K, Hiraoka K, Inagaki A, Takahashi M, Kushibiki T, Hontani K, et al. Therapeutic activity of retroviral replicating vector-mediated prodrug activator gene therapy for pancreatic cancer. Cancer Gene Ther 2018; 25(7-8):184-95.

152. Galanis E, Carlson SK, Foster NR, Lowe V, Quevedo F, McWilliams RR, et al. Phase I trial of a pathotropic retroviral vector expressing a cytocidal cyclin G1 construct (Rexin-G) in patients with advanced pancreatic cancer. Mol Ther 2008; 16(5):979-84.

Original Article

Laminin: A new biomarker for gastric cancer

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ABSTRACT

Objective: To evaluate the diagnostic and prognostic utility of serum laminin levels in gastric cancer
Design: A prospective and observational study
Setting: Department of General Surgery, Van Yuzuncu Yil University Medical Faculty, Van, Turkey
Subjects: Between June 2018 and September 2018, eighty patients who were diagnosed with gastric cancer as the patient group, and forty volunteers as the control group were included in the study.
Intervention: From each patient, 3 ml of peripheral blood sample was collected.
Main outcome measures: Enzyme-linked immunosorbent assay kit for human laminin were used for the detection of laminin in samples of serum. Data on patient demographics (age, gender) and serum laminin levels were recorded in both control and gastric cancer groups.

In gastric cancer patients, serum laminin levels were further analyzed with respect to tumor stages and tumor size.
Results: Serum laminin levels were significantly higher in gastric cancer patients [median (min-max): 205 (165-483) vs. 12 (9-18) ng/mL, $P<0.001$]. Laminin levels were higher in patients with advanced invasion depth, distant organ metastasis and lymph node metastasis ($P<0.001$). The sensitivity and specificity determined from the receiver operating characteristic curves at cut-off level of 70 were 95% and 97% for serum laminin, respectively.
Conclusion: The serum concentration of laminin can be used as a biomarker at the time of diagnosis for gastric cancer with high sensitivity and specificity. In addition, laminin can be used to discriminate between earlier, advanced or metastatic stages of gastric cancer.

KEY WORDS: biomarker, gastric cancer, laminin, metastasis

INTRODUCTION

Gastric cancer is a major health issue all over the world. In the light of Cancer Statistics 2021 report of Siegel *et al*, 26,560 new gastric cancer cases are expected to be seen in USA. In addition, the same report also predicted that approximately 11,180 people will die due to gastric cancer in the USA[1].

According to Globocan 2020, gastric cancer ranks 5th in the world due to the number of new cases in both sexes of all ages in 2020 with 5.6%. On the other hand, gastric cancer ranks 4th in the world due to the number of deaths of both sexes of all ages with 7.7%[2].

According to NCCN Guideline v4 (2020), in elective conditions after tumoral evaluation with a multidisciplinary approach, primary treatment is decided according to algorithms[3]. Unfortunately, because the mechanism of gastric cancer is not known

completely, patients present in advanced stage. Currently, biomarkers which can be helpful at the time of diagnosis (like p27, cyclin E, E-cadherin, c-erbB2, c-myc, tumor suppressor gene p53 etc.) have been reported, but there is no exact biomarker for diagnosis. Hence, new diagnostic biomarkers are urgently needed[4].

In epithelial tissues, as in gastric epithelia, the basal surface of cells is surrounded by the basement membrane, which is mainly composed of laminins, collagen type-IV and other glycoproteins[5]. Laminin proteins contain three major proteins, an α-chain, a β-chain, and a γ-chain, found in five, four, and three genetic variants respectively. In gastric mucosa, laminin chains were shown to have differential expression, with laminin α1 chain found at the basement membrane of both surface and glandular

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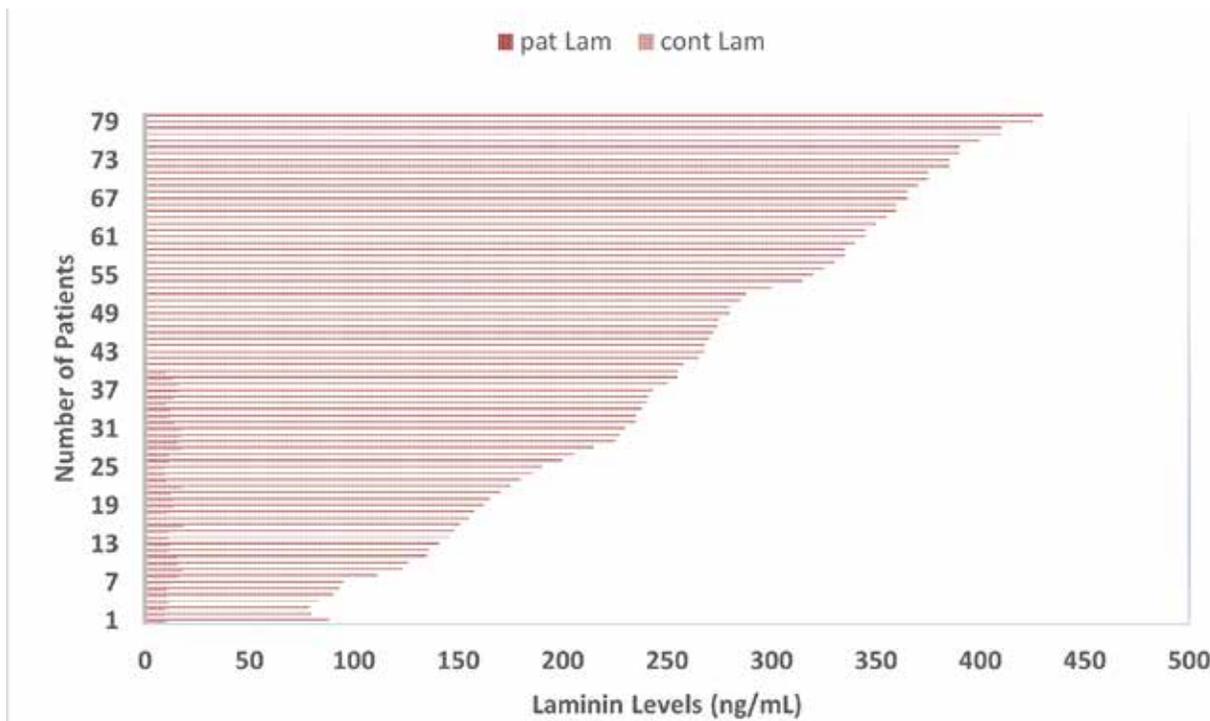
Figure 1: The histogram of the laminin levels of the control group and patients' group.

epithelia, while laminin $\alpha2$ and $\alpha3$ chains were grossly mutually exclusive. Specifically, $\alpha2$ chains were mostly detected in the glandular basement membrane and $\alpha3$ at basement membranes underneath the surface epithelium. The composition of the surface epithelial basement membrane was associated with rapid recovery capacity of the gastric surface epithelium following chemical injury, but also suggested specific programs towards surface or glandular cell differentiation[6]. Laminin proteins were studied in human studies before at different cancer types such as breast, colon and melanoma[7,8]. However, there are no human studies showing laminin protein being used as a prognostic biomarker in gastric cancer.

Therefore, the present study was aimed to evaluate the diagnostic potential of serum levels of laminin in gastric cancer patients. The level of laminin was also correlated to the clinicopathological variables in the cancer patients.

SUBJECTS AND METHODS
Study population
This study was a prospective observational study. Between June 2018 and September 2018, eighty patients were diagnosed with gastric cancer using the pathological samples. These patients were new who did not receive chemotherapy, radiotherapy, and operation history. A group of forty healthy volunteers served as the control group in the study.

Assessments
Data on patient demographics (age, gender) and serum laminin levels were recorded in both control and gastric cancer groups. In gastric cancer patients, serum laminin levels were further analyzed with respect to gastric cancer staging based on TNM staging system and tumor size. In patients who underwent surgery, pathological diagnosis was performed.

Sample collection
From each patient, 3 ml of peripheral blood samples were collected and transferred to another tube and allowed to settle at 24-25 °C temperature for 15 minutes. From clotted blood samples, 1 ml of supernatant serum was collected and transferred to a

Table 1: Patient demographics and serum laminin levels in study groups

Characteristics	Control group (n=40)	Patient group (n=80)	P-value
Age (mean±sd, year)	60.3±3.8	58.8±4.99	0.128[1]
Gender (n, %)			0.590[2]
Female	13 (32.5%)	30 (37.5%)	
Male	27 (67.5%)	50 (62.5%)	
Laminin level (ng/mL) (mean ± sd)	12.98±2.88	254±98.85	<0.001[1]

[1]Mann-Whitney U Test; [2]Chi-Square Test

centrifuge tube (1 ml). Following steps were completed within 1 hour (at 25-24 °C temperature) or 2 hours (at 4 °C): centrifugation (relative centrifugal force: 413, NUVE-NF 1200R) for 20 min at 4°C, collection of the supernatant and storage at -80°C for future use.

Analysis method

Two ELISA kits for Human Laminin (LN)-YLA1827HU were used for the detection of laminin in the serum samples (YLbiont, Shanghai YL Biotech Co, Shanghai). It is based upon the principle of a sandwich assay which can detect levels of laminin as low as 0.49 ng/ml (detection range: 1-500 ng/mL) (intra-assay: CV <8%; inter-assay: CV <10%).

Statistical analysis

Normality control was done by drawing Shapiro Wilk and Kolmogorov Smirnov tests, histogram, Q-Q plot and box plots. The data are given in the form of mean +/- sd. According to Shapiro Wilk results, data were analyzed by Mann-Whitney U test. Groups containing three or more categories were compared using Kruskal Wallis tests with post-hoc Dunn's test. Receiver operating characteristic (ROC) curves were found for specificity and sensitivity. Significance limit was accepted as $P <0.05$. Analyzes were performed with NCSS 10 (2015, Kaysville, Utah, USA).

Written informed consent was obtained in accordance with the ethical principles stated in the Declaration of Helsinki. This study was started after ethical permission from Ethical Committee at Van Yuzuncu Yil University, Faculty of Medicine, Van, Turkey (Decision Number: 05, Decision Date: 06.06.2018).

RESULTS
Patient demographics and serum laminin levels in study groups

Thirty women (37.5%) and 50 men (62.5%) with gastric cancer (n=80) without a history of chemotherapy, radiotherapy or surgery as the patient group, and forty normal [13 female (32.5%) and 27 male (67.5%)] served as a control group (n=40) in this study. The mean age ± standard deviation (SD) of 58.8±4.99 years (45-71 years) for gastric cancer patients and 60.3±3.8 years (49-68 years) for the control group were not statistically different (P=0.128).

Laminin levels were calculated in both groups. The mean ± SD laminin level was 12.98±2.88 ng/mL (range: 9 to 18) in normal control group. On the other hand, the mean laminin level was 254±98.85 ng/mL (range: 165 to 483) in gastric cancer group. A highly significant difference was found in the median laminin levels between groups (P<0.001; Table 1 and Figure 1).

Preoperative serum laminin levels

There was significant relationship between laminin level and clinicopathologic parameters. Laminin levels at advanced stages were found higher (especially at T3 and T4 stage; P<0.001). Also, laminin levels were significantly higher at lymph node metastasis positive group (P<0.001) and advanced TNM stage group (stage III or IV; P<0.001). Another
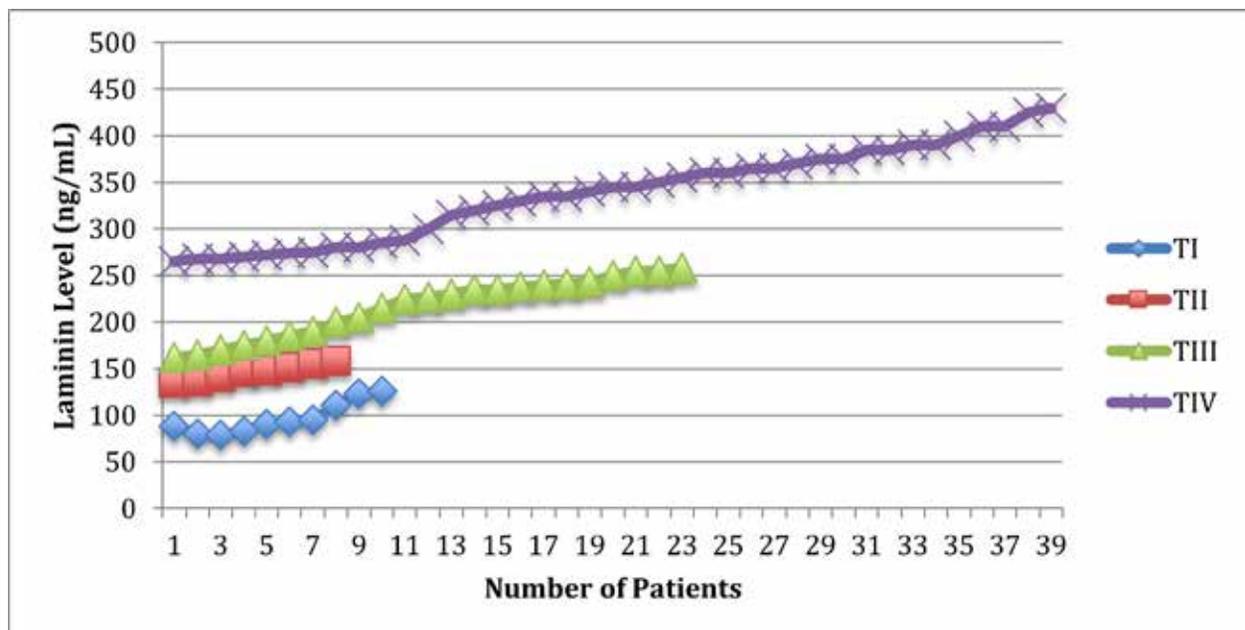

Figure 2: The histogram of the TNM stages.

Table 2: The association of laminin with the clinicopathologic parameters of the gastric cancer patients.

Clinicopathologic Variables	n (%)	Laminin (ng/mL) mean ± sd	P-value
Tumor differentiation			<0.001[1]
Well	5 (6.25)	84 ± 4.84	
Moderate	7 (8.75)	117 ± 17.78	
Poor	68 (85)	280.78 ± 81.52	
TNM stage			<0.001[1]
Stage I	10 (12.5)	96.8 ± 17.22	
Stage II	8 (10)	146.25 ± 8.44	
Stage III	23 (28.75)	216.48 ± 31.7	
Stage IV	39 (48.75)	338.85 ± 49.61	
Depth of invasion			<0.001[1]
T1	7 (8.75)	86.86 ± 6.3	
T2	12 (15)	141 ± 15	
T3	8 (10)	183.75 ± 14.07	
T4	53 (66.25)	312.49 ± 61.72	
Lymph node invasion			<0.001[2]
Negative	7 (8.75)	86.86 ± 6.3	
Positive	73 (91.25)	270.19 ± 87.95	
Distant metastasis			<0.001[2]
Yes	8 (10)	89.88 ± 10.34	
No	72 (90)	272.40 ± 86.5	

[1]Kruskal Wallis test with post-hoc Dunn's test; [2]Mann-Whitney U Test

result of our study is that high preoperative serum laminin levels correlated with metastasis (Table 2 and Figure 2).

The cut-off values and sensitivity and specificity of serum laminin were determined with ROC curves. The sensitivity and specificity determined from the ROC curves at cut-off level of 70 were 95% and 97% for serum laminin, respectively.

DISCUSSION

Gastric cancer, a major health problem with a poor 5-year survival all over the world, is poorly understood at molecular carcinogenesis level[9]. Due to the lack of information on the mechanism of gastric cancer, approximately 50% of patients present advanced stage of gastric cancer which precludes a curative treatment[10].

Gastric cancer treatment choice depends on the location and extent of the cancer[11]. There are many biomarkers including alpha-fetoprotein, carbohydrate antigen (CA) 72-4, and CA 12-5, carcinoembryonic antigen and CA 19-9 commonly used in clinical practice[12]. These markers are not specific, as these markers lack the sensitivity and specificity required to assess the diagnosis and prognosis of gastric cancer. On the other hand, the positive expression of monoclonal gastric cancer 7 antigen (MG7-Ag) shows a high risk of gastric cancer. Furthermore, the sensitivity and specificity

of MG7Ag as a single marker in the diagnosis of gastric cancer may not be sufficient[13]. Therefore, the screening value of these biomarkers for early gastric cancer is limited and there is a need for biomarkers with high sensitivity and specificity. In this study, it was investigated whether serum laminin level can be used in the early diagnosis of gastric cancer by finding both sensitivity and specificity.

Laminin molecule was first described in Engelbreth-Holm-Swan tumor, a murine fibrosarcoma by Timpl *et al*[14]. Laminin molecule is a high molecular weight protein of the extracellular matrix, and is an important component of basal membrane[15]. Laminin molecules are secreted and incorporated into cell-associated extracellular matrices, and are vital for the maintenance and survival of tissues[16].

In epithelial tissues, as in gastric epithelia, the basal surface of cells is surrounded by the basement membrane, which is mainly composed of laminins, collagen type-IV and other glycoproteins[5]. In gastric mucosa, laminin chains were shown to have differential expression at a molecular level. The composition of the surface epithelial basement membrane was associated with rapid recovery capacity of the gastric surface epithelium following chemical injury, but also suggested specific programs towards surface or glandular cell differentiation[6]. In an in vitro study, it was shown that laminin γ-2 subgroup mediates invasion of gastric cancer cells via Wingles and Int-1 pathway[17]. This makes the gastric cancer clinic more aggressive. In addition, as shown in studies, the ability of cancer cells to bind to laminin molecules has been associated with their metastatic potential, and highly metastatic cancer cells appear to express significantly more laminin receptors on their surface than their much less metastatic or benign counterparts[18]. However, there is no exact mechanism for the relationship between laminin molecules and gastric cancer. Considering previous studies, we hypothesized that the higher the level of laminin levels in the blood, the more aggressive and metastatic the cases of gastric cancer, and we designed this study and we aimed to test our hypothesis.

Laminin plays a major role in multiple cell functions such as migration, adhesion, cellular growth and differentiation and inflammatory response. Laminin receptors are also found at the surface of platelets, hepatocytes, muscle cells and endothelial cells[19]. Laminin binding proteins have been identified in *Helicobacter pylori*, *Staphylococcus aureus* and *Escherichia coli*[20].

Aghcheli *et al* evaluated laminin levels in upper gastrointestinal tract cancers, and divided into two

as cardia cancer and non-cardia gastric cancer. Serum laminin levels were higher in both groups compared to the control group. However, a relationship between tumor size and tumor stage and laminin levels has not been evaluated. Sensitivity of laminin was 90% for cut-off point of 62 ng/ml and 80% for cut-off point of 67 ng/ml; the respective specificity of laminin was 60% and 68%[21]. However, in our study, the sensitivity and specificity determined from the ROC curves at cut-off level of 70 ng/mL were 95% and 97%, respectively.

Higher serum levels of laminin could be a bad prognostic factor in breast cancer[22]. Serum laminin levels were also useful for colon cancer at the time of diagnosis. Serum laminin level of colon cancer group was significantly higher than control group. No significant differences were seen in serum laminin levels due to age, gender, tumor location, tumor size, histological type, peritoneal metastasis and lung metastasis. However, serum laminin levels were only higher in patients with hepatic metastasis[7]. In another study by Wurz et al, serum laminin levels were significantly higher in patients with advanced stage gynaecological cancers than in healthy controls[23].

Laminin levels were higher in patients with advanced stage melanoma compared to healthy group[8]. On the other hand, Matteoni et al showed that serum laminin levels could also be a tumor marker for malignant ascites with 75% sensitivity, 100% specificity and 91% accuracy[24]. In another study, serum laminin levels might be useful to evaluate histological differentiation and aggressiveness of oral squamous cell carcinoma[25].

There were some limitations in this study. First, due to the cross-sectional design, it is impossible to establish any cause and effect relationship. Secondly, given the relatively small sample size and the ethnic and racial diversity in gastric cancer stage at diagnosis, our findings may not be generalized. Third, serum laminin levels were measured only once at the time of initial diagnosis. Nevertheless, despite limitations, given the restricted amount of data available on the prognostic role of serum laminin levels in gastric cancer, providing evidence regarding the utility of laminin as an oncofetal protein in diagnosis and identification of metastatic potential and poor oncological outcome among early stage gastric cancer patients, our findings represent a valuable contribution to the literature.

CONCLUSION

In conclusion, the serum concentration of laminin can be used as a predictive biomarker at the time of diagnosis for gastric cancer. Also, laminin can be used for cancer stage discrimination (earlier, advanced or metastatic). The availability of laminin should be supported by more patients and more studies. This is the first study which evaluates the correlation between tumor stages and serum laminin levels.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin 2021; 71(1):7-33.
2. Stomach Source: GLOBOCAN 2020. (Accessed: March 19, 2021, at https://gco.iarc.fr/today/data/factsheets/cancers/7-Stomach-fact-sheet.pdf).
3. NCCN Clinical Practice Guidelines in Oncology Gastric Cancer version 2.2021 -March 9, 2021. (Accessed: March 19, 2021, at https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf).
4. Thrumurthy SG, Chaudry MA, Hochhauser D, Mughal M. The diagnosis and management of gastric cancer. BMJ 2013; 347:f6367.
5. Domogatskaya A, Rodin S, Tryggvason K. Functional diversity of laminins. Annu Rev Cell Dev Biol 2012; 28:523-53.
6. Virtanen I, Tani T, Bäck N, Häppölä O, Laitinen L, Kiviluoto T, et al. Differential expression of laminin chains and their integrin receptors in human gastric mucosa. Am J Pathol 1995; 147(4):1123-32.
7. Saito N, Kameoka S. Serum laminin is an independent prognostic factor in colorectal cancer. Int J Colorectal Dis 2005; 20(3):238-44.
8. Burchardt ER, Hein R, Bosserhoff AK. Laminin, hyaluronan, tenascin-C and type VI collagen levels in sera from patients with malignant melanoma. Clin Exp Dermatol 2003; 28(5):515-20.
9. Matsuoka T, Yashiro M. Biomarkers of gastric cancer: current topics and future perspective. World J Gastroenterol 2018; 24(26):2818-32.
10. Correa P. Gastric cancer: overview. Gastroenterol Clin North Am 2013; 42(2):211-17.
11. Orditura M, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM, et al. Treatment of gastric cancer. World J Gastroenterol 2014; 20(7):1635-49.
12. Shimada H, Noie T, Ohashi M, Oba K, Takahashi Y. Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association. Gastric cancer 2014; 17(1):26-33.

13. Ren J, Chen Z, Juan SJ, Yong XY, Pan BR, Fan DM. Detection of circulating gastric carcinoma-associated antigen MG7-Ag in human sera using an established single determinant immuno-polymerase chain reaction technique. Cancer 2000; 88(2):280-5.

14. Timpl R, Rohde H, Robey PG, Rennard SI, Foidart J-M, Martin GR. Laminin–a glycoprotein from basement membranes. J Biol Chem 1979; 254(19):9933-7.

15. Durbeej M. Laminins. Cell Tissue Res 2010; 339(1):259-68.

16. Yurchenco PD, Patton BL. Developmental and pathogenic mechanisms of basement membrane assembly. Curr Pharm Des 2009; 15(12):1277-94.

17. Yamamoto H, Kitadai Y, Oue N, Ohdan H, Yasui W, Kikuchi A. Laminin γ2 mediates Wnt5a-induced invasion of gastric cancer cells. Gastroenterology 2009; 137(1):242-52. e1-6.

18. de Manzoni G, Guglielmi A, Verlato G, Tomezzoli A, Pelosi G, Schiavon I, et al. Prognostic significance of 67-kDa laminin receptor expression in advanced gastric cancer. Oncology 1998; 55(5):456-60.

19. Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. Dev Dyn 2000; 218(2):213-34.

20. Valkonen KH, Ringner M, Ljungh Å, Wadström T. High-affinity binding of laminin by Helicobacter pylori: evidence for a lectin-like interaction. FEMS Immunol Med Microbiol 1993; 7(1):29-37.

21. Aghcheli K, Parsian H, Qujeq D, Talebi M, Mosapour A, Khalilipour E, et al. Serum hyaluronic acid and laminin as potential tumor markers for upper gastrointestinal cancers. Eur J Intern Med 2012; 23(1):58-64.

22. Sidhom G, Imam M. Evaluation of serum laminin as a tumor marker in breast cancer. Int J Clin Lab Res 1999; 29(1):26-9.

23. Würz H, Crombach G. Radioimmunoassay of laminin P1 in body fluids of pregnant women, patients with gynaecological cancer and controls. Tumor Biol 1988; 9(1):37-46.

24. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity.

Original Article

Risk factors for the development of acute kidney injury in patients with COVID-19 pneumonia receiving ICU care

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ABSTRACT

Objective: The present study aimed to investigate the role of factors affecting critical and severe COVID-19 pneumonia in patients with and without acute kidney injury (AKI) who were followed up in the intensive care unit (ICU).
Design: Retrospective study
Setting: Atatürk Chest Diseases and Chest Surgery Education and Research Hospital
Subjects: Three hundred and twenty-one critical and severe COVID-19 patients who were followed up in ICU were retrospectively evaluated in terms of AKI and one-month independent mortality.
Interventions: Critical and severe COVID-19 pneumonia
Main outcome measures: Serum troponin, D-dimer, ProCT, C-reactive protein, glomerular filtration rate (GFR), uric acid, albumin, LDH, IgA, IgM, IgG, CBC, creatinine, and 48-hour urine creatinine levels were assessed and short-term (30-day) independent mortality.

Results: Short-term (30-day) mortality occurred in 146 (45.5%) patients, among whom 55 (19.5%) had AKI. In patients with AKI, the APACHE II and SOFA scores and the ferritin, troponin, 48-hour urine creatinine, uric acid, and LDH levels were significantly higher ($P<0.05$), whereas age and GFR values were significantly lower ($P=0.017$ and $P<0.001$, respectively). Multiple logistic regression analysis indicated that the presence of AKI and elevated 48-hour urine creatinine levels increased the risk of ICU admission by 1.257 (odds ratio [OR]: 1.357) and 3.986 times (OR: 3.986), respectively.
Conclusions: Based on our findings, we suggest that monitoring renal function and serum ferritin, troponin, LDH and GFR levels during the first 48 hours and then at regular intervals can help reduce mortality in these patients.

KEY WORDS: acute kidney injury, COVID-19, intensive care unit, mortality

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was renamed as coronavirus disease 2019 (COVID-19) and was declared a pandemic by the World Health Organization in 2020. Nucleic acid amplification tests such as real-time reverse transcriptase polymerase chain reactions (RT-PCR) are frequently used in diagnosis of COVID-19[1]. This disease primarily affects the respiratory system and causes acute respiratory distress syndrome (ARDS). Additionally, it may also lead to cytokine storm due to systemic inflammation and involve multiple organs and systems, such as liver, kidney and nervous system. Clinical manifestations of COVID-19 comprise four categories: mild, moderate, severe and critical. Acute kidney injury (AKI) is a major complication of the disease, leading to increased mortality and morbidity. The mechanism of AKI remains unclear, although it has been associated with the detection of fragments of viral proteins in the renal system by PCR and the increased expression of angiotensin-converting enzyme 2 (ACE2) receptors in the renal system[2]. Some previous studies indicated that AKI developed within an average period of 20 days in patients with coronavirus-induced Severe Acute Respiratory Syndrome and also noted that AKI was a negative prognostic factor[1]. In contrast, Wang *et al*[3] evaluated 116 COVID-19 patients and reported that the

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Table 1: Demographic and clinical characteristics

Variables	Mean±SD	Median (Min-Max)
Age (years)	66.8±12.7	68 (20-94)
APACHE II score	21.2±6.1	20 (10-38)
SOFA score	6.0±3.1	5 (2-18)
Time (days)	4.5±3.3	3 (1-30)
Hospital stay (days)	14.7±9.7	12 (1-59)
ICU stay (days)	7.8±7.2	5 (1-36)

Variables	N	%
Gender		
Male	200	62.3
Female	121	37.7
Mortality(30-day)		
No	175	54.5
Yes	146	45.5
Comorbidity		
No	110	34.3
Yes	211	65.7
HT		
No	179	55.8
Yes	142	44.2
DM		
No	241	75.1
Yes	80	24.9
CAD		
No	254	79.1
Yes	67	20.9
CHF		
No	300	93.5
Yes	21	6.5
COPD		
No	267	83.2
Yes	54	16.8
Malignancy		
No	302	94.1
Yes	18	5.6
Neurological disorder		
No	303	94.4
Yes	18	5.6
Bronchial asthma		
No	317	98.8
Yes	4	1.2
IMV		
No	239	74.5
Yes	82	25.5
NIMV		
No	259	80.7
Yes	62	19.3
HFNO		
No	163	50.8
Yes	158	49.2
OMR		
No	174	54.2
Yes	147	45.8
AKI (KDIGO criteria)		
No	227	80.5
Yes	55	19.5
Renal replacement therapy (IHD or CRTT)		
No	310	96.6
Yes	11	3.4

APACHE II: Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; TIME: time from the onset of symptoms to hospital admission; ICU: intensive care unit; HT: hypertension; DM: diabetes mellitus; CAD: coronary artery disease; CHF: congestive heart failure; COPD: chronic obstructive pulmonary disease; IMV: invasive mechanical ventilation; NIMV: noninvasive mechanical ventilation; HFNO: high-flow nasal oxygen therapy; OMR: non-rebreather oxygen masks with reservoir bags; AKI: acute kidney injury; KDIGO criteria: Kidney Disease: Improving Global Outcomes; IHD: intermittent hemodialysis; CRTT: continuous renal replacement therapy; SD: standard deviation

prevalence of AKI was remarkably low, and that AKI was not associated with mortality. On the other hand, literature suggests that daily evaluation of renal function is recommendable, and that AKI increases in-hospital mortality and morbidity in critical and severe COVID-19 patients followed up in the intensive care unit (ICU). Moreover, although the incidence of AKI is not clear, it has been reported to be approximately 37%[4].

In COVID-19 infection, laboratory abnormalities can be observed as a result of cytokine storm caused by exaggerated systemic inflammation, particularly in severe and critical cases, and these abnormalities have been shown to be associated with kidney functions and mortality. Of note, these abnormalities have mostly been reported in complete blood count, neutrophil count (NE), white blood cell count, lymphocyte count (LY), C-reactive protein (CRP), D-dimer, procalcitonin (ProCT), troponin, ferritin and lactate dehydrogenase (LDH). Moreover, it has also been reported that serum CRP, D-dimer, ferritin and troponin levels increase particularly in relation to disease severity and mortality[5]. In a retrospective study, Haung et al[6] evaluated a cohort of 2,623 COVID-19 patients and found that serum albumin level was associated with disease severity and poor prognosis. In contrast, another study reported that serum ProCT level was remarkably low and increased in the presence of secondary infections[6,7]. On the other hand, Feng et al[7] evaluated COVID-19 patients and reported that serum immunoglobulin M (IgM) levels decreased in critical and severe patients, while no significant difference was found between mild and severe patients with regard to IgG and IgA levels. The authors also noted that the troponin levels increased and albumin levels decreased in severe patients.

Considering that COVID-19 is a relatively recent disease, it is tempting to consider that it has many unknown aspects. In particular, the effect of inflammatory biomarkers, acute phase reactants, and risk factors on the development of AKI in COVID-19 patients is not clear. In the present study, the primary aim was to determine the role of factors affecting critical and severe COVID-19 pneumonia in patients with and without AKI who were followed up in ICU. The secondary endpoint of the study was one-month independent mortality.

SUBJECTS AND METHODS

After obtaining ethical approval, patients who were admitted to ICU due to positive SARS-COV-2 PCR results and the signs of pneumonia and hypoxemic respiratory failure between March 2020 and January 2021 were retrospectively evaluated (Ethics committee, date:12/31/2020, date:707). All the patients included in the study met the Infectious Disease Society of America and American Thoracic Society ICU admission criteria: radiological (posterior/anterior X-ray or thoracic computed tomography) infiltration >50%, tachypnea (respiratory rate >30 breaths/min), and the ratio of the partial pressure of oxygen in arterial blood (PaO_2) to the inspired oxygen fraction (FiO_2) (PaO_2/FiO_2 ratio) <300 mmHg[8]. Patients were divided into four groups according to the respiratory support received during ICU admission: invasive mechanical ventilation (IMV), non-invasive mechanical ventilation, high-flow nasal oxygen therapy and non-rebreather oxygen masks with reservoir bags. In all the patients included in the study, serum troponin, D-dimer, ProCT, CRP, glomerular filtration rate (GFR), uric acid, albumin, LDH, IgA, IgM, IgG, complete blood count, creatinine and 48-hour urine creatinine levels were assessed and short-term (30-day) independent mortality was monitored. AKI was defined retrospectively using the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (increase in serum creatinine by 0.3mg/dL or more within 48 hours) within the first 72 hours after ICU admission. Before classifying the patients according to KDIGO guidelines, patients were evaluated for the presence of AKI during the first 48 hours after ICU admission. However, due to the retrospective nature of the study, hourly urine output, hematuria and proteinuria could not be evaluated. Intermittent hemodialysis (IHD) and continuous renal replacement therapy (CRRT) performed during the one-month period in the ICU were recorded for each patient[9,10]. Time from the onset of symptoms to hospital admission and the duration of hospital and ICU stays were recorded. Acute Physiology and Chronic Health Evaluation (APACHE) II score and Sequential Organ Failure Assessment (SOFA) score were used for predicting ICU mortality and prognosis[11]. Exclusion criteria were as follows: pregnancy, age under 18 years, active infection other than COVID-19, ongoing renal replacement therapy due to chronic kidney disease, a history of AKI within the last three months, collagen tissue disease, use of drugs that could affect the concentrations of uric acid and Ig, and a history of iron, vitamin B12 and folic acid supplementation.

All blood parameters were measured using a Mindray BC-6800 hematology analyzer (Mindray Medical International Ltd., China) with the photometric method. The normal ranges of white blood count, NE, LY, hemoglobin, hematocrit, platelet count, mean platelet volume, plateletcrit (PCT), platelet distribution width, and red blood cell distribution width were 4.6-10.2x10^3/μL, 2-7x10^3/μL, 0.8-4x10^3/μL, 12-16 g/dl, 40-54%, 142-424x10^3/μL, 7.8-11 fL, 0.19-0.36%, 14-18%, and 11.6-17.2%, respectively. The normal ranges of CRP, blood urea nitrogen, creatinine, albumin, uric acid, LDH, ferritin, troponin, D-dimer, IgA, IgM and IgG were 0-5 mg/L, 8-20 mg/dL, 0.81-1.44 mg/dL, 3.5-5.2 g/dL, 3.5-7.2 mg/dL, 0-248 IU/L, 22-322 ng/ml, 0-58.5 ng/L, 0-0.44 mg/L, 0.7-4 g/L, 0.4-2.3 g/L, and 7-16 g/L, respectively. ProCT levels below 0.5 ng/mL represented a low risk and the levels over 2 ng/ml indicated a high risk. Troponin, ferritin and PCT were measured with the immunoassay test using a Siemens ADVIA Centaur XP immunoassay device. CRP, blood urea nitrogen, creatinine, albumin, uric acid and LDH levels were measured using a Beckman Coulter autoanalyzer and D-dimer was measured using a SYSMEX automatic hematology analyzer with the turbidimetric method.

Table 2: Laboratory parameters

Variables	Mean±SD	Median (Min-Max)
Ferritin (ng/ml)	678.3±548.4	50.5 (4.3-1650)
Troponin (ng/L)	258.5±14.63	13.8 (0.018-19223.6)
D-Dimer (mg/L)	5.13±12.36	1.27 (0.19-80)
Procalcitonin (ng/ml)	1.99±8.38	0.18 (0.01-100)
CRP (mg/L)	125.6±94.12	115.8 (0.37-562.9)
Creatinine (mg/dL) (on ICU admission)	1.09±0.41	1 (0.26-3)
Creatinine at 48th hour (mg/dL)	1.19±0.78	0.64 (0.44-4.96)
GFR	72.0±58.3	72.3 (4.5-101)
Uric acid (mg/dL)	6.04±4.89	5.3 (0.3-77.9)
Albumin (g/L)	4.12±17.18	3.13 (1.47-4)
LDH (mg/dL)	502.12±312.87	420 (106-2518)
IgA (g/L)	2.98±1.69	2.7 (0.52-12.38)
IgM (g/L)	1.13±0.66	0.95 (0.26-4.27)
IgG (g/L)	10.51±2.78	10.35 (2.2-22.68)
WBC (X10^3/μL)	10.49±5.5	9.43 (2.3-35.93)
NE (X10^3/μL)	8.98±5.4	8.01 (1.31-34.87)
LY (X10^3/μL)	1.06±1.19	0.81 (0.05-16.49)
PLT (X10^3/μL)	249.17±108.42	227 (58-797)
HGB (g/dL)	13.38±1.78	13.5 (7.1-18.9)
HCT (%)	40.38±5.84	40.8 (3.21-61.5)
RDW (%)	14.29±1.96	13.9 (11.7-30.8)
MPV (fL)	9.69±2.05	9.5 (6.25-40.3)
PCT (%)	0.23±0.10	0.21 (0.02-0.73)
PDW (%)	16.27±0.48	16.2 (14-18.3)

ICU: intensive care unit; CRP: C-reactive protein; GFR: glomerular filtration rate; LDH: lactate dehydrogenase; IgA: Immunoglobulin A; IgM: Immunoglobulin M; IgG: Immunoglobulin G; WBC: white blood cell count; NE: neutrophil count; LY: lymphocyte count; PLT: platelet count; HGB: hemoglobin; HCT: hematocrit; RDW: red blood cell distribution width; MPV: mean platelet volume; PCT: plateletcrit; PDW: platelet distribution width; SD: standard deviation

Table 3: Effects of demographic and clinical characteristics on AKI development

Variables	AKI (n=227)	No AKI (n=55)	P
Age (years)	67 (20-94)	20 (10-38)	0.017
APACHE II score	20 (10-38)	25 (13-38)	<0.001
SOFA score	5 (2-14)	8 (2-18)	<0.001
Time from first symptoms to admission (days)	3 (1-18)	5 (1-10)	0.117
Hospital stay (days)	14 (2-59)	9 (2-42)	<0.001
ICU stay (days)	6 (2-34)	6 (2-36)	0.510
Ferritin (ng/ml)	488 (4.3-1650)	612.6 (20-1650)	0.008
Troponin (ng/L)	12.42 (0.5-14533.95)	25.45 (0.018-19223.6)	<0.001
D-Dimer (mg/L)	1.28 (0.19-80)	1.54 (0.22-80)	0.134
Procalcitonin (ng/ml)	0.17 (0.01-100)	0.24 (0.01-75)	0.078
IgA (g/L)	2.72 (0.52-12.38)	2.45 (0.9-12.24)	0.223
IgG (g/L)	10.6±2.6	10.1±3.4	0.351
IgM (g/L)	0.94 (0.28-4.27)	0.94 (0.26-3.66)	0.235
CRP (mg/L)	115.8 (0.37-395.74)	125 (1.81-562.9)	0.179
GFR	75.3 (15.8-118.9)	51.5 (4.5-108)	<0.001
Creatinine (mg/dL)(on ICU admission)	0.98 (0.5-3)	1.08 (0.26-2.94)	0.018
Creatinine at 48th hour (mg/dL)	0.85 (0.44-3)	1.96 (0.9-4.96)	<0.001
Uric acid (mg/dL)	4.6 (0.3-15.4)	8.6 (1.7-77.9)	<0.001
Albumin (g/L)	3.13 (1.68-5.31)	3.04 (1.7-309)	0.132
LDH (mg/dL)	416 (106-2518)	491 (131-2483)	0.012
WBC (X10^3/μL)	9.39 (2.38-35.93)	10.5 (2.3-28.82)	0.178
NE (X10^3/μL)	8 (1.31-34.87)	9.23 (1.34-27.33)	0.139
LY (X10^3/μL)	0.82 (0.09-16.49)	0.65 (0.05-5.07)	0.055
PLT (X10^3/μL)	227 (0.58-797)	217 (23-558)	0.370
HGB (g/dL)	13.3±1.8	13.5±1.9	0.519
HCT (%)	40.6 (3.21-61.5)	40.9 (26.6-55.4)	0.711
RDW (%)	13.9 (11.7-25)	14.1 (11.8-30.8)	0.419
MPV (fL)	9.67±2.34	9.74±1.09	0.834
PCT (%)	0.21 (0.04-0.73)	0.20 (0.02-0.49)	0.419
PDW (%)	16.2 (15.2-18.3)	16.2 (14-17.2)	0.854

AKI: acute kidney injury; APACHE II: Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; ICU: intensive care unit; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; CRP:C-reactive protein; GFR: glomerular filtration rate; LDH: lactate dehydrogenase; WBC: white blood cell count; NE: neutrophil count; LY: lymphocyte count; PLT: platelet count; HGB: hemoglobin; HCT: hematocrit; RDW: red blood cell distribution width; MPV: mean platelet volume; PCT: plateletcrit; PDW: platelet distribution width.

Statistical analysis

Data were analyzed using SPSS for Windows version 23.0 (Armonk, NY: IBM Corp.). Categorical variables were expressed as frequencies (n) and percentages (%) and continuous variables were expressed as mean, standard deviation. Normal distribution of data was determined using Shapiro-Wilk test. Categorical variables were compared using Pearson's chi-squared test and Fisher's exact test. Continuous variables with normal distribution were compared using Student's t-test and the variables with nonnormal distribution were compared using Mann-Whitney U test. Multiple logistic regression analysis was performed to determine the independent variables affecting the dependent variables. A P-value of <0.05 was considered significant.

RESULTS

The study included 321 patients, comprising 200 (62.3%) men and 121 (37.7%) women, with a mean age of 66.8±12.7 years. Short-term (30-day) mortality occurred in 146 (45.5%) patients, among whom 55 (19.5%) had AKI according to KDIGO guidelines (increase in serum creatinine by 0.3mg/dL or more within 48 hours) and 11 (3.4%) had received CRRT for one month. Table 1 presents the demographic and clinical characteristics of the patients. For the treatment of COVID-19, all the 321 patients (100%) received favipiravir, vitamin C, and low-molecular-weight heparin, 72 (22.5%) patients received methylprednisolone, 21 (6.5%) patients received tocilizumab, 3 (0.93%) patients received anakinra, 111 (34.5%) patients received dexamethasone, and 18 (5.6%) patients received immune (convalescent) plasma. Table 2 presents the mean values of laboratory parameters of the patients.

The frequency of mortality and CRRT during the one-month period was significantly higher in patients with AKI (P<0.001 and P<0.05, respectively). Similarly, the APACHE II and SOFA scores and the ferritin and troponin levels assessed on ICU admission and the 48-hour urine creatinine, uric acid and LDH levels were significantly higher in patients with AKI (P<0.05). In contrast, age and GFR values were significantly lower

in patients with AKI (*P*=0.017 and *P*<0.001, respectively). Multiple logistic regression analysis indicated that the presence of AKI and elevated 48-hour urine creatinine levels increased the risk of ICU admission by 1.257 (odds ratio [OR]: 1.357) and 3.986 times (OR: 3.986), respectively. Table 3 presents the effects of demographic and clinical characteristics on AKI development.

DISCUSSION

SARS-CoV-2 enters the target cell by binding to the ACE2 molecule via the S protein. The ACE2 protein is released in large amounts in intestinal epithelial, renal tubular, alveolar, cardiac and smooth muscle cells. However, the mechanism of AKI is unclear and is considered to be multifactorial, although it has been associated with the detection of fragments of viral proteins in the renal system by PCR and the increased expression of ACE2 proteins in the renal system[2,10]. Studies have shown that a cytokine storm results from exaggerated systemic inflammation, thereby leading to AKI or microthrombi[2,12]. A previous study detected proteinuria, hematuria and elevated creatinine levels in COVID-19 patients[12]. A systematic review by Lotfi et al[13] indicated that COVID-19 patients had a higher incidence of AKI and renal abnormalities on radiological examinations and these occurrences were associated with increased mortality. The authors also noted that 2/3 of the hospitalized patients had deteriorated renal function and this condition persisted in 25% of them after discharge[13,14]. The incidence of AKI in COVID-19 patients with ARDS was reported as 25% and 0.5% in two different studies[15,16]. In a previous review, Ahmed et al[17] reported a mortality rate of 50% for COVID-19 patients with AKI that were hospitalized in ICU. On the other hand, critical and severe COVID-19 patients have been shown to have a mortality rate of over 60%, regardless of respiratory support and medical treatment in ICU[18]. In our study, the rate of short-term (30-day) independent mortality was 45.5%. This rate was lower than those reported in the literature, which could be attributed to our low IMV rate and APACHE II and SOFA scores and the short duration of ICU stay. Additionally, this rate could be explained by the fact that COVID-19, since it is a relatively recent disease, has not been standardized and its effectiveness has not been proven and the effect of respiratory support and medical treatment performed in ICU on the disease has not yet been fully understood[19-20].

In our study, AKI was detected in 19.5% and renal replacement therapies (IHD, CRTT) were administered in 3.4% of the patients. The incidence of AKI in COVID-19 patients followed up in ICU is reported to be approximately 37%[3,4]. However, a previous study reported that although initial examinations showed mild impairment in renal function, this impairment did not meet the AKI criteria and was found to be caused by hypoxemia[3,4]. In a previous review, Ahmed et al[17] reported the incidence of AKI in COVID-19 patients as 30%. Hirsch et al[21] evaluated hospitalized COVID-19 patients and reported that the incidence of AKI was higher particularly in patients that developed ARDS and also noted that AKI occurred within the first 24 hours after admission. By contrast, a previous meta-analysis indicated that AKI occurred within an average of 9 days after admission[22]. The differences among the findings presented by these studies could be attributed to the lack of effective antiviral agents in COVID-19 treatment, particularly in severe and critical cases, and the administration of different treatment trials. In our study, however, we found a lower incidence of AKI, which could be explained by the fact that AKI was defined retrospectively using the KDIGO guidelines (increase in serum creatinine by 0.3mg/dL or more within 48 hours) within the first 72 hours after ICU admission[9]. Nevertheless, due to the retrospective nature of our study, hourly urine output, hematuria, proteinuria and radiological findings of the patients could not be evaluated, which are commonly evaluated in other studies[9,21]. In our study, in a similar way to the study by Hirsch et al[21], AKI was evaluated during the first 48 hours, when the highest degree of hypoxemia and the greatest hemodynamic changes are seen[9]. In our study, renal replacement therapies (IHD, CRTT) were administered in 3.4% of the patients regardless of the duration of AKI. Although the role of genetics, ethnicity, systemic inflammation, and immune dysfunction in the development of AKI in COVID-19 patients remains unclear, secondary bacterial infections and the administration of positive pressure ventilation in ICU pose a risk for AKI development[21]. In our patients, the IMV rate was lower than those reported in the literature, which could be the reason for our low AKI incidence.

Symptoms of COVID-19 typically emerge within 2-14 days after exposure, while approximately 81% of the patients remain asymptomatic or develop mild symptoms such as dry cough, fever, myalgia and weakness. The severe disease develops in approximately 14% of all cases and 5% of them are admitted to ICU. Moreover, most of the patients admitted to ICU develop ARDS and acute hypoxemic respiratory failure[23,24]. In our study, time from the onset of symptoms to hospital admission was found to be an insignificant factor for AKI development in COVID-19 patients hospitalized in ICU (*P*>0.05). Nevertheless, a comparison could not be made due to the lack of studies in the newly defined critical and severe COVID-19 disease. To date, an effective antiviral therapy for COVID-19 disease has not yet been

identified worldwide. The use of favipiravir before hospitalization in our study may have reduced the severity of the disease. In our study, AKI incidence may have decreased due to this. Due to limited data in the literature, the effectiveness of antiviral agents (favipiravir, remdesivir) against AKI is unknown[25]. Accordingly, further multicentric, randomized, controlled studies are needed on this subject.

Previous studies evaluated patients with moderate to severe COVID-19 pneumonia and reported that in patients aged over 52 years, CRP, LDH, elevated D-dimer, low albumin, and APACHE II and SOFA scores were associated with disease progression and suggested that LY and PCT levels could be used as prognostic factors[17,26]. In COVID-19 patients, neutrophilia, lymphopenia, and increase in acute phase reactants (CRP, PCT, D-dimer, ferritin) have been identified as risk factors for abnormal kidney function[17]. In a retrospective study, He et al[27] evaluated 204 COVID-19 patients and found higher IgG, NE, D-dimer, LDH, CRP and PCT values and lower IgM, LY and platelet count values in severe patients. Another study reported that serum levels of acute phase reactants including LDH, PCT, CRP and ferritin were higher in COVID-19 patients with AKI than in the group without AKI[28]. On the contrary, Casas-Aparicio et al[29] reported that obesity, age and IMV were identified as risk factors for AKI in patients with severe COVID-19 pneumonia, while no significant difference was established with regard to serum levels of acute phase reactants. In the present study we, in a similar way to the limited number of studies on COVID-19, found that age, APACHE II and SOFA scores, duration of ICU stay, LDH, ICU admission, creatinine, 48-hour urine creatinine, GFR, troponin and ferritin levels were significant risk factors, while the other acute phase reactants were not established as risk factors.

Our study was limited in several ways. First, it was designed as a retrospective single-center study. Second, the study only included patients with critical and severe COVID-19 pneumonia that were hospitalized in ICU and excluded patients with mild and moderate COVID-19. Third, data on serum levels of acute phase reactants and inflammatory biomarkers were drawn from single measurements. Finally, only one criterion (increase in serum creatinine within the first 48 hours) was used for the diagnosis of AKI.

CONCLUSION

The results indicated that early diagnosis of AKI is highly important for reducing mortality and morbidity in severe and critical COVID-19 patients followed up in ICU. Based on our findings, we suggest that monitoring renal function and serum ferritin, troponin, LDH and GFR levels during the first 48 hours and then at regular intervals can help reduce mortality in these patients. Further studies are needed to investigate the role of inflammatory biomarkers and acute phase reactants as risk factors for the development of AKI in COVID-19 patients, which is a recent disease and has many unknown aspects.

REFERENCES

1. Chu KH, Tsang WK, Tang CS, Lam MF, Lai FM, To KF, et al. Acute renal impairment in coronavirus-associated severe acute respiratory syndrome. Kidney Int 2005; 67(2):698-705.
2. Pan XW, Xu D, Zhang H, Zhou W, Wang LH, Cui XG. Identification of potential mechanism of acute kidney injury during the COVİD-19 outbreak: a study based on single-cell transcriptome analysis. Intensive Care Med 2020; 46(6):1114-6.
3. Wang L, Li X, Chen H, Yan S, Li D, Li Y, et al. Coronavirus disease 19 infection does not result in acute kidney injury: An analysis of 116 hospitalized patients from Wuhan, China. Am J Nephrol 2020; 51(5):343-8.
4. Ng JH, Bijol V, Sparks MA, Sise ME, Izzedine H, Jhaveri KD. Pathophysiology and pathology of acute kidney injury in patients with COVID-19. Adv Chronic Kidney Dis 2020; 27(5):365-76.
5. Lim AYH, Goh JL, Chua MCW, Heng BH, Abisheganaden JA, George PP. Temporal changes of haematological and radiological findings of the COVID-19 infection-a review of literature. BMC Pulm Med 2021; 21(1):37.
6. Huang W, Li C, Wang Z, Wang H, Zhou N, Jiang J, et al. Decreased serum albumin level indicates poor prognosis of COVID-19 patients: hepatic injury analysis from 2,623 hospitalized cases. Sci China Life Sci 2020; 63(11):1678-87.
7. Feng Y, Ling Y, Bai T, Xie Y, Huang J, Li J, et al. COVID-19 with different severities: A multicenter study of clinical features. Am J Respir Crit Care Med 2020; 201(11):1380-8.
8. Mandell LA, Wunderink RG, Anzueto A, Barlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America; American Thoracic Society. Infectious

Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007; 44(Suppl 2):S27-72.

9. Kellum JA, Lameire N, KDIGO AKI Guideline Work Group. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). Crit Care 2013; 17(1):204.

10. Nadim MK, Forni LG, Mehta RL, Connor MJ, Liu KD, Ostermann M, et al. COVID-19-associated acute kidney injury: consensus report of the 25th Acute Disease Quality Initiative (ADQI) Workgroup. Nat Rev Nephrol 2020; 16(12):747-64.

11. Keegan MT, Gajic O, Afessa B. Severity of illness scoring systems in the intensive care unit. Crit Care Med 2011; 39(1):163-9.

12. Cheng Y, Luo R, Wang K, Zhang M, Wang Z, Dong L, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. Kidney Int 2020; 97(5):829-38.

13. Lotfi B, Farshid S, Dadashzadeh N, Valizadeh R, Rahmini MM. Is Coronavirus Disease 2019 (COVID-19) associated with renal involvement? A review of century infection. Jundishapur Journal of Microbiology 2020; 13(4):e102899.

14. Ng JJ, Luo Y, Phua K, Choong AMTL. Acute kidney injury in hospitalized patients with coronavirus disease 2019 (COVID-19): Meta-analysis. J Infect 2020; 81(4):647-79.

15. Fanelli V, Fiorentino M, Cantaluppi V, Gesualdo L, Stallone G, Ronco C, et al. Acute kidney injury in SARS-CoV-2 infected patients. Crit Care 2020; 24(1):155.

16. Guan WJ, Ni ZY, Hu Y, Liang WH, Qu CQ, He JX, et al. Clinical characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020; 382(18):1708-20.

17. Ahmed AR, Ebad CA, Stoneman S, Satti MM, Conlon PJ. Kidney injury in COVID-19. World J Nephrol 2020; 9(2):18-32.

18. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med 2020; 46(5):846-8.

19. Tobaiqy M, Qashqary M, Al-Dahery S, Mujallad A, Hershan AA, Kamal MA, et al. Therapeutic management of patients with COVID-19: a systematic review. Infect Prev Pract 2020; 2(3):100061.

20. Edalatifard M, Akhtari M, Salehi M, Naderi Z, Jamshidi A, Mostafaei S, et al. Intravenous methylprednisolone pulse as a treatment for hospitalised severe COVID-19

patients: results from a randomised controlled clinical trial. Eur Respir J 2020; 56(6):2002808.

21. Hirsch JS, Ng JH, Ross DW, Sharma P, Shah HH, Barnett RL, et al. Acute kidney injury in patients hospitalized with COVID-19. Kidney Int 2020; 98(1):209-18.

22. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020; 8(5):475-81.

23. Hajjar LA, Costa IB, Rizk SI, Biselli B, Gomes BR, Bittar CS, et al. Intensive care management of patients with COVID-19: a practical approach. Ann Intensive Care 2021; 11(1):36.

24. Jutzeler CR, Bourguignon L, Weis CV, Tong B, Wong C, Rieck B, et al. Comorbidities, clinical signs and symptoms, laboratory findings, imaging features, treatment strategies, and outcomes in adult and pediatric patients with COVID-19: A systematic review and meta-analysis. Travel Med Infect Dis 2020; 37:101825.

25. Agrawal U, Raju R, Udwadia AF. Favipiravir: A new and emerging antiviral option in COVID-19. Med J Armed Forces India 2020; 76(4):370-6.

26. Wang D, Li R, Wang J, Jiang Q, Gao C, Yang J, et al. Correlation analysis between disease severity and clinical and biochemical characteristics of 143 cases of COVID-19 in Wuhan, China: a descriptive study. BMC Infect Dis 2020; 20(1):519.

27. He R, Lu Z, Zhang L, Fan T, Xiong R, Shen X, et al. The clinical course and its correlated immune status in COVID-19 pneumonia. J Clin Virol 2020; 127:104361.

28. Zheng X, Zhao Y, Yang L. Acute kidney injury in COVID-19: The Chinese experince. Semin Nephrol 2020; 40(5):430-42.

29. Casas-Aparicio GA, León-Rodríguez I, Alvarado-de la Barrera C, Gonzalez-Navarro M, Peralto- Prado AB, Luna-Villalobos V, et al. Acute kidney injury in patients with severe COVID-19 in Mexico. PLoS One 2021; 16(2):e0246595.

Original Article

Evaluation of the reproducibility of MS Candidate genes using genome wide data

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ABSTRACT

Objectives: Evaluation of the reproducibility of multiple sclerosis (MS) susceptibility single nucleotide polymorphisms (SNPs) in Iranian MS patients
Design: Case-control study
Setting: Department of Genetics, North Tehran Branch, Islamic Azad University, Tehran, Iran and Department of Medical Genetics, Urmia University of Medical Sciences, Urmia, Iran.
Subjects: A total of 129 relapsing-remitting multiple sclerosis (RRMS) patients and 200 healthy controls were recruited in the study.
Intervention(s): None
Main outcome measure(s): Validation of genetic risk factors associated with MS in Iranian MS patients
Results: The SNP genotyping was performed using allele-specific polymerase chain reaction (PCR). Allele and genotype single locus regression analysis was performed.

Three polymorphisms showed significant association (*P*<0.05) with MS. In particular, rs4810485 of CD40 (*P*=0.002, OR=2.676, 95%CI=1.406 - 5.095), rs1077667 of TNFSF14 (*P*=0.007, OR=2.461, 95%CI=1.264 - 4.789) and rs4976646 of RGS14 (*P*=0.001, OR=2.763, 95%CI=1.474 - 5.176) were identified as susceptibility risk factors in our group. We also correlated associated SNPs with MS clinical phenotypes like visual impairment and ataxia. Two SNPs, rs4810485 and rs4976646, were significantly associated with ataxia and impaired vision.
Conclusion: The current study implies the existence of some similarities between the MS genetic structure of the genome-wide association studies populations and the studied Iranian population and outlined the importance of confirmed genes to understand signaling pathways in MS disease and clarified their utility to develop new drug targets and proper treatment of the disease.

KEY WORDS: CD40, multiple sclerosis, RGS14, single nucleotide polymorphism, TNFSF14

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated demyelinating and neurodegenerative disease of the central nervous system characterized by inflammation, demyelination, axonal degeneration and neurodegeneration[1]. MS is usually diagnosed between the third and fourth decade of life that affects more than two million individuals worldwide[2], and it occurs more often in women than men.

Based on past epidemiological studies, Iran has the maximum prevalence of MS in Asia and the Middle East[3]. The prevalence of MS in Iran is in the range of 7.4 to 89 per 100,000 in different provinces[4]. Previous studies from Sweden, Canada, Norway, UK and India indicated that the prevalence of MS in Iranian immigrants is higher than the native population and the prevalence was varied from 21 per 100,000 people in Bombay, India in 1985 to 433 per 100,000 people in British Columbia, Canada in 2012[3].

There are three main features during the progression of MS: (i) formation of lesions (plaques)

in the central nervous system; (ii) inflammation; and (iii) destruction of myelin sheaths of neurons, and diffusion of global tissue injury. The plaque affects the white matter in the brain stem, optic nerve and spinal cord[5,6]. These focal plaques are the main features of the diagnosis of MS pathology. As MS progresses, myelin loss results in the breakdown of the axons of neurons and can no longer conduct electrical signals[7,8]. MS is difficult to diagnose because there is no single test that can diagnose the disease. The diagnosis of MS is made through the collection of evidence from a clinical examination, medical history, lab tests and magnetic resonance imaging (MRI) of the brain and sometimes the spinal cord. The clinical course of MS can be relapsing-remitting MS (RRMS), primary-progressive MS, secondary progressive MS and progressive relapsing MS. RRMS is the common type of MS. Patients with RRMS will experience new symptoms or worsening symptoms (relapses) which can last for days to weeks and months also[9,10]. Hence, screening tests can be very helpful in diagnosing people prone to MS.

The pathogenesis of MS is complex. It results from interactions between several environmental factors and alleles of many susceptibility loci across the genome[11]. Some factors may increase the risk of MS, like age, some viruses, smoking, exposure to more sunlight and vitamin-D. MS can affect any person at any age, but statistics have shown that the ratio of women with MS to men is 2:1, and people between the age of 15 to 60 years are affected more. An important question is how many variants explicate the genetic part of MS. Recent studies declare that there are many hundred common and rare alleles across the genome with very mild effect sizes that altogether influence MS risk. Additionally, environmental factors and interactions (both gene-gene and gene-environment) may describe the variable character of this complex disease[12].

Recent investigations of the genetics of MS have resulted in completion of the first genome-wide association studies (GWAS)[11]. Classical linkage studies and GWAS have yielded a remarkable number of non-major histocompatibility complex genes with modest effects, involved in MS susceptibility[13,14]. In the 1970s, there was success with the finding of variants in the major histocompatibility complex, which were associated with MS, particularly the HLA-DRB1*1501 allele[12].

Complex clinical presentation in MS suggests a heterogeneous genetic etiology. So, it is clear that HLA by itself cannot explain the whole genetic portion of MS and more genetic factors had to be discovered[15]. The identification of several non-major histocompatibility complex MS susceptibility loci has been gained through recent GWAS. Single nucleotide polymorphisms (SNPs) can be used as genetic biomarkers for the screening test.

On the other hand, genetic studies including GWAS have yielded inconsistent findings and low reproducibility with limited replication of MS loci in different studies due to genetic heterogeneity among different ethnic groups[16]. Therefore, it is important to validate genetic variants, which were formerly reported to be associated with MS by the past GWASs in different ethnic groups. In fact, none of the previous GWASs has included Iranian MS patients[17-19].

Objectives

In the present case-control study, we genotyped twenty SNPs in Iranian MS patients. SNPs were selected from previous GWASs, which have a major effect (high amount of odds ratio (OR) and 95% confidence interval (CI)) and the possibility of association with multiple sclerosis[17-19].

MATERIALS AND METHODS
Patients

A total of 129 MS patients and 200 healthy controls from the Iranian population, matched by age, gender and ethnicity were compared in a case-control study. Patients were included in the study if they had a clinically definite diagnosis of RRMS according to the criteria of McDonald[20] or Poser[21]. The control group consisted of healthy subjects without a history of neurological disease and was matched with cases for age, gender and ethnicity. The demographic data of the samples are presented in Table 1. Females represented 82 of our MS patients with a female: male ratio of 1.74. The study was approved by the Ethics Committee of the Urmia University of Medical Sciences, Urmia, Iran (Approval ID: IR.UMSU.REC.1397.131). Written informed consent was taken from all patients and controls included in the study. The sample size was calculated through Cochran's formulas:

$$n_0 = Z^2pqN / d^2(N-1) + Z^2pq$$
$$n_0 = (1.96)^2 \times 0.5 \times 0.5 \times 2200 / (0.05)^2 (2199) + (1.96)^2 \times 0.5 \times 0.5 = 327$$

Table 1: The demographic data

Characteristics	Controls	Total patients
Female, n (%)	134(67%)	82(63.56%)
Male, n (%)	66 (33%)	47(36.44%)
Total, n	200	129
Female: male ratio	2.03	1.74
Age at time of analysis, mean±SD (range)	37.54±8.55 (20-60)	37.24 ±7.95 (22-57)

Table 2: Association analysis of the MS risk variants in the Iranian replication case-control (recessive and dominant model).

SNP[a]	Gene[b]	AF (Case)[c]	AF (Control)[d]	H-W P (exact) control/case[e]	Analysis	OR (95% CI)[f]	P-value[g]	Power[h]
rs7200786	CLEC16A	A:0.293 G:0.706	A:0.178 G:0.821	0.314/0.009	Recessive model AA vs AG+GG	2.6515 (0.6103-11.5192)	0.1932	0.6476
					Dominant model AA+AG vs GG	1.5789 (0.5009-4.977)	0.4355	0.5205
rs4810485	CD40	T:0.32 C:0.67	T:0.22 C:0.77	0.098/0.001	Recessive model TT vs GT+GG	2.676 (1.406 - 5.095)*	0.002	0.7329
					Dominant model TT+GT vs GG	1.394 (0.886 – 2.192)	0.15	0.3117
rs1077667	TNFSF14	T:0.24 C:0.75	T:0.21 C:0.78	0.089/0.0001	Recessive model TT vs CT+CC	2.461 (1.264 – 4.789)*	0.007	0.5830
					Dominant model TT+CT vs CC	1.163 (0.734 – 1.842)	0.52	0.1018
rs4976646	RGS14	C:0.360 T:0.639	C:0.302 T:0.697	0.982/0.005	Recessive model CC vs CT+TT	2.763 (1.474 -5.176)*	0.001	0.893
					Dominant model CC+CT vs TT	0.946 (0.608 -1.473)	0.806	0.056

Information regarding the association analysis of the MS risk variants in the Iranian replication case-control under the recessive and dominant model.
[a]dbSNP rs-number; [b]gene name; [c,d]allele frequency of SNP in case and control in our study population; [e]Hardy-Weinberg Equilibrium; [f]odds ratios (OR) for the corresponding risk alleles and the 95% confidence interval (C.I.) of the corresponding OR; [g]P-values calculated with one degree of freedom (df=1); [h]statistical power for their given ORs and allele frequencies using QUANTO.
* indicate statistical significance.

In this study, statistical power for each minor allele frequency and OR combination was calculated using Quanto and Online Sample Size Estimator (osse.bii.a-star.edu.sg).

Clinical and para-clinical data, *i.e.* MRI, were obtained during medical examinations in our university clinic. The clinical data contain visual impairment arising from optic neuritis, and ataxia measured by the functional scale scores for Expanded Disability Status Scale. These measures were correlated with associated SNPs.

Isolation of DNA, SNP selection and genotyping

Genomic DNA was isolated from peripheral white blood cells using the salting-out standard method. The concentration and purity of the extracted DNA were assayed by Eppendorf Bio Photometer. DNA samples were diluted to working solutions of 50-100 ng/μl.

Twenty SNPs that had suggestive evidence of association with MS were selected from the previous GWASs[17-19]. Based on the comparison between case and control populations, reference alleles and wild-type genotypes were determined.

The SNP genotyping was performed using allele-specific polymerase chain reaction. Primers were designed by web-based allele-specific primer design application, bioinfo.biotec.or.th/WASP/. As a quality control, a random 15% of samples were re-genotyped and no contradictions were observed.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was evaluated separately in the groups of patients with MS and healthy controls using the Fisher exact test to check the model of association. To evaluate the SNP effects on MS susceptibility, OR and 95% CI and significance were calculated using the IBM SPSS Statistics (Version 23). The level of statistical significance was set at *P*<0.05.

RESULTS

Twenty MS risk variants were genotyped and analyzed. Regression analysis for alleles and genotypes under the recessive model, dominant model and multiplicative model were done and statistically significant association were found for three polymorphisms, rs4810485 of CD40, rs1077667 of TNFSF14 and rs4976646 of RGS14. These associations remained significant after Bonferroni correction (*P*<0.00256) except rs1077667 of TNFSF14.

Regression analysis for genotypes under the recessive and dominant model

HWE evaluation of rs7200786 of CLEC16A, rs1077667 of TNFSF14, rs4810485 of CD40, and rs4976646 of RGS14 showed that, control group was under the HWE but case group was not. So we analyzed association of these SNPs under recessive and dominant model[22].
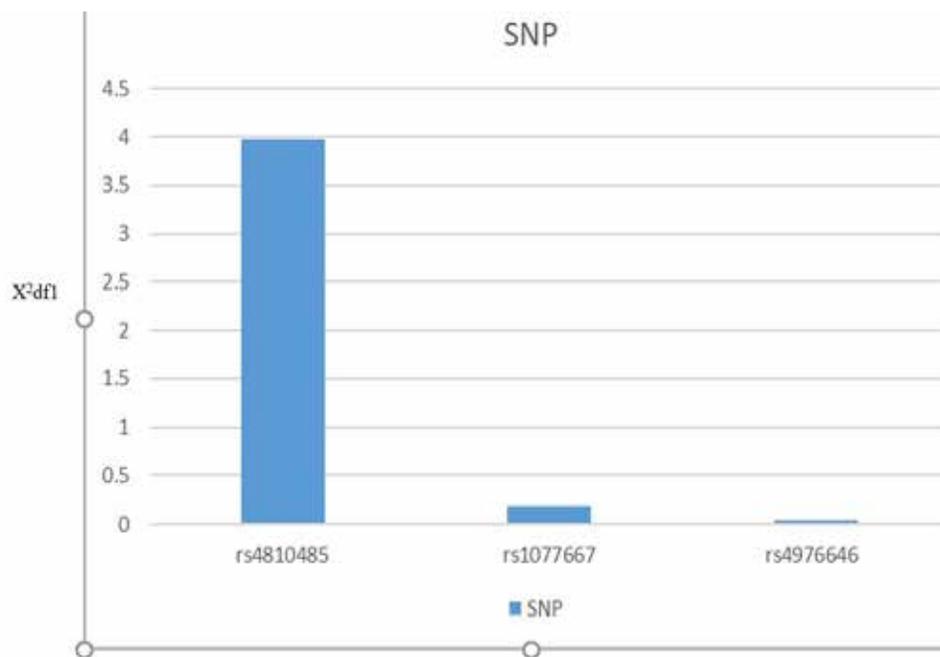
Fig 1: Effect modification analysis of gender on the MS-associated SNPs. Effect modification analysis of sex (female and male as subgroups) demonstrated a significant statistical difference between the total odds ratio and subgroups odds ratios of rs4810485(X^2df1=3.98).

Regression analysis for genotypes under the recessive model identified rs4810485 of CD40 (P=0.002, OR=2.676, 95%CI=1.406 -5.095), rs1077667 of TNFSF14 (P=0.007, OR=2.461, 95%CI=1.264 -4.789) and rs4976646 of RGS14 (P=0.001, OR=2.763, 95%CI=1.474 - 5.176) as significant MS susceptibility risk factors in our group (Table 2). rs4810485 of CD40 and rs4976646 of RGS14 remained significant after Bonferroni correction (P<0.00256).

Regression analysis for alleles under the multiplicative model

The SNPs listed in Table 3 were in HWE in both case and control groups, so their association were analyzed under the multiplicative model. None of them were in association with MS in our study population (Table 3).

Sex effects

We have studied the effect of gender on the MS-associated SNPs. Interestingly, effect modification analysis of sex (female and male as subgroups) demonstrated a significant statistical difference between the total OR and subgroups OR of rs4810485 (X^2=3.98). The effect modification results of MS-associated SNPs are presented in Figure 1.

Association of ataxia and impaired vision with associated SNPs

We have studied the correlation of the rs4810485, rs1077667 and rs4976646 with MS clinical phenotypes like visual impairment and ataxia. We found two SNPs, rs4810485 and rs4976646, in significant association with ataxia and impaired vision (Table 4).

DISCUSSION

In the present study, we have performed a replication study of twenty susceptibility SNPs in Iranian RRMS patients and we found significant associations for three of them, namely rs4810485, rs1077667 and rs4976646.

rs4810485, which reached the statistical significance threshold in genotypic comparison under the recessive model (TT vs GT+GG) in our group, is an intronic variant of tumor necrosis factor (TNF) receptor superfamily member 5 (CD40). CD40 is an important co-stimulatory molecule and it expresses on the surface of antigen-presenting cells including dendritic cells, B-lymphocytes, macrophages and microglia[23]. CD40 interacts with CD40 ligand and this interaction is essential for the regulation of the immune system and homeostasis[24]. Some studies show significant associations between CD40 variants and MS, although some have not found significant associations. In the mouse model of MS, treatment with a neutralizing antibody to CD40L prevented experimental autoimmune encephalomyelitis[24]. Furthermore, RRMS patients represented boosted the proliferation of Memory B cells with CD40 stimulation compared to healthy controls because of NFκB cascade dysregulation[24]. CD40 stimulation leads to activation of MAP kinases and phosphoinositide 3-kinase[25],

Table 3: Association analysis of the MS risk variants in the Iranian replication case-control. (Multiplicative model)

SNP[a]	Gene[b]	AF (Case)[c]	AF (Control)[d]	H-W P (exact) control/case[e]	Multiplicative model[f]	Power[g]
rs2283792	MAPK1	T: 0.44 G: 0.56	T:0.25 G:0.75	1/1	T vs G OR: 2.333 95% CI: 0.999-5.448 P-value: 0.0502	0.94
rs11810217	EVI5	T: 0.33 C: 0.67	T:0.13 C:0.87	0.251/0.107	T vs C OR: 0.6022 95% CI: 0.178-2.0344 P-value: 0.4142	0.99
rs1014486	IL12A	C: 0.2 T: 0.8	C: 0.09 T: 0.91	0.704/0.334	C vs T OR: 2.395 95% CI: 0.772-7.430 P-value: 0.1304	0.79
rs3135388	HLA	A: 0.13 G: 0.87	A:0.07 G:0.93	0.66/1	A vs G OR: 2 95% CI: 0.567-7.053 P-value: 0.281	0.42
rs354033	ZNF746	A: 0.13 G: 0.87	A: 0.08 G: 0.92	0.669/1	A vs G OR: 1.923 95% CI: 0.544-6.789 P-value: 0.309	0.30
rs2744148	SOX8	G: 0.28 A: 0.72	G: 0.26 A: 0.74	0.85/1	G vs A OR: 1.129 95% CI: 0.493-2.585 P-value: 0.773	0.059
rs1131265	TIMMDC1	C: 0.14 G: 0.86	C:0.15 G: 0.85	1/1	C vs G OR: 0.880 95% CI: 0.304-2.541 P-value: 0.813	0.044
rs2255214	CD86	G: 0.48 T: 0.52	G: 0.43 T: 0.57	1/1	G vs T OR: 1.237 95% CI: 0.602-2.542 P-value: 0.561	0.142
rs13333054	IRF8	T: 0.27 C: 0.73	T: 0.17 C: 0.83	1/0.25	T vs C OR: 1.813 95% CI: 0.751-4.374 P-value: 0.185	0.571
rs2104286	IL2RA	C: 0.08 T: 0.92	C: 0.14 T: 0.86	1/0.7	C vs T OR: 0.526 95% CI: 0.161-0.171 P-value: 0.287	0.396
rs1920296	IQCB1	G: 0.13 A: 0.87	G: 0.09 A: 0.91	0.46/0.57	G vs A OR: 1.457 95% CI: 0.433-4.900 P-value: 0.542	0.203
rs1335532	CD58	G: 0.1 A: 0.9	G: 0.15 A: 0.85	0.61/0.21	G vs A OR: 0.634 95% CI: 0.204-1.968 P-value: 0.431	0.997
rs12148050	TRAF3	A: 0.42 G: 0.58	A: 0.26 G: 0.74	1/1	A vs G OR: 2.087 95% CI: 0.903-4.824 P-value: 0.085	0.852
rs12087340	BCL10	T: 0.43 C: 0.57	T:0.29 C:0.71	1/1	T vs C OR: 1.816 95% CI: 0.836-3.945 P-value: 0.131	0.735
rs7552544	VCAM1	T: 0.17 C: 0.83	T:0.2 C:0.8	0.08/0.31	T vs C OR: 0.833 95% CI: 0.315-2.202 P-value: 0.713	0.80
rs34383631	CD6	T: 0.19 C: 0.81	T: 0.11 C: 0.89	0.21/0.31	T vs C OR: 1.818 95% CI: 0.610-5.416 P-value: 0.283	0.509

Information regarding the association analysis of the MS risk variants in the Iranian replication case-control under the multiplicative model. [a]dbSNP rs-number; [b]gene name; [c,d]allele frequency of SNP in case and control in our study population; [e]Hardy-Weinberg Equilibrium; [f]odds ratios (OR) for the corresponding risk alleles and the 95% confidence interval (C.I.) of the corresponding OR and the P-values calculated with one degree of freedom (df=1); [g]statistical power for their given ORs and allele frequencies using Online Sample Size Estimator (OSSE) (osse.bii.a-star.edu.sg).

Table 4: Association of clinical phenotypes with associated SNPs.

Clinical phenotypes	rs4810485	rs1077667	rs4976646
Ataxia	P-value= 0.003*	P-value= 0.662	P-value= 0.01*
	OR= 3.667*	OR= 1.22	OR= 3.514*
	95%CI= 1.495-8.994*	95%CI= 0.5 – 2.977	95%CI= 1.302- 9.484*
Impaired vision	P-value = 0.013*	P-value= 0.546	P-value= 0.033*
	OR= 2.49*	OR= 1.25	OR= 5.568*
	95%CI = 1.207 -5.138*	95%CI= 0.606 – 2.58	95%CI= 1.106-28.026*

The odds ratio and 95% CI of the associated SNPs with ataxia and visual impairment.
*indicate statistical significance.

activation of canonical nuclear factor kappa B (NFκB) [26], and non-canonical NFκB signaling[27]. NFκB and MAP kinases are the essential components of signaling pathways downstream of CD40 engagement in B cells[24]. Furthermore, another study revealed that CD40 -1C>T (rs1883832) is associated with an increased risk of MS[28]. GWASs have identified 97 variants associated with MS, which are either within or near to NFκB signaling gene[17,19].

We additionally identified an association for rs1077667 (TT genotype) with MS risk under the recessive model. rs1077667 is an intronic variant of the TNF ligand superfamily member 14 (TNFSF14) gene. In our study, we compared case and control population genotype and we found TT (rs1077667) as wild type genotype and CC (rs1077667) as mutant genotype.

TNFRSF14 gene encodes a protein which is a member of the TNF ligand family and, it is a costimulatory factor for the activation of lymphoid and proliferation of T cells. Related pathways with the TNFRSF14 gene are the innate immune system and ERK signaling. Gene ontology annotations related to the TNFRSF14 gene are signaling receptor binding and TNF receptor binding. TNFSF14 and its receptor, TNFRSF14, are newly identified as a risk gene for MS. The membrane-bound TNFSF14, a co-stimulatory molecule, is essential for CD4+ memory T-cell survival and has diverse stimulatory effects on the immune system. Over-expression of the membrane-bound TNFSF14 leads to autoimmunity in mice. The expression of TNFSF14 and serum level of its protein was found to be significantly decreased in MS patients compared with controls[29].

The CC genotype of variant rs4976646 in the regulator of G protein signaling 14 (RGS14) gene was associated with MS risk in genotypic comparison under the recessive model in our group. RGS14 encodes a member of the regulator of G-protein signaling family, which is required for the nerve growth factor-mediated neurite outgrowth and involved in stress resistance.

Also, association analysis after sex stratification showed sex-specific effects for rs4810485. We found a remarkable statistical difference between the total OR and subgroups OR of rs4810485.

Given that the genetic risk of MS is defined by many prevalent genetic variants, each with a small effect, it is obvious that every MS patient genomes contain a higher burden of disease-associated SNPs than comparative normal controls in the same population[13].

In our study, we analyzed the correlation of MS clinical phenotypes like visual impairment and ataxia with MS-associated SNPs. We found two SNPs, rs4810485 and rs4976646, in association with the intended clinical phenotypes. Nevertheless, the clinical association data needs to be clarified with precaution, with relevance to statistical robustness.

CONCLUSIONS

Our study replicated a number of susceptibility SNPs on the RRMS patients and represented some similarities between the Iranian population and the MS genetic structure of the GWAS populations. However, these data need to confirm the role of these variations on expression level and their pathological effects on MS susceptibility.

Also, the results of associated SNPs from our replication study suggest that larger, population-specific GWASs are needed to find all potentially MS-associated genetic variants in Iranian MS patients.

REFERENCES

1. Hadjigeorgiou GM, Kountra PM, Koutsis G, Tsimourtou V, Siokas V, Dardioti M, *et al*. Replication study of GWAS risk loci in Greek multiple sclerosis patients. Neurol Sci 2019; 40(2):253-60.

2. Baranzini SE, Oksenberg JR. The genetics of multiple sclerosis: from 0 to 200 in 50 years. Trends Genet 2017; 33(12):960-70.

3. Nasr Z, Majed M, Rostami A, Sahraian MA, Minagar A, Amini A, *et al*. Prevalence of multiple sclerosis in Iranian emigrants: review of the evidence. Neurol Sci 2016; 37(11):1759-63.

4. Etemadifar M, Izadi S, Nikseresht A, Sharifian M, Sahraian MA, Nasr Z. Estimated prevalence and incidence of multiple sclerosis in Iran. Eur Neurol 2014; 72(5-6):370-4.

5. Dyment DA, Ebers GC, Sadovnick AD. Genetics of multiple sclerosis. Lancet Neurol 2004; 3(2):104-10.

6. Huang WJ, Chen WW, Zhang X. Multiple sclerosis: pathology, diagnosis and treatments. Exp Ther Med 2017; 13(6):3163-6.

7. Bifulco M, Malfitano A. Advances in flow cytometry investigation of cannbinoid CB2 receptor agonists in multiple sclerosis: Commentary. J Mult Scler 2014; 2:1.

8. Rosati G. The prevalence of multiple sclerosis in the world: an update. Neurol Sci 2001; 22(2):117-39.

9. Lassmann H, van Horssen J, Mahad D. Progressive multiple sclerosis: pathology and pathogenesis. Nat Rev Neurol 2012; 8(11):647-56.

10. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, *et al*. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018; 17(2):162-73.

11. Sanna S, Pitzalis M, Zoledziewska M, Zara I, Sidore C, Murru R, *et al*. Variants within the immunoregulatory CBLB gene are associated with multiple sclerosis. Nat Genet 2010; 42(6):495-7.

12. Zuvich RL, Bush WS, McCauley JL, Beecham AH, de Jager PL, Ivinson AJ, *et al*. Interrogating the complex role of chromosome 16p13. 13 in multiple sclerosis susceptibility: independent genetic signals in the CIITA–CLEC16A–SOCS1 gene complex. Hum Mol Genet 2011; 20(17):3517-24.

13. Cavanillas M, Fernandez O, Comabella M, Alcina A, Fedetz M, Izquierdo G, *et al*. Replication of top markers of a genome-wide association study in multiple sclerosis in Spain. Genes Immun 2011; 12(2):110-5.

14. Donnelly P. Progress and challenges in genome-wide association studies in humans. Nature 2008; 456(7223):728-31.

15. Munoz-Culla M, Irizar H, Otaegui D. The genetics of multiple sclerosis: review of current and emerging candidates. Appl Clin Genet 2013; 6:63-73.

16. Bashinskaya VV, Kulakova OG, Boyko AN, Favorov AV, Favorova OO. A review of genome-wide association studies for multiple sclerosis: classical and hypothesis-driven approaches. Hum Genet 2015; 134(11-12):1143-62.

17. International Multiple Sclerosis Genetics Consortium (IMSGC); Beecham AH, Patsopoulos NA, Xifara DK, Davis MF, Kemppinen A, Cotsapas C, *et al*. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet 2013; 45(11):1353-60.

18. Akkad DA, Olischewsky A, Reiner F, Hellwig K, Esser S, Epplen JT, *et al*. Combinations of susceptibility genes are associated with higher risk for multiple sclerosis and imply disease course specificity. PLoS One 2015; 10(5):e0127632.

19. International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium; Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, *et al*. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 2011; 476(7359):214-9.

20. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, *et al*. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001; 50(1):121-7.

21. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, *et al*. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983; 13(3):227-31.

22. Lewis CM. Genetic association studies: design, analysis and interpretation. Brief Bioinform 2002; 3(2):146-53.

23. Field J, Shahijanian F, Schibeci S, Australia and New Zealand MS Genetics Consortium (ANZgene), Johnson L, Gresle M, *et al*. The MS risk allele of CD40 is associated with reduced cell-membrane bound expression in antigen presenting cells: implications for gene function. PLoS One 2015; 10(6):e0127080.

24. Chen D, Ireland SJ, Remington G, Alvarez E, Racke MK, Greenberg B, *et al*. CD40-mediated NF-κB activation in B cells is increased in multiple sclerosis and modulated by therapeutics. J Immunol 2016; 197(11):4257-65.

25. Dadgostar H, Zarnegar B, Hoffmann A, Qin XF, Truong U, Rao G, *et al*. Cooperation of multiple signaling pathways in CD40-regulated gene expression in B lymphocytes. Proc Natl Acad Sci USA 2002; 99(3):1497-502.

26. Craxton A, Shu G, Graves JD, Saklatvala J, Krebs EG, Clark EA. p38 MAPK is required for CD40-induced gene expression and proliferation in B lymphocytes. J Immunol 1998; 161(7):3225-36.

27. Hostager BS, Bishop GA. CD40-mediated activation of the NF-κB2 pathway. Front Immunol 2013; 4:376.

28. Qin J, Xing J, Liu R, Chen B, Chen Y, Zhuang X. Association between CD40 rs1883832 and immune-related diseases susceptibility: A meta-analysis. Oncotarget 2017; 8(60):102235-43.

29. Jernås M, Malmeström C, Axelsson M, Nookaew I, Wadenvik H, Lycke J, *et al*. MicroRNA regulate immune pathways in T-cells in multiple sclerosis (MS). BMC Immunol 2013; 14(1):32.

Original Article

Reliability of the projection area per length squared for quantifying the degree of scoliosis on X-ray films

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ABSTRACT

Objectives: Precise measurement of spinal curvature is the cornerstone for assessment of and intervention for spinal scoliosis. This study provides an alternative and reliable approach for spinal curvature measurement.

Design: This retrospective study was authorized and conducted in the Orthopedics and Traumatology Department of Ondokuz Mayis University, Turkey.

Setting: Orthopedics and Traumatology Department, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey.

Subject: The anteroposterior digital X-rays of 46 patients diagnosed with idiopathic scoliosis were examined. The straight length between the superomedial and inferomedial corners of the upper and lower end vertebral bodies and the semilunar area on the concave side of the curvature were estimated using the planimetry technique.

Intervention: The percentage of the semilunar area was estimated as the projection area per length squared (PAL). Measurements were taken on the radiographs twice by three observers independently using the PAL approach and the Cobb method. The diagnostic performance of the PAL method was tested against the Cobb method on the evaluation of the scoliotic deformity.

Main Outcomes Measures: High correlation between the PAL estimations and Cobb angle measurements existed for the first and second sessions (r=0.840; *P*<0.001 and r=0.855; *P*<0.001, respectively).

Results: The optimal cut-off values of the PAL were calculated as 5.53% and 9.67% corresponding to Cobb angles of 20° and 40°, respectively. Both methods showed high intra-observer and inter-observer reliability (all ICC values >0.929).

Conclusion: The PAL method is more reliable than the Cobb method for measuring the degree of scoliosis and could replace the Cobb method, which showed a high degree of variability in the measurements. It could be used as an alternative and robust diagnostic criterion in the determination of the severity of scoliosis.

KEY WORDS: Cobb angle, length squared, planimetry, radiography, scoliosis

INTRODUCTION

Scoliosis is defined as an abnormal lateral curvature of the spine in the coronal plane, generally accompanied by spinal rotation. It can cause serious postural deformities such as chest wall deformity, shoulder asymmetry, rib prominence or truncal shift[1]. Delays in the diagnosis and treatment of lateral curvature can lead not only to postural asymmetry, but also to pain, muscular weakness, cardiopulmonary dysfunction and psychological stress[2]. The measurement of spinal curvature is important in the evaluation of curve severity and progression in patients with scoliosis. These measurements are essential in the planning of surgical approaches, monitoring of disease progression and the evaluation of the effectiveness of interventions[3].

The Cobb method is the most commonly used technique in clinical practice and is considered the gold standard for the quantitative evaluation of the coronal curvature in scoliosis[4]. However, the Cobb angle

measurements may include serious systematic errors that undermine its reliability, such as the direct effect of endplate architecture, which is irregularly shaped. Using the Cobb approach in examination, a variation of the line drawn parallel to the superior and inferior endplates of the reference vertebrae could be defined, resulting in various angles being measured[5]. Another limitation is the inclination angle of the superior and inferior endplates of the reference vertebrae. Therefore, the Cobb method is prone to measurement errors in clinical practice[6]. Many studies have focused on the reliability and reproducibility of the Cobb method and some have reported high intra- and inter-observer variability[7-10].

Several alternative approaches have been described for measuring coronal spinal curvatures in scoliosis[11-14]. Ferguson defined three or more vertebrae references for assessing scoliosis curves[11]. However, this method is time-consuming, subjective and requires measurement skills to precisely determine the scoliotic angle. Vrtovec et al stated that the Ferguson method is not accurate, is complex and cannot be compared with the Cobb method[6]. Scholten et al reported that the Ferguson method generally shows smaller values because the method is influenced by axial vertebral rotation[15]. In addition, the method developed by Diab et al is influenced by vertebral body morphology and the exact location of the central point of the vertebra may not reflect the exact geometric center point of the vertebral image[12].

The main criticism of the lateral tangent method is the difficulty in determining the straight lines of the concave-shaped lateral margin of the vertebral body. The observer may select different lines drawn against the lateral margins of the upper end and lower end of the vertebrae. Therefore, this method is highly subjective and causes intra- and inter-observer measurement variances[16].

The centroid method requires four reference vertebrae and several bony landmarks on the reference vertebrae for the measurement of the degree of scoliosis[14]. This makes it a very complex and difficult to apply, time-consuming approach[6]. Hong et al reported that osteophytes on the vertebral margin may produce high measurement variability when using the centroid approach[17]. Hwang et al also stated that when using the centroid method, convexity in the lateral margin of the vertebral body and the blurred image of the corners of the vertebra increase the errors of measurement[16].

As mentioned above, the Cobb angle and other suggested methods to measure the spinal curvatures are subjective, prone to error and may cause high intra- and inter-observer variations in the measurement of scoliosis. Therefore, the proposed projection area per length squared (PAL) method is a more practical,

reliable approach that is not affected by the vertebral body morphology for the measurement of spinal coronal curvature of individuals with scoliosis in a clinical setting.

The purpose of this study was to examine the reliability and practicality of the PAL approach in quantifying the degree of scoliosis on anteroposterior X-ray films and to compare it with the gold standard Cobb method.

SUBJECTS AND METHODS
Study design
The study was approved by the Ethics Committee of Ondokuz Mayis University, Samsun. A total of 46 whole spine anteroposterior digital radiographs of patients (31 male and 15 females with a mean age of 14.26±1.79 years) with varying degrees of adolescent idiopathic scoliosis, were randomly selected from the archives of the Orthopedics and Traumatology Department. A total of 73 images were examined; 34 thoracic, 22 lumbar and 17 thoracolumbar scoliosis curves. Postoperative radiographs were not included in the study.

Three independent observers with different levels of measurement experience were involved in this study. Observer 1 had four years of experience using the PAL technique, but no measurement experience with the Cobb method. Observer 2 had experience measuring the Cobb angle for eight years, but no prior experience with the PAL technique. Observer 3 had no measurement experience with either the PAL or the Cobb methods. It was anticipated that 20 spinal X-rays would be more than sufficient to acquire the skills for using the PAL method and Cobb method. Therefore, on the same day one week before starting the study, the three observers independently measured 20 spinal X-ray images using the PAL and Cobb methods.

Radiographic measurements
PAL method
The planimetry technique was used to estimate the PAL of each scoliosis curve on the digital images. Planimetry is the most widely preferred method for surface area measurement of irregularly shaped structures[18]. All anteroposterior digital X-ray films were stored in Digital Imaging and Communications in Medicine (DICOM) format. All measurements were obtained by using ImageJ software. ImageJ is a freely available, open-source Java image-processing and analysis program that was developed by the National Institution of Health (USA). The PAL estimation of scoliotic curves in the spine was applied as follows: DICOM files were transferred to the ImageJ software. The upper and lowermost tilted vertebrae of each curve were identified independently by each observer. The superomedial corner of the upper-end vertebra and
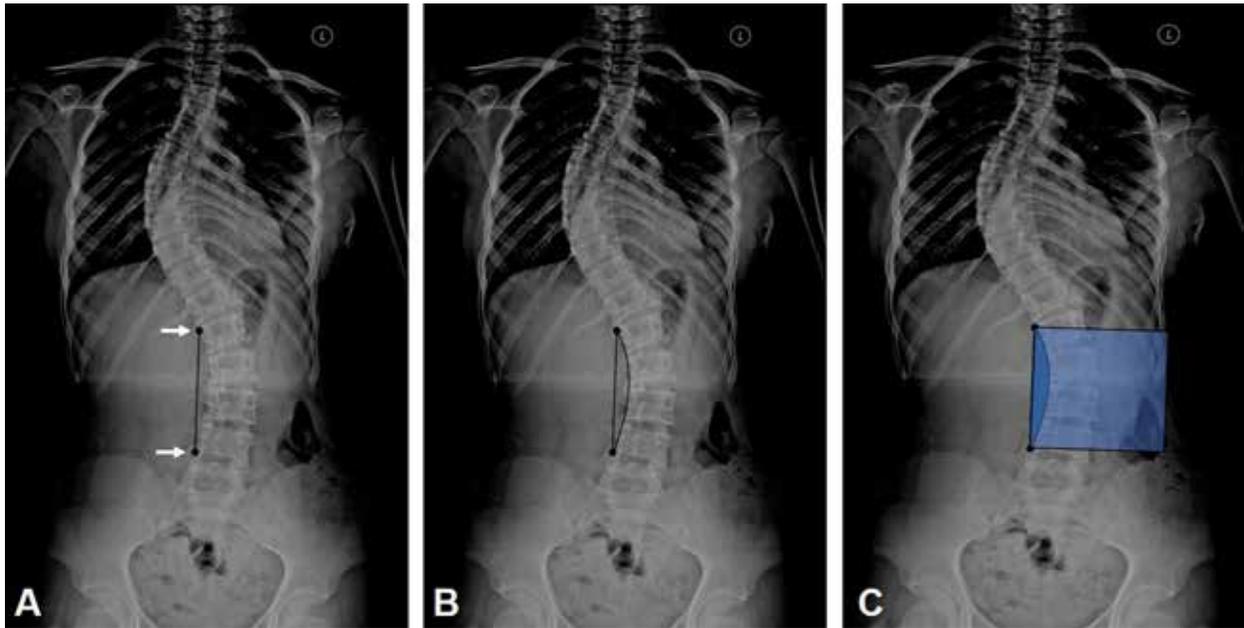
Fig. 1: (A) White arrows show the superomedial and inferomedial corners of the vertebral bodies of the superior and inferior end vertebrae; **(B)** Anteroposterior X-ray images showing the semilunar area drawn for the estimation of the projection area per length squared; **(C)** The PAL of the curvature expresses the surface area proportion of the semilunar area within the projection area of the square, which is the virtual reference surface area obtained from the square of the length.

the inferomedial corner of the lower-end vertebra were marked as landmarks. The landmarks were connected with a straight line (Fig. 1A). The medial boundaries of all the vertebrae between the superomedial and inferomedial corners were drawn along the curvature, and the upper and lower ends of the elliptical-shaped line were connected to the beginning and end of the straight line. Finally, a semilunar area was obtained on the concave side of the curvature (Fig. 1B).

Both the semilunar region projection area and the length of the straight line were calculated using the ImageJ program. Finally, the PAL was calculated as a percentage using the following formula[19]:

$$PAL = \frac{A}{l^2} \times 100$$

Where (A) denotes the semilunar region area and (l) represents the estimated length of the straight line between the superior and inferior end vertebrae. The PAL of the curvature expresses the surface area proportion of the semilunar area within the projection area of the square that is the virtual reference surface area obtained from the square of the length (Fig. 1C).
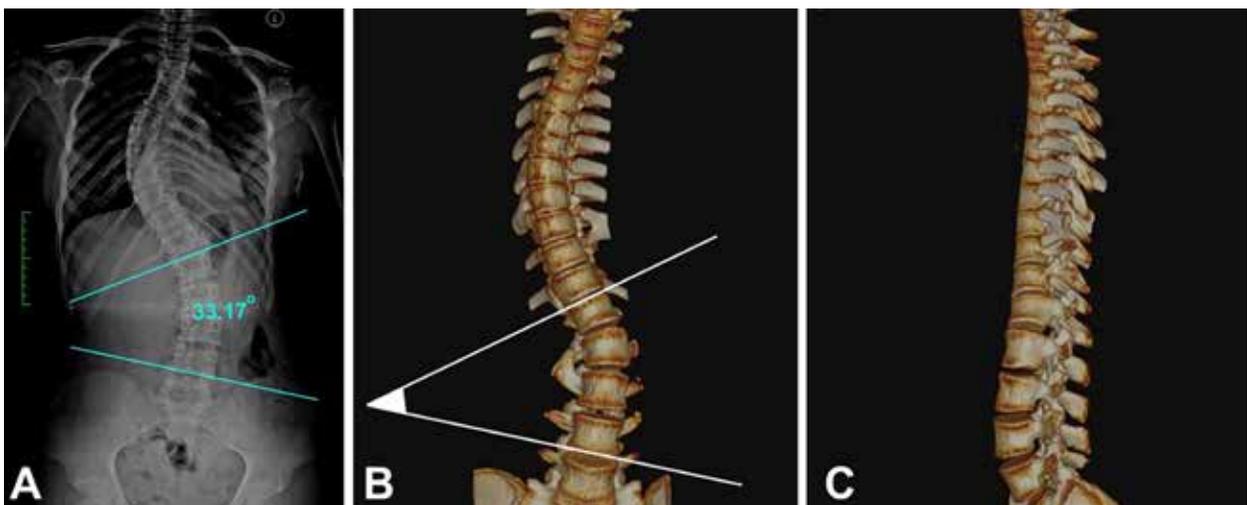

Fig. 2: (A) Computer-assisted Cobb angle measurement on anteroposterior radiographs using OsiriX software; **(B)** 3D analysis and Cobb angle measurement from 3D reconstructed CT image; **(C)** 3D reconstructed image of the spine in the sagittal plane.
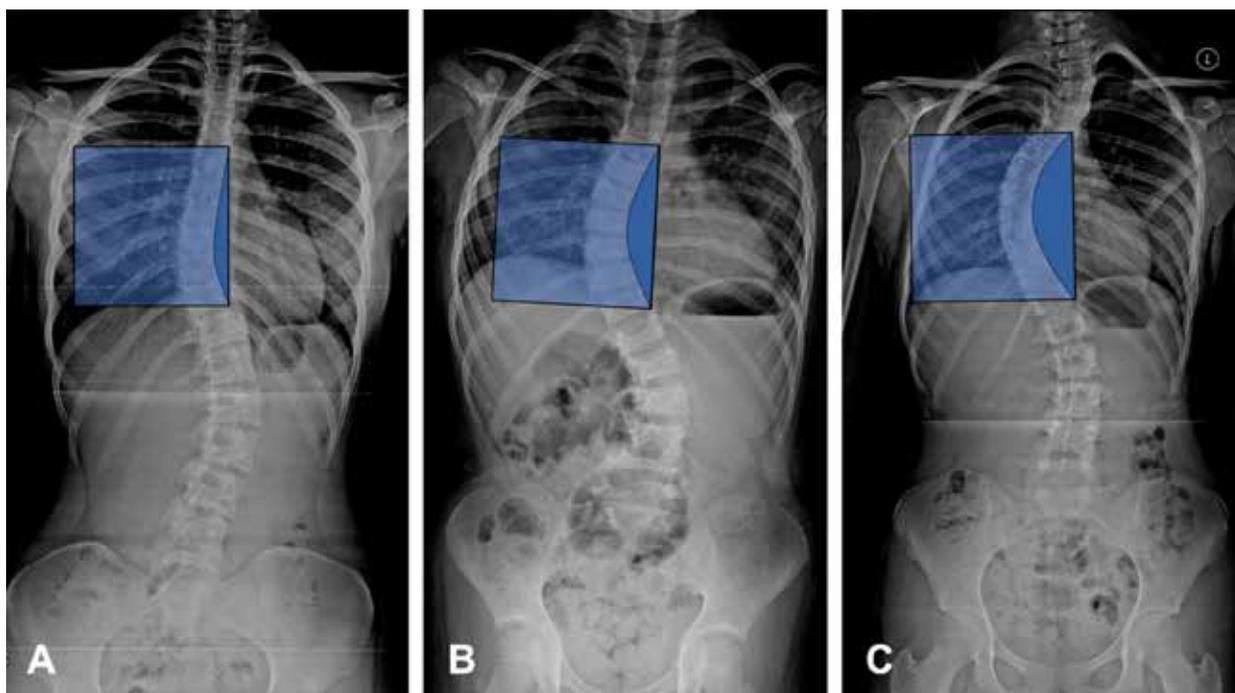
Fig. 3: Three subjects with (A) minimum, (B) medium and (C) maximum projection area per length squared (3.09%, 7.84%, and 16.20%, respectively). The PAL values of the three subjects corresponded to Cobb angles of 13.43º, 31.70º and 58.38º, respectively.

All calculation values and other related data were recorded on a spreadsheet using Microsoft Excel. After initial setup and preparation of the formulae, surface area and length measurements and related formulae were entered for each subject and final data were calculated automatically.

Cobb method

The Cobb angle measurements were obtained by using OsiriX software (OsiriX v.3.8.1 32 bit, Pixmeo SARL, Bernex, Switzerland). All digital X-ray images were transferred and stored in OsiriX software. The software permits the observers to change the contrast, brightness and magnification of the digital images to improve image quality. After opening the images, the examiners independently defined the superior and inferior end vertebrae of each scoliotic curvature in two separate sessions. Lines were drawn through and parallel to the superior and inferior endplates of the upper and lower end vertebrae using the software tools. Finally, the program estimated the Cobb angle automatically (Fig. 2A). In addition, 3D anatomical reconstruction of the normal spine was provided and the Cobb method was demonstrated with the OsiriX software (Fig. 2B and 2C).

The three observers independently measured the scoliosis curves on radiographs using the PAL approach and the Cobb method twice at an interval of one month to reduce bias. The observers were blinded to the results of the others and to their own previous measurements of the same images for each measurement method. In both the PAL and Cobb methods, the end vertebrae were not pre-selected to be able to determine intra- and inter-observer variability of the PAL estimations and Cobb angle measurements.

Statistical analysis

The statistical analysis of obtained data was performed using the Statistical Package for the Social Sciences for Windows, version 22 software (SPSS, Chicago, IL, USA).

The intraclass correlation coefficient (ICC) (two-way mixed model on absolute agreement) was used to define the intra and interobserver reliability of each method. The mean absolute difference (MAD) was also estimated to evaluate the intra- and inter-observer variability in PAL estimations and Cobb angle measurements. According to the Shapiro-Wilk normality test, the Spearman correlation test was used to analyze the degree of the relationship between the PAL approach and the Cobb method.

Receiver operating characteristic (ROC) analysis was performed to assess the actual correctness of the diagnostic performance of the PAL method as compared to the Cobb method in the evaluation of the severity of the coronal curvature in scoliosis. The optimal cut-off values of PAL were also determined for classification of scoliosis as mild, moderate or severe scoliosis. The Cobb angle measurement was used as the gold standard to determine the optimal cut-

off scores of PAL. The area under the curve (AUC), sensitivity, specificity, positive and negative predictive values, and likelihood ratio (+) values were calculated to analyze the diagnostic utility of the PAL method and to estimate the most appropriate cut-off scores on PAL measurement. Two cut-off scores of the PAL, corresponding to Cobb angles of 20° and 40°, were calculated to categorize the severity of scoliosis. The AUC was evaluated as 0.9-1: Excellent, 0.8-0.9: Good, 0.7-0.8: Fair, 0.6-0.7: Poor and 0.5-0.6: Fail.

RESULTS

The mean Cobb angle (±SD) on 46 digital radiographs was 32.70±10.75°. The mean PAL (±SD) obtained using the planimetry method was 8.26±2.70%. Three subjects with minimum, medium and maximum PAL values are shown in Figure 3.

The analysis of intra-observer variability in the PAL and Cobb methods is shown in Table 1. In intra-observer comparisons, the PAL and Cobb methods showed excellent ICC values (higher than 0.983 and 0.987, respectively). The MAD values were similar in the estimation results of the three observers in both sessions for each measurement method. The results of the intra-observer analysis indicated that PAL and Cobb methods show a high degree of intra-observer agreement among all observers.

Table 1: Intra-observer reliability of the PAL approach and Cobb method

Methods	Mean±SD	ICC	95% CI	MAD
First observer				
PAL (%)	8.24±2.81	0.984	0.975-0.990	0.41
Cobb (%)	33.41±10.82	0.989	0.976-0.994	1.38
Second observer				
PAL (%)	8.27±2.66	0.983	0.973-0.989	0.39
Cobb (%)	32.97±10.88	0.992	0.959-0.997	1.13
Third observer				
PAL (%)	8.26±2.64	0.988	0.981-0.993	0.33
Cobb (%)	32.35±10.69	0.987	0.919-0.995	1.30

Data are presented as mean+standard deviation (SD) values.
ICC: intraclass correlation cofficient; CI: confidence interval; MAD: mean absolute difference; PAL: projection area per length squared

The analyses of inter-observer variability in the PAL and Cobb methods are shown in Tables 2 and 3. In the inter-observer comparisons, the ICC values of the PAL method in both sessions were more than 0.970. The combined ICCs among all observers for the PAL approach in both sessions were >0.931, demonstrating excellent reliability. The MAD values of the PAL method were similar between each pair of observers for the first and second sessions (less than 0.56 and 0.54, respectively). The combined MAD values of all the observers for the PAL method also showed
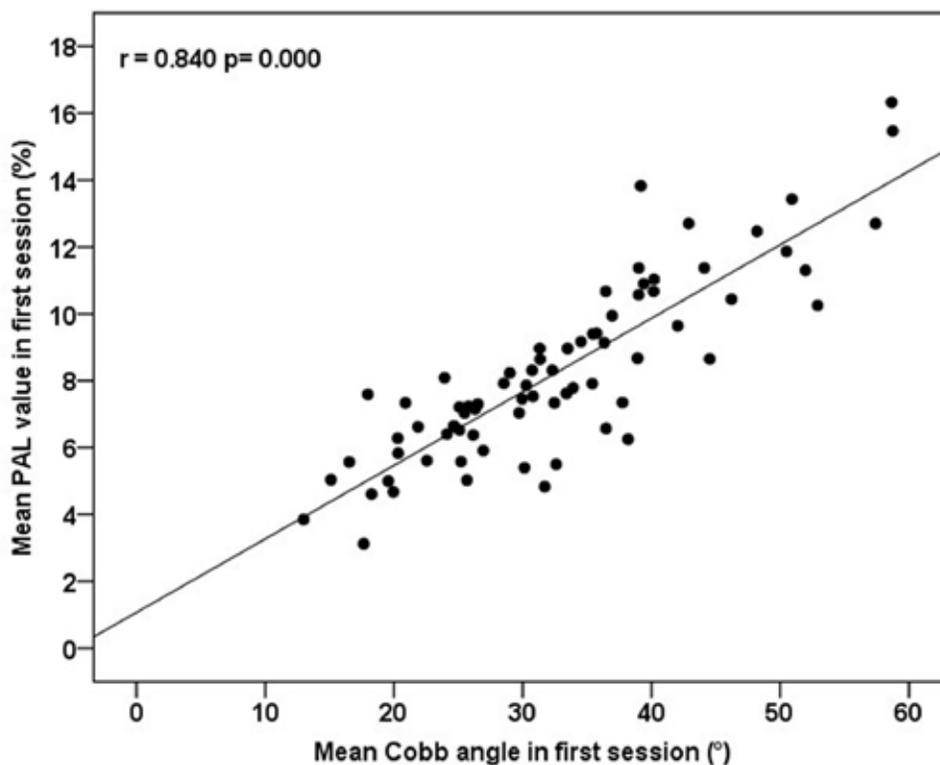

Fig. 4: Correlations between the PAL estimations and Cobb angle measurements obtained by the three observers in the first session.

Table 2: Inter-observer reliability of the PAL approach and Cobb method in the first session

Methods	Mean±SD	ICC	95% CI	MAD
First vs. second observer				
PAL (%)	8.28±2.77	0.974	0.959-0.983	0.53
Cobb (%)	32.79±10.74	0.958	0.933-0.973	2.68
First vs. third observer				
PAL (%)	8.26±2.74	0.970	0.953-0.981	0.56
Cobb (%)	32.99±10.73	0.954	0.927-0.971	2.72
Second vs. third observer				
PAL (%)	8.29±2.64	0.974	0.959-0.983	0.49
Cobb (%)	32.73±10.73	0.962	0.941-0.976	2.51
First vs. second vs. third observer				
PAL (%)	8.28±2.71	0.973	0.960-0.982	0.79
Cobb (%)	32.83±10.72	0.958	0.939-0.972	3.95

Data are presented as the mean+standard deviation (SD) values.
ICC: intraclass correlation cofficient; CI: confidence interval; MAD: mean absolute difference

similarity in the first and second sessions (less than 0.79 and 0.75 respectively). According to these results, the PAL approach showed a very good strength of agreement among all observers.

The Cobb method had excellent ICCs for all the comparisons in the first and second sessions (more than 0.954 and 0.929, respectively). The MAD values of the Cobb method showed similarity between each pair of observers in both sessions (less than 2.72 and 3.41, respectively). Although the combined MAD values of all three observers for the Cobb method were slightly higher than those between each pair of observers, the estimated values were within the range of clinically acceptable error (<5°). These results indicated that the Cobb method showed excellent inter-observer agreement for both measurement sessions.

The correlation analyses between the PAL estimations and the Cobb angle measurements for the first and second sessions are shown in Figures 4 and 5. To minimize bias, the average PAL and Cobb angle values obtained by all observers for each session were used for comparison of the PAL and Cobb methods. According to the estimation results of the measurement methods in both sessions, the PAL estimates had high linear correlations with the Cobb angle measurements.

The ROC curves, presented in Fig 6, indicate graphically the diagnostic performance of the PAL approach in the quantitative evaluation of the scoliosis. Based on the ROC curves, the PAL method had excellent diagnostic performance as compared to the Cobb method for assessing the coronal curvature in scoliosis. The ROC analysis results of PAL estimations to determine the curve severity in scoliosis are presented in Table 4. According to the ROC analysis, the optimal cut-off values of PAL were calculated to be 5.53% (sensitivity: 95.4%, specificity: 87.5%) and 9.67% (sensitivity: 93.3%, specificity: 89.7%), corresponding to Cobb angles of 20° and 40°,
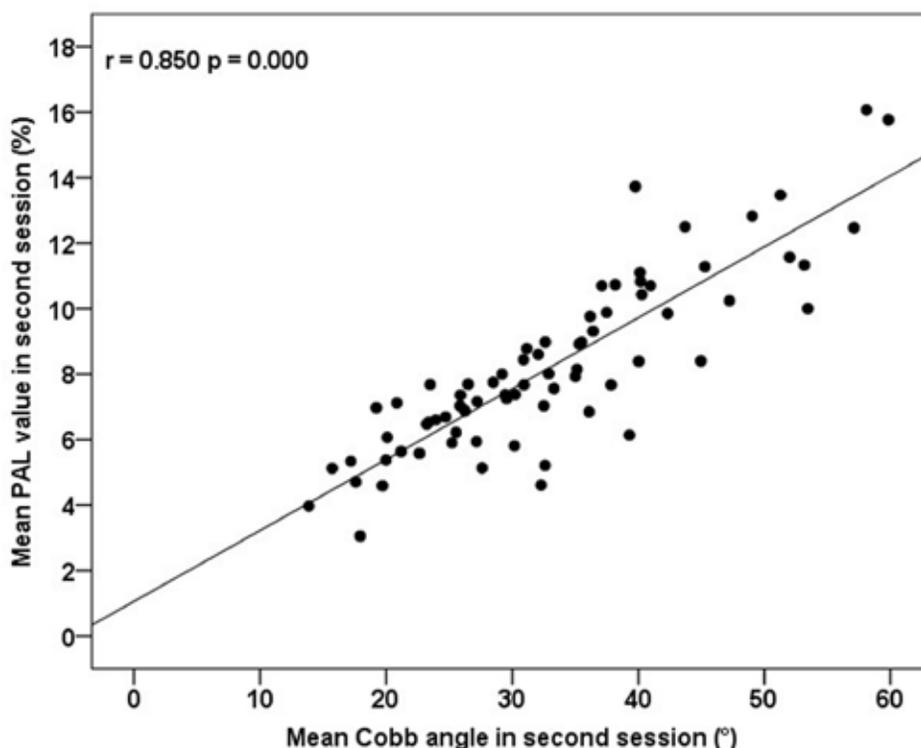

Fig. 5: Correlations between the PAL estimations and Cobb angle measurements obtained by the three observers in the second session.

Table 3: Inter-observer reliability of the PAL approach and Cobb method in the second session

Methods	Mean±SD	ICC	95% CI	MAD
First vs. second observer				
PAL (%)	8.22±2.71	0.970	0.952-0.981	0.51
Cobb (%)	33.60±10.95	0.952	0.925-0.970	2.97
First vs. third observer				
PAL (%)	8.24±2.72	0.971	0.955-0.982	0.54
Cobb (%)	32.77±10.81	0.929	0.838-0.964	3.41
Second vs. third observer				
PAL (%)	8.23±2.66	0.977	0.964-0.986	0.45
Cobb (%)	32.59±10.85	0.943	0.882-0.969	2.67
First vs. second vs. third observer				
PAL (%)	8.23±2.69	0.973	0.906-0.982	0.75
Cobb (%)	32.99±10.86	0.942	0.906-0.964	4.42

Data are presented as the mean+standard deviation (SD) values. ICC: intraclass correlation cofficient; CI: confidence interval; MAD: mean absolute difference

Table 4: The area under the receiver operating characteristic (ROC) curve, confidence interval, sensitivity, specificity, positive and negative predictive and likelihood ratio (+) values of the PAL indicators in evaluating the severity of scoliotic curvature.

Characteristics	Cut off 1	Cut off 2
AUC (95 % CI)	94.5% (86.5%-100%)	95.0% (90.2%-99.8%)
P values	<0.001	<0.001
Cut off	5.53%	9.67%
Sensitivity	95.4% (86.2%-98.8%)	93.3% (66.0%-99.7%)
Specificity	87.5% (46.7%-99.3%)	89.7% (78.2%-95.7%)
PPV	98.4% (90.3%-99.9%)	70.0% (45.7%-87.2%)
NPV	70.0% (35.4%-91.9%)	98.1% (88.6%-99.9%)
LR +	7.63 (1.22-47.8)	9.02 (4.18-19.5)

AUC: area under curve; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio

respectively. The AUC was significant for the first and second cut-off scores in the discrimination (*P*<0.001). ROC analysis results indicated that both cut-off points are robust predictive indicators with high level of sensitivity and specificity for assessment of the pathological degree of the scoliosis, performing well against the Cobb angle measurements. According to the ROC analysis findings, the PAL cut-off value of 5.53% is the optimal discrimination between mild and moderate curvature of the scoliosis, and the PAL cut-off value of 9.67% is the optimal discrimination between moderate and severe curvature of the scoliosis (Table 4).

DISCUSSION

The Cobb method is the most widely utilized and accepted technique for assessing the severity of scoliosis by clinicians. However, it has several limitations which may result in measurement variability[20]. Therefore, various alternative methods have been proposed to examine the coronal curvature in scoliosis and those have been compared with the Cobb method.

Previously suggested methods are significantly influenced by irregularly shaped vertebral bodies and are prone to error in angular measurements. Furthermore, the proposed methods involve multiple troublesome steps and complex measurement protocols for the quantification of scoliotic deformities[6,11,13,14]. In the PAL technique, the observer
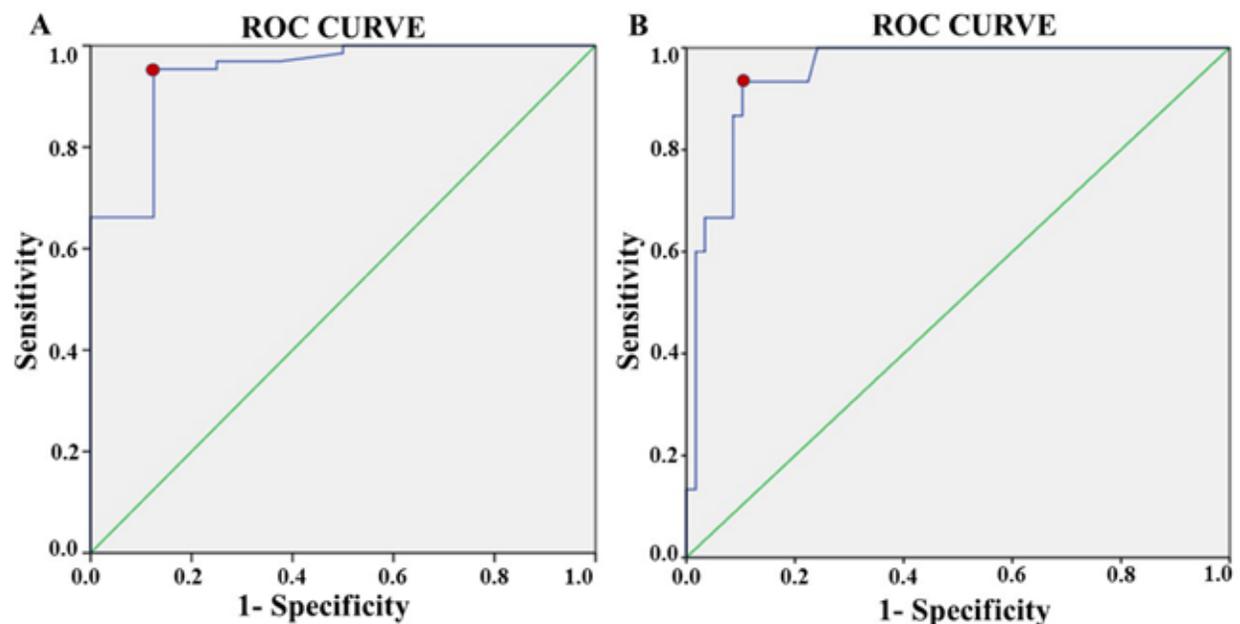

Fig. 6: The ROC curves of the PAL for prediction of the curve severity in scoliosis. The closed circles indicate the PAL cut-off values of 5.53% (A) and 9.67% (B) in the determination of the pathological degree of the scoliosis.

can easily identify the reference point landmarks on the superior and inferior end vertebrae in adolescent scoliosis. Degenerative changes such as marginal osteophytes or spur formation in adult scoliosis may make it difficult to define reference points on the vertebral body corners of superior and inferior end vertebrae in the PAL approach. However, identification of the different reference points on the superomedial and inferomedial corners of upper and lower end vertebrae can generate negligible measurement differences. Therefore, the PAL method may provide more reliable data to assess the degree of the scoliosis. Unlike previously described angular measurement methods, the PAL method describes the deformity as percentages by quantifying the semilunar area, which is formed by the scoliotic curvature. Therefore, the PAL method may provide more representative results about magnitude of scoliotic curvature than those previously presented in literature. Tangential radiographic methods used for assessing scoliosis, are influenced by irregularity in vertebral endplates or the lateral walls of the vertebral body[5,16]. Degenerative changes in the scoliotic curve make the vertebral endplates less distinct landmarks for the Cobb method[21]. As a result, the obtained value by the Cobb method may be greatly influenced by the surface pathologies of the reference vertebrae. Another limitation of the Cobb method is that the Cobb angle reflects changes in the inclination of the end vertebrae rather than changes in the magnitude of the scoliotic curve[6]. In contrast to tangential radiographic approaches, the estimated PAL value is not influenced by the irregular shape of the vertebral body because the PAL technique is based on manual delineation of the medial margins of the vertebral bodies in the curvature. The PAL technique provides quantitative information independent of the vertebral endplate architecture or marginal convexity in the vertebra body. Thus, with use of the PAL method, an irregularly shaped curvature of the spine may be able to be assessed.

The PAL method directly reflects changes in the severity of the scoliosis because it depends on surface area measurements of the semilunar area, which are closely related to the magnitude of the scoliotic curvature. An increase in the PAL will result from an increase in the degree of the scoliosis. The method described in this study could overcome all the handicaps and limitations of the previous methods, and the values obtained by the PAL approach are closely related to the magnitude of the curvature in scoliosis.

To date, comprehensive analysis of the reliability and reproducibility of the Cobb angle measurements in patients with scoliosis have been well studied by various studies[22-25]. In a recent study of Guo et al, it has been reported that the intra- and inter-observer ICC values of the Cobb method in degenerative lumbar scoliosis were in the range of 0.959-0.964 and 0.969-0.972, respectively[22]. Similarly, Pan et al stated that the ICCs of intra- and inter-observer reliability analysis for manual measurement of the Cobb angle on chest X-rays was 0.941 and 0.887, respectively[23]. Additionally, in a study evaluating patients with idiopathic scoliosis, Wong et al reported that intra- and inter-observer reliability of Cobb angle measurements using ICCs were 0.988 and 0.949, respectively[24]. In our study, the intra- and inter-observer ICC values of the Cobb method in the first and second sessions were more than 0.954 and 0.929, respectively. These results showed that the Cobb method shows excellent intra- and inter-observer agreement for both measurement sessions. We also examined the reliability of the PAL approach for measuring scoliosis curvature, and our findings indicated that the intra- and inter-observer ICC values of the PAL approach ranged from 0.983-0.989 and 0.970-0.977, respectively. According to these findings, the PAL approach may provide accurate and reliable data for evaluating the severity of scoliosis.

The current literature evidently shows that none of the suggested methods can completely replace the Cobb method for the assessment of scoliosis. However, according to the findings of our study, the correlation coefficients (r) between the Cobb method and the PAL approach obtained in the first and second sessions were 0.84 and 0.85, respectively. The PAL estimation results were seen to be highly correlated with the Cobb angle measurements for the assessment of the scoliosis curve magnitude. Therefore, the PAL approach may be a favorable alternative to the Cobb technique for assessment of scoliosis.

The PAL approach, like the Cobb method, can also be used for evaluation of the sagittal spinal curvatures. Previously, Kuru et al measured the degree of lumbar lordosis by using first and fifth vertebral bodies as referrals for the PAL method, and concluded that the PAL method could provide reliable and reproducible data for measuring the degree of lumbar lordosis on lateral X-ray images[19]. Therefore, the PAL method can be used for assessing cervical lordosis, thoracic kyphosis or lumbar lordosis in plain X-ray images, and obviously the PAL approach may represent a robust alternative to the Cobb method for various types of spinal curvatures.

Cobb angle measurement still remains the gold standard in current clinical diagnosis for quantifying the severity of scoliosis. Therefore, in our study, for analyzing the utility of the PAL approach in the clinical diagnosis of scoliosis, we optimized the PAL

cut-off values by comparing them with the Cobb angle measurements. According to analysis results, the PAL technique has high diagnostic performance with a high level of sensitivity (95.4% and 93.3%) and specificity rates (87.5% and 89.7%) and performed well against the Cobb method in the diagnosis of the severity of scoliosis. PAL value <5.53% could be considered as mild scoliosis, PAL values between 5.53%-9.67% could be considered as moderate, and PAL value >9.67% could indicate severe scoliosis. In clinical practice, PAL values of 5.53% and 9.67% could be used as reliable threshold values in the diagnosis of scoliotic deformity and for therapeutic decision-making.

The Ferguson and Centroid methods are difficult to apply, very complex and time-consuming approaches on measuring scoliotic deformities, as both methods use multiple bony landmarks on three or four defined vertebrae[6,14]. These methods are examiner dependent and require advanced skills, which can be obtained by time. The Cobb method and lateral tangent methods are more easily applicable than the above-mentioned methods. However, as a result of concave-shaped and blurred vertebral surfaces, straight lines cannot be considered[5,16]. However, when both methods are preferred, it is hard to obtain straight lines for analyzing the curvature angles due to the concave-shaped and blurred vertebral surfaces. The examiner could define a variety of lines parallel to the surface of the reference vertebrae. Therefore, both methods are highly subjective and prone to measurement error, whereas the PAL method is based on defining only two certain landmarks and drawing medial boundaries of vertebral bodies. According to our study findings, the PAL method showed high intra- and inter-observer reliability with all ICC values >0.970. Therefore, this method provides accurate, reproducible and objective data for evaluating the degree of scoliosis.

Several limitations of the present study should be noted. First, since each observer was free to select the end vertebrae, as encountered in the Cobb method, defining different end vertebrae may lead to measurement variability in the PAL method. Second, the cut-off score of PAL corresponding to the Cobb angles of 10 degrees were not defined because the study group was composed of adolescent scoliosis patients with scoliosis ranging from mild to severe. Thus, a wide range of examinations including less severe scoliosis cases is required for more extensive evaluation of the severity of the scoliosis with the PAL approach. In addition, future studies should include application of the PAL method on degenerative scoliosis and the largest structural curvature to be able to provide more detailed information about the applicability and efficiency of the PAL method.

The present study has several strengths which simulate clinical conditions. First, all the observers had different levels of experience in measuring scoliosis using the PAL and Cobb methods. Second, the end vertebrae were not pre-selected in the PAL and Cobb methods. Third, the statistical power of the present study was relatively high, because the 73 scoliotic curves evaluated in the present study provided over 200 intra-observer comparisons for each examiner and radiographic parameter, and over 300 inter-observer comparisons per radiographic parameter. 3D reconstructions of the spine allow the quantitative assessment of the deformity, the objective anatomical assessment of treatment, and the detection of progressive scoliosis and the surgical anatomy for planning and simulation[25]. A quasi-automated three-dimensional reconstruction method of the spine from biplanar X-rays can be used in addition to this method.

CONCLUSION

In conclusion, the method described here is a reliable and reproducible technique that provides an objective criterion for measuring coronal curvature in scoliosis. The PAL method provides quantitative data independent of the vertebral surface pathologies of the reference vertebrae. An irregularly shaped curvature in scoliosis can be evaluated with this method. Therefore, the PAL approach could be used as an alternative and robust criterion to determine the severity of scoliosis on anteroposterior radiographs.

REFERENCES

1. Altaf F, Gibson A, Dannawi Z, Noordeen H. Adolescent idiopathic scoliosis. BMJ 2013; 346:f2508.
2. He JW, Yan ZH, Liu J, Yu ZK, Wang XY, Bai GH, *et al*. Accuracy and repeatability of a new method for measuring scoliosis curvature. Spine 2009; 34(9):E323-9.
3. Kuklo TR, Potter BK, Polly Jr DW, O'Brien MF, Schroeder TM, Lenke LG. Reliability analysis for manual adolescent idiopathic scoliosis measurements. Spine 2005; 30(4):444-54.

4.   Cobb J. Outline for the study of scoliosis. Instr Course Lect AAOS 1948; 5:261-75.

5.   Gstoettner M, Sekyra K, Walochnik N, Winter P, Wachter R, Bach CM. Inter-and intraobserver reliability assessment of the Cobb angle: manual versus digital measurement tools. Eur Spine J 2007; 16(10):1587-92.

6.   Vrtovec T, Pernuš F, Likar B. A review of methods for quantitative evaluation of spinal curvature. Eur Spine J 2009; 18(5):593-607.

7.   Morrissy RT, Goldsmith GS, Hall EC, Kehl D, Cowie GH. Measurement of the Cobb angle on radiographs of patients who have scoliosis. Evaluation of intrinsic error. J Bone Joint Surg Am 1990; 72(3):320-7.

8.   Loder RT, Spiegel D, Gutknecht S, Kleist K, Ly T, Mehbod A. The assessment of intraobserver and interobserver error in the measurement of noncongenital scoliosis in children≤ 10 years of age. Spine 2004; 29(22):2548-53.

9.   Wills BPD, Auerbach JD, Zhu X, Caird MS, Horn BD, Flynn JM, *et al*. Comparison of Cobb angle measurement of scoliosis radiographs with preselected end vertebrae: traditional versus digital acquisition. Spine 2007; 32(1):98-105.

10. Ritter R, Nagasse Y, Ribeiro I, Yamazato C, Oliveira FMd, Kusabara R. Comparison of Cobb angle measurement in scoliosis by residents and spine experts. Coluna/Columna 2016; 15(1):13-6.

11. Ferguson AB. The study and treatment of scoliosis. South Med J 1930; 23(2):116-20.

12. Diab KM, Sevastik JA, Hedlund R, Suliman IA. Accuracy and applicability of measurement of the scoliotic angle at the frontal plane by Cobb's method, by Ferguson's method and by a new method. Eur Spine J 1995; 4(5):291-5.

13. Harrison DE, Harrison DD, Cailliet R, Troyanovich SJ, Janik TJ, Holland B. Cobb method or Harrison posterior tangent method: which to choose for lateral cervical radiographic analysis. Spine 2000; 25(16):2072-8.

14. Chen YL. Vertebral centroid measurement of lumbar lordosis compared with the Cobb technique. Spine 1999; 24(17):1786-90.

15. Scholten PJ, Veldhuizen AG. Analysis of Cobb angle measurements in scoliosis. Clin Biomech (Bristol, Avon) 1987; 2(1):7-13.

16. Hwang J-H, Hong J-Y, Suh S-W, Yang J-H, Lee J-M. A comparative analysis of 4 curvature measurement methods in early-onset scoliosis. Spine 2012; 37(20):E1273-81.

17. Hong J-Y, Suh S-W, Modi HN, Lee J-M, Park S-Y. Centroid method: an alternative method of determining coronal curvature in scoliosis. A comparative study versus Cobb method in the degenerative spine. Spine J 2013; 13(4):421-7.

18. Jørgensen LB, Sørensen JA, Jemec GB, Yderstræde KB. Methods to assess area and volume of wounds–a systematic review. Int Wound J 2016; 13(4):540-53.

19. Kuru O, Sahin B, Kaplan S. Alternative approach to evaluating lumbar lordosis on direct roentgenograms: projection area per length squared. Anat Sci Int 2008; 83(2):83-8.

20. Lechner R, Putzer D, Dammerer D, Liebensteiner M, Bach C, Thaler M. Comparison of two-and three-dimensional measurement of the Cobb angle in scoliosis. Int Orthop 2017; 41(5):957-62.

21. Zhang J, Lou E, Shi X, Wang Y, Hill DL, Raso JV, *et al*. A computer-aided Cobb angle measurement method and its reliability. J Spinal Disord Tech 2010; 23(6):383-7.

22. Guo J, Deng X-C, Ling Q-J, Yin Z-X, He E-X. Reliability analysis of Cobb measurement in degenerative lumbar scoliosis using endplate versus pedicle as bony landmarks. Postgrad Med 2017; 129(7):762-7.

23. Pan Y, Chen Q, Chen T, Wang H, Zhu X, Fang Z, *et al*. Evaluation of a computer-aided method for measuring the Cobb angle on chest X-rays. Eur Spine J 2019; 28(12):3035-43.

24. Wong Y-S, Lai KK-L, Zheng Y-P, Wong LL-N, Ng BK-W, Hung AL-H, *et al*. Is radiation-free ultrasound accurate for quantitative assessment of spinal deformity in idiopathic scoliosis (IS): a detailed analysis with EOS radiography on 952 patients. Ultrasound Med Biol 2019; 45(11):2866-77.

25. Lechner R, Putzer D, Dammerer D, Liebensteiner M, Bach C, Thaler M. Comparison of two- and three-dimensional measurement of the Cobb angle in scoliosis. Int Orthop 2017; 41(5):957-62.

Original Article

Clinical utility of high sensitivity cardiac troponin-I measurement in COVID-19 infection: a predictor of major adverse events

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ABSTRACT

Objective: Effective patient classification relies on the identification of highest-risk patients who may require more intensive supervision and support. The aim of this study was to investigate the association between in-hospital major adverse events (MAEs) and high sensitivity cardiac troponin-I (hs-cTnI) in hospitalized patients with COVID-19 infection.
Design: Retrospective observational study
Setting: Sakarya Education Hospital, Turkey
Subjects: A cohort of 123 patients hospitalized with a diagnosis of COVID-19 was analysed.
Intervention: The demographic, clinical and admission laboratory findings were collected.
Main outcome measures: In-hospital MAEs were determined as the primary outcome.
Results: MAEs occurred in 56.2% of the elevated hs-cTnI group and in 3.3% of the normal hs-cTnI group ($P<0.001$).

The rates of acute pulmonary oedema ($P=0.001$), the need for invasive ventilation ($P<0.001$) and death ($P<0.001$) were significantly higher in the elevated hs-cTnI group. Moreover, patients with elevated hs-cTnI were more likely to have acute renal failure, acute arrhythmia and to require intensive care unit admission, non-invasive oxygen support and treatment with inotropic agents ($P<0.001$ for all). The multivariate regression analysis indicated that hs-cTnI independently predicts MAEs in hospitalized patients with COVID-19 (odds ratios: 35.077, 95% confidence interval: 9.035-136.181, $P<0.001$). In the receiver operating characteristic curve analysis, a cut-off hs-cTnI value of 21.8 was determined to predict MAEs with 81% sensitivity and 79% specificity ($P<0.001$).
Conclusions: The hs-cTnI is an independent predictor for adverse outcomes in patients with COVID-19.

KEY WORDS: COVID-19, high-sensitive troponin I, major adverse events, myocardial injury

INTRODUCTION

The coronavirus disease 2019 (COVID-19) outbreak is an alarming international public health emergency. The potentially lethal respiratory illness is caused by a newly identified coronavirus first recognized in Wuhan, China, in December 2019[1,2]. Most of the previous studies associated with COVID-19 are primarily focused on epidemiological and clinical features[3-6]. There are only a few studies in the literature that investigate risk factors associated with clinical outcomes[7,8]. The mortality rates for COVID-19 infection were found to be highest in the elderly (14.8% of those over 80 years old) and those with a history of underlying cardiovascular disease[6].

Cardiac troponin is a marker of myocardial injury, including but not limited to myocardial infarction or myocarditis. Elevated cardiac troponin concentrations in COVID-19 disease are common in hospitalized patients, and increased cardiac troponin in these patients indicates myocardial injury due to non-ischemic causes other than myocardial infarction. Although the literature shows that marginal elevation of cardiac troponin I (cTnI) is common in most patients infected with COVID-19, only about 8-12% of cases

show values above the 99th percentile upper reference limit. Among patients diagnosed with COVID-19, cTnI values were found to be higher in patients with severe disease than in patients with milder disease[9]. Moreover, a significant increase in cTnI values was noted in patients with severe disease; major complications occurred in these patients[10].

To classify patients effectively, it is crucial to identify highest-risk patients who may require more intensive supervision and support. Therefore, the aim of this study is to evaluate the relationship between in-hospital major adverse events (MAEs) and cardiac high-sensitive troponin I (hs-cTnI) in hospitalized patients with a diagnosis of COVID-19.

SUBJECTS AND METHODS

The current study was a single-centre retrospective cohort design study. Between 29 March 2020 and 15 April 2020, a total of 123 patients who were diagnosed with COVID-19 infection according to the WHO provisional guidance, had measurment of hs-cTnI in the emergency department, and hospitalization were included in the study. Exclusion criteria were the presence of acute coronary syndrome, haematological disease and cancer. An additional exclusion criterion was lack of relevant patient-related data regarding chronic diseases and laboratory information. We could not include all COVID-19 patients consecutively, because hs-cTnI value was not found among routine tests in many patients at the time of admission. The study took place at Sakarya University Education and Research Hospital, Sakarya, Turkey. Positivity was assessed with a reverse-transcriptase–polymerase-chain-reaction assay from nasopharynx sample for COVID-19 infection. This study complied with the Declaration of Helsinki and was approved by the independent medical ethics committee of Sakarya University Education and Research Hospital. Clinical signs and symptoms, laboratory values, outcome and complication data were collected from electronic medical records.

Laboratory testing at hospital admission included complete blood count, creatinine, aspartate transaminase, alanine transaminase, creatinine kinase, lactate dehydrogenase (LDH), international normalized ratio, prothrombin time (PT), D-dimer and hs-cTnI (ARCHITECT hs-cTnI assay, Abbott). A panel of acute-phase reactants, including serum ferritin and C-reactive protein (CRP), was performed routinely. Elevated hs-cTnI was defined as an hs-cTnI concentration higher than the 99th percentile of normal.

The primary outcome of the study was the composite of in-hospital MAEs, which were defined as acute pulmonary oedema, the need for invasive ventilation and death. The secondary outcomes included acute arrhythmia (atrial fibrillation, ventricular fibrillation, ventricular tachycardia), acute renal failure, admission to the intensive care unit and the need for inotropes in the follow-up period.

Ethics approval was granted by the Sakarya University Education and Research Hospital Ethics Committee (application number 71522473/050.01.04/243)

Statistical analysis

For the statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 16.0 for Windows (SPSS Inc., Chicago, IL) was used. Continuous data were expressed as mean ± standard deviation and the categorical data were expressed as percentages. The normal distribution of the data was assessed by the Kolmogorov-Smirnov test. Comparisons between groups were performed using a chi-square or Fisher's exact test for qualitative variables, as appropriate. Where suitable, an independent t-test was used for normally distributed continuous variables, and the Mann-Whitney U test was conducted for non-normally distributed continuous variables. For the prediction

Table 1: Comparison of elevated hs-cTnI and normal hs-cTnI groups in terms of clinical features.

Clinical features	Total (n=123)	Elevated hs-cTnI (n=32)	Normal hs-cTnI (n=91)	P-value
Age (years)	56.99±18.87	73.22±10.48	51.29±17.84	<0.001
Male gender, n (%)	51 (41.5)	10 (31.2)	41 (45.1)	0.166
Hypertension, n (%)	38 (30.9)	21 (65.6)	17 (18.7)	<0.001
Diabetes mellitus, n (%)	24 (19.5)	15 (46.9)	9 (9.9)	<0.001
Active smoker, n (%)	25 (20.3)	2 (6.2)	23 (25.3)	0.003
Coronary artery disease, n (%)	10 (8.1)	9 (28.1)	1 (1.1)	<0.001
Chronic heart failure, n (%)	9 (7.3)	6 (18.8)	3 (3.3)	0.040
COPD or asthma, n (%)	12 (9.8)	5 (15.6)	7 (7.7)	0.270
Chronic renal failure, n (%)	9 (7.3)	7 (21.9)	2 (2.2)	<0.001
Stroke, n (%)	4 (3.3)	3 (9.4)	1 (1.1)	0.024
Atrial Fibrillation, n (%)	3 (2.4)	2 (6.2)	1 (1.1)	0.106

Data presented as mean ± standard deviation or number (%).
hs-cTnI: high-sensitive cardiac troponin I; COPD: chronic obstructive pulmonary disease.

Table 2: Comparison of elevated hs-cTnI and normal hs-cTnI groups in terms of laboratory values.

Laboratory Parameter	Total (n=123)	Elevated hs-cTnI (n=32)	Normal hs-cTnI (n=91)	P-value
White blood cells (K/uL)	8.18±3.87	10.71±4.83	7.28±3.03	0.001
Neutrophils (K/uL)	5.38±3.08	8.12±4.11	4.36±1.75	<0.001
Lymphocytes (K/uL)	1.83±1.23	1.76±1.98	1.85±0.84	0.802
Hemoglobin (g/dL)	12.86±1.87	11.6±2.1	13.2±1.5	<0.001
Platelets (K/uL)	212.1±82.4	213.0±94.6	211.7±78.3	0.940
Serum ferritin (µg/L)	296.4±617.0	523.4±1083.1	212.8±270.6	0.027
C-reactive protein (mg/L)	45.76±83.84	81.9±145.3	33.0±40.3	0.003
D-dimer (µgFEU/L)	1563.8±3513.4	3760.4±6141.9	791.4±1191.8	<0.001
Aspartate aminotransferase (U/L)	33.45±25.82	44.2±42.4	29.6±14.9	0.065
Alanineaminotransferase (U/L)	31.54±30.78	33.5±46.8	30.8±22.9	0.677
Serum creatinine (mg/dL)	0.93±0.56	1.08±0.50	0.78±0.23	0.007
Creatine kinase (U/L)	151.0±201.6	202.4±281.3	135.2±168.8	0.239
Lactate dehydrogenase (U/L)	274.1±118.5	342.1±154.6	249.7±91.9	0.003
International normalised ratio	1.15±0.14	1.25±0.16	1.12±0.12	<0.001
Prothrombin time (s)	12.54±1.63	13.5±1.86	12.1±1.33	<0.001
hs-cTnI (ng/L)	768.4±5236.0	1985.7±8368.4	4.72±4.71	<0.001

Data presented as mean ± standard deviation.
hs-cTnI: high-sensitive cardiac troponin I.

of MAEs by hs-cTnI, the cut-off, corresponding sensitivity and specificity values were estimated by receiving operator characteristic (ROC) curve analysis. Multivariate logistic regression analysis was performed to assess the independent predictors of MAEs. All variables that were found to be significant in univariate analysis ($P \leq 0.1$) were included as covariates in the multivariate logistic regression model. Results are reported as odds ratios (OR) with 95% confidence interval (CI). A P-value less than 0.05 was considered statistically significant in all tests.

RESULTS

The study population included a total of 123 patients with a mean age of 56.99±18.87 years, of whom 51 (41.5%) were men and 72 (58.5%) were women. Of the 123 patients, 49 (39.8%) had fever, 70 (56.9%) had dry cough, 27 (22%) had fatigue, 32 (26%) had muscle soreness or chest tightness, 48 (39%) had shortness of breath and 6 (4.9%) experienced loss of smell and/or taste. There were 7 (5.6%) cases of headache and 4 (3.3%) cases of diarrhoea. Among the overall population, 60.2% had at least one coexisting medical condition. History of hypertension (HT, 30.9%), diabetes type 2 (DM, 19.5%), coronary artery disease (CAD, 8.1%), chronic heart failure (7.3%), asthma or chronic obstructive pulmonary disease (COPD, 9.8%), chronic renal failure (CRF, 7.3%), atrial fibrillation (2.4%) and stroke (3.3%) were the most common comorbidities. The ratio of active smokers was 20.3%. In our cohort, the level of D-dimer increased in 65 (52.8%), ferritin increased in 41 (33.3%) and LDH increased in 61 (49.6%) of the patients.

The patients were divided into two groups according to the elevation of hs-cTnI. On admission, 32 of the 123 patients had elevated hs-cTnI. It was observed within normal limits in 91 patients. Clinical characteristics at the time of admission of the groups are shown in Table 1. The groups were similar regarding gender, atrial fibrillation and history of COPD or asthma, whereas

Table 3: Comparison of elevated hs-cTnI and normal hs-cTnI groups in terms of in-hospital outcomes.

In-hospital outcomes	Total (n=123)	Elevated hs-cTnI (n=32)	Normal hs-cTnI (n=91)	P-value
Intensive care unit, n (%)	36 (29.3)	29 (90.6)	7 (7.7)	<0.001
Noninvasive oxygen support, n (%)	53 (43.1)	31 (96.9)	22 (24.2)	<0.001
Invasive ventilation, n (%)	7 (5.7)	6 (18.8)	1 (1.1)	<0.001
Acute pulmonary edema, n (%)	6 (4.9)	5 (15.6)	1 (1.1)	0.001
Usage of inotropic agents, n (%)	11 (8.9)	8 (25.0)	3 (3.3)	<0.001
Acute arrhythmia,n (%)	5 (4.1)	5 (15.6)	0 (0)	<0.001
Acute renal failure,n (%)	8 (6.5)	7 (21.9)	1 (1.1)	<0.001
Death, n (%)	10 (8.1)	9 (28.1)	1 (1.1)	<0.001
MAEs, n (%)	21 (17.1)	18 (56.2)	3 (3.3)	<0.001

Data are n (%).
hs-cTnI: high-sensitive cardiac troponin I; MAE: major adverse events.

statistically significant difference was detected with regard to age, smoking status, history of HT, DM, CAD, chronic heart failure, CRF and stroke.

On admission, as shown in Table 2, there was no difference in the lymphocyte count, platelet count, aspartate transaminase levels, alanine transaminase levels and creatinine kinase between the two groups. However, the white blood cell counts, neutrophil counts and haemoglobin levels of the elevated hs-cTnI group were higher than those of the normal hs-cTnI group. Moreover, the elevated hs-cTnI group also had significantly higher CRP, ferritin, D-dimer, LDH and creatinine levels.

The outcomes of the patients during the follow-up period are summarized in Table 3. At the in-hospital follow-up, MAEs occurred in 56.2% of the elevated hs-cTnI group and in 3.3% of the normal hs-cTnI group ($P<0.001$). The clinical outcomes in hospital, including acute pulmonary oedema ($P=0.001$), need for invasive ventilation ($P<0.001$) and death ($P<0.001$) were also significantly higher in the elevated hs-cTnI group. Moreover, acute arrhythmia ($P<0.001$) and acute renal failure ($P<0.001$) rates, as well as the need for non-invasive oxygen support ($P<0.001$), inotropic agents ($P<0.001$) and the intensive care unit ($P<0.001$) were found to be significantly higher in the elevated hs-cTnI group compared to the normal hs-cTnI group.

Univariate and multivariate regression analysis results for MAEs are shown in Table 4. Age, DM, COPD or asthma, haemoglobin, CRP, PT and hs-cTnI achieved statistical significance in the univariate logistic analysis. Multivariate logistic regression
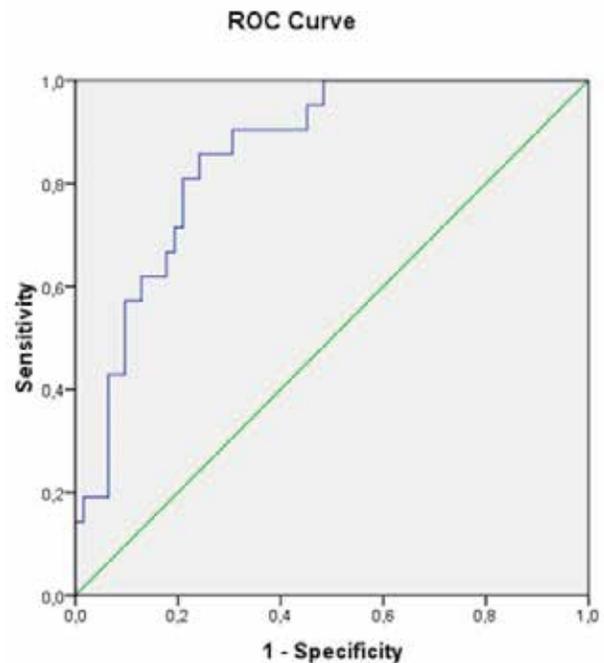
Figure 1: Receiver operator characteristic (ROC) curve analysis of high-sensitive cardiac troponin I (hs-cTnI) for the prediction of major adverse events (MAEs) in hospitalized patients with Covid-19.

analysis revealed that only elevated hs-cTnI (OR:35.077, 95% CI:9.035-136.181, $P<0.001$) was an independent predictor of MAEs. Furthermore, univariate logistic regression analysis indicated that age, history of HT and CAD, count of neutrophil, level of D-dimer, PT and elevated hs-cTnI were predictors of death.

Table 4: Logistic regression analysis in the prediction of MAEs in patients with COVID-19.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Gender	0.416	0.019 - 8.895	0.574			0.117
Age	1.242	1.034 - 1.492	0.020			0.501
Diabetes Mellitus	19.589	0.678-566.078	0.083			
Hypertension	1.352	0.089-20.610	0.828			
CRF history	0.439	0.002-78.108	0.756			0.661
COPD or asthma	0.031	0.001-1.523	0.080			
CHF history	2.630	0.099-70.141	0.564			
CAD history	0.027	0.001-2.140	0.106			
Active smoker	0.119	1004-75.134	0.518			
Neutrophils	1.00	1.000-1.001	0.329			0.060
Hemoglobin	0.453	0.211 - .971	0.042			
Ferritin	0.999	0.997 - 1.000	0.152			0.417
C-reactive protein	0.948	0.909 - .988	0.012			
D-dimer	1.000	0.999 - 1.001	0.982			
Creatinine	0.187	0.008-4.271	0.293			
Lactate dehydrogenase	1.000	0.987-1.014	0.999			0.085
Prothrombin time	2.297	0.900-5.861	0.082			
hs-cTnI	124.636	3.557-4367.43	0.008	35.077	9.035-136.181	<0.001

OR: odds ratio; CI: confidence interval; CAD: coronary artery disease; CHF: chronic heart failure; CRF: chronic renal failure; COPD: chronic obstructive pulmonary disease; hs-cTnI: high-sensitive cardiac troponin I.

However, multivariate regression analysis indicated that only elevated hs-cTnI independently predicted death in patients with COVID-19 (OR: 29.864, 95% CI: 3.584-248.864, *P*:0.002).

In the ROC curve analysis, a cut-off hs-cTnI value of 21.8 ng/L was determined to predict MAEs with 81% sensitivity and 79% specificity (ROC area: 0.856; 95% CI: 0.774–0.938; *P*<0.001) (Figure 1).

DISCUSSION

Our study evaluated the impact of elevated hs-cTnI on in-hospital MAEs in patients with COVID-19. The results of this present study show that elevated hs-cTnI is an independent predictor of MAEs in patients with COVID-19. The study data demonstrated that hs-cTnI ≥21.8ng/L resulted in 81% sensitivity and 79% specificity for the prediction of MAEs.

Acute respiratory syndrome coronavirus 2 is a novel pathogen responsible for the now widely recognized disease, COVID-19. Although the spectrum of the disease ranges from asymptomatic to fatal multi-organ failure, the mechanisms underlying these interpersonal differences in the course of the disease are not well understood. Therefore, it is of great importance to explore clinical features and factors that affect the prognosis of COVID-19 patients. In previous studies, the most common symptoms were fever (in up to 88.7% of patients during hospitalization) and cough (in 67.8% of patients), followed by dry cough, headache, fatigue or shortness of breath[3,11]. In our study, the most common symptoms were dry cough, fever and shortness of breath.

The lung is the main target organ of COVID-19 infection, but severe cases also include dysfunction of other organs. Concurrent cardiac injury was shown in approximately 7.2% of patients, and even higher, approximately 22.2%, among intensive care unit patients[12]. In our study involving hospitalized patients, this rate was found to be 26%. In studies that previously identified critically ill patients with COVID-19, an increase in troponin levels has often been reported without a clear association to myocardial dysfunction or myocarditis in intensive care unit patients[13]. Myocardial injury related to COVID-19 infection could be caused by several mechanisms, including myocarditis (direct viral damage of myocardium), hyperinflammatory syndromes, pro-coagulant conditions, destabilization of coronary plaque and hypoxia[8,14,15]. Elevated troponin values have been reported to be associated with disease severity, a high risk of acute respiratory distress syndrome, hepatic dysfunction, acute renal injury and even an increased risk of death[16-18]. The patient group with elevated troponin had 59.6% mortality, compared to 8.9% in the patient group with normal troponin values[16].

Likewise, it was shown that elevated troponin was an independent risk factor for death after adjusting for other confounders. Patients with elevated hs-cTnI had a 51.2% probability of death compared to 4.5% in patients with normal values[14]. Hua Fan reported that patients with elevated hs-cTnI levels on admission had a shorter duration from symptom onset to death. In the same study, death risk increased by 20.8% when the hs-cTnI level increased by one unit[19]. A meta-analysis of four studies that included a total of 341 patients in China found the values of cTnI to be significantly more increased in cases of severe disease than in milder cases[9]. Similarly, Atallah *et al* reviewed the results of previous COVID infection and cardiac-related studies; showed that, in addition to the increase in systemic inflammatory markers, the increase in hs-cTnI is also associated with adverse events and mortality in COVID-19 patients[20]. In the present study, we found that only elevated hs-cTnI levels on admission independently predicted death and MAEs.

The effects of age and comorbidities have been addressed in other studies related to COVID-19[4,12]. The presence of DM was related to a higher mortality rate or adverse outcome in previous studies[12]. Our current data demonstrate that patients with DM had higher MAE risk, but greater risk of death was not observed. Li *et al* reported that the presence of COPD, CRF, cardiovascular disease or HT was related to a higher mortality rate[21]. In the current study, age, history of HT and CAD were observed as risk factors for death. However, multivariate logistic regression analysis revealed that they were not independent predictors of death. In terms of laboratory tests, a high neutrophil count was found to be an independent predictor of poor outcomes[22]. In the present study, the count of neutrophil was found to be a risk factor, but not an independent predictor of death.

Study limitations

The major limitation of this study was the small sample size. A prospective study is needed to determine the predictive value of hs-cTnI with a greater patient population. Furthermore, it was a single-center study, and this study did not include long-term follow-up.

CONCLUSION

Determining the risk of MAE is very important for the prevention of adverse outcomes in patients with COVID-19. Cardiomyocyte injury, as quantified by cardiac troponin concentrations, may occur in COVID-19 and the level of this biomarker correlates with disease severity and mortality. Similarly, the present study revealed that hs-cTnI is an independent predictor of MAEs in patients with COVID-19. The use of hs-cTnI assay can help to identify patients at risk

of MAEs and give information about the prognosis in patients diagnosed with COVID-19 at admission. More studies in larger population are warranted to confirm this finding.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020; 382(8):727-33.

2. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, *et al*. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020; 382(13):1199-207.

3. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395(10223):497-506.

4. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395(10223):507-13.

5. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: Summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020; 323(13):1239-42.

6. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, *et al*; China Medical Treatment Expert Group for COVID-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020; 382(18):1708-20.

7. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, *et al*. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020; 8(5):475-81.

8. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395(10229): 1054-62.

9. Lippi G, Lavie CJ, Sanchis-Gomar F. Cardiac troponin I in patients with coronavirus disease 2019 (COVID-19): Evidence from a meta-analysis. Prog Cardiovasc Dis 2020; 63(3):390-1.

10. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med 2020; 58(7):1131-34.

11. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, *et al*. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020; 395(10224):565-74.

12. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020; 323(11):1061-9.

13. Alatassi A, Habbal M, Tamim H, Sadat M, Al Qasim E, Arabi YM. Association between troponin-I levels and outcome in critically ill patients admitted to non-cardiac intensive care unit with high prevalence of cardiovascular risk factors. BMC Anesthesiol 2018; 18(1):54.

14. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, *et al*. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 2020; 5(7):802-10.

15. Blackburn R, Zhao H, Pebody R, Hayward A, Warren-Gash C. Laboratory-confirmed respiratory infections as predictors of hospital admission for myocardial infarction and stroke: Time-series analysis of English data for 2004-2015. Clin Infect Dis 2018; 67(1):8-17.

16. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, *et al*. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol 2020; 5(7):811-8.

17. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med 2020; 46(5):846-8.

18. Mueller C, Giannitsis E, Jaffe AS, Huber K, Mair J, Cullen L, *et al*; ESC Study Group on Biomarkers in Cardiology of the Acute Cardiovascular Care Association. Eur Heart J Acute Cardiovasc Care 2021; 10(3):310-9.

19. Fan H, Zhang L, Huang B, Zhu M, Zhou Y, Zhang H, *et al*. Cardiac injuries in patients with Coronavirus Disease 2019: not to be ignored. Int J Infect Dis 2020; 96:294-7.

20. Atallah B, Mallah SI, AbdelWareth L, AlMahmeed W, Fonarow GC. A marker of systemic Inflammation or direct cardiac injury: Should cardiac troponin levels be monitored in COVID-19 Patients? Eur Heart J Qual Care Clin Outcomes 2020; 6(3):204-207.

21. Li P, Chen L, Liu Z, Pan J, Zhou D, Wang H, *et al*. Clinical features and short-term outcomes of elderly patients with COVID-19. Int J Infect Dis 2020; 97:245-50.

22. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, *et al*. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med 2020; 217(6):e20200652.

Case Report

An approach to sensitized kidney transplant using molecular techniques in Northwest Region, Saudi Arabia

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ABSTRACT

When compared to dialysis, patients with end-stage renal disease and renal transplant provide significant survival and quality-of-life benefits. However, for patients pursuing transplants who are highly sensitized, waiting time has traditionally been long with limited options. Waiting for a kidney is a smart tactic for candidates who have a good chance of finding a suitable deceased donor promptly. Desensitization on the waiting list is considered for candidates who do not have a living donor and have a poor chance of having a deceased donor match. The transplant center, the recipient and the referring nephrologist must all work together to tailor a treatment plan for the highly sensitized kidney transplant candidate.

These case reports are aimed to discuss some of the unique challenges of transplanting highly sensitized patients presenting at a high-volume transplant center with desensitization experience, as well as to evaluate existing and emerging strategies to assist the patient population.

KEY WORDS: desensitization, human leukocyte antigen, panel reactive antibodies

INTRODUCTION

Histocompatibility testing is necessary for two reasons in a successful kidney transplant program. To begin, human leukocyte antigens (HLA) play a critical role in the cellular and humoral immune responses that control the transplant outcome. Second, HLA polymorphism is a significant impediment to successful transplantation. The role of HLA matching in renal transplantation is changing as immunology progresses and technology improves our ability to differentiate HLA antigens and antibodies reactive to them[1].

The presence of the antibodies against the HLA molecules can be directed against HLA class I or/and class II antigens, which are known as the risk factor for acute rejections and graft loss. Moreover, panel reactive antibody (PRA) estimation identifies sensitized patients before transplantation and also forms the basis of cadaver organ allocation[1-3].

People with high PRA can therefore spend longer waiting for an organ to which they have no pre-existing antibodies. These antibodies develop following previous transplants, blood transfusions and pregnancy. Transplanting organs into recipients who are highly sensitized to the organs significantly increases the risk of rejection, resulting in higher immunosuppressant requirements and shorter organ survival. Wide-ranging efforts have been made to identify treatment regimens to reduce PRA in sensitized transplant candidates. In certain conditions, plasma exchange, intravenous immunoglobulin, rituximab and other "antibody-directed" immune therapies may be employed[3-4]. Other factors that affect graft outcome include race, age, diabetes, sensitization, previous recipient transplant, donor age, cause of death, ischemia time, transplant center and year[5].

CASE REPORT
Case 1

A 68-year-old multiparous female with end-stage renal disease (ESRD) secondary to systemic lupus erythematosus was referred for a kidney transplant.

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Figure 1: Patient 1 and donors HLA typing, crossmatch and PRA results

Figure 2: Patient 2 and donors HLA typing, crossmatch and PRA results

She had been on regular hemodialysis for the last 5 years. She was found to have class II donor-specific antibodies (DSA) against her 1st donor (son) DR4 (MFI 2959), DQ8 (MFI2981) with a negative T cell complement-dependent cytotoxicity (CDC) cross-match (70% match). In this scenario, a transplantation was considered high risk for antibody-mediated rejection (AMR), 2 subsequent donors had similar results. She was advised to seek other donors and after several months her grandson was found to be a zero HLA mismatch with a negative cross-match (80%). She underwent a successful transplant on 12/02/18, discharged home on tacrolimus and mycophenolate, without steroids. At the last follow-up, her serum creatinine was 59 μmols/L.

HLA typing and PRA were performed on Luminex, followed by crossmatching CDC method at tissue typing section of Laboratory Medicine department at Tertiary Care Hospital, in Tabuk, Saudi Arabia (PRA), as described in Figure 1.

Case 2

A 54-year-old female with ESRD secondary to diabetes had undergone a history of two previous kidney transplants and restarted hemodialysis after the second transplant failed 2 years ago. The first was 26 years ago, from a deceased donor, and the second from an unrelated donor. As expected, she had class II positive with DSA to the donor son (DQ7 MFI5638) with a negative T cell CDC XM, she further had DSA against the same antigen (DQ7 MFI 19763), and negative XM. Another two daughters had the same antigen (DQ7) and DSA and were not considered for donation. Several months later, an older son was found not to possess DQ7, but DQ6 to which she had DSA but only (1123MFI). Another related donor was scheduled for a tissue typing workup and he was the son of the patient. PRA was Class one positive with no DSA and Class two positive with DSA DQ6 (1123) MFI, and with a negative XM, she underwent a successful third transplant on 20/06/16 and was discharged home on tacrolimus and mycophenolate. Serum creatinine at last follow-up was 68 μmols/L as illustrated in the Figure 2.

DISCUSSION

The immune system of the recipient recognizes antigens on the donor's tissues as "foreign bodies" which leads to graft rejection[6]. The degree of immune reaction, and thus the degree and pace of graft rejection, is also affected by donor-recipient histocompatibility, as HLA-matched grafts survive longer than HLA-mismatched grafts[7-8].

Highly sensitized patients find themselves at a great disadvantage because the high level of DSA is a risk factor for AMR and graft loss and generally precludes transplantation. The principal goal in transplantation is to find a donor/recipient pair with the minimum immunological risk to achieve graft longevity. The best option for highly sensitized patients is to find a donor who does not have the HLA antigens that correspond to be specific to the recipient's antibodies. The search for this donor must be relentless and unceasing to achieve best outcomes, as in our reported cases. Desensitization to lower the levels of preformed antibodies to avoid early, hyperacute and accelerated acute rejection may be the only feasible choice for transplant in highly

sensitized recipients, where some studies have shown hyperacute rejection is uncommon in desensitizing patients[9-10].

CONCLUSION

In areas where immunological tests are compromised or strong evidence of graft rejection factors is common, healthcare professionals need to keep these factors in mind, as it can increase the possibility of graft failure. On the other hand, multiple graft failure can always have a hope to find a possible donor with the correct mode of follow-up that can reduce the burden to families and health care setups. Overall, these types of conditions can be challenging for clinicians along with families and require a multidisciplinary approach to enable the best outcomes.

REFERENCES

1. Feucht HE, Opelz G. The humoral immune response towards HLA class II determinants in renal transplantation. Kidney Int 1996; 50(5):1464-75.
2. Schonemann C, Groth J, Leverenz S, May G. HLA class I and class II antibodies: monitoring before and after kidney transplantation and their clinical revelance. Transplantation 1998; 65(11):1519-23.
3. Josephson MA. Monitoring and managing graft health in the kidney transplant recipient. Clin J Am Soc Nephrol 2011; 6(7):1774-80.
4. Mahoney RJ, Taranto S, Edwards E. B-cell cross matching and kidney allograft outcome in 9031 United States transplant recipients. Hum Immunol 2002; 63(4):324-35.
5. Takemoto SK, Terasaki PI, Gjertson DW, Cecka JM. Twelve years' experience with national sharing of HLA-matched cadaveric kidneys for transplantation. New Engl J Med 2000; 343(15):1078-84.
6. Vo AA, Petrozzino J, Yeung K, Sinha A, Kahwaji J, Peng A, *et al*. Efficacy, outcomes, and cost-effectiveness of desensitization using IVIG and rituximab. Transplantation 2013; 95(6):852-8.
7. Jordan SC, Tyan D, Stablein D, McIntosh M, Rose S, Vo A, *et al*. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. J Am Soc Nephrol 2004; 15(12):3256-62.
8. Takemoto, Terasaki PI, Gjertson DW, Cecka JM. Twelve years' experience with national sharing of HLA-matched cadaveric kidneys for transplantation. New Engl J Med 2000; 343(15):1078-84.
9. Ingulli E. Mechanism of cellular rejection in transplantation. Pediatr Nephrol 2010; 25(1):61-74.
10. Sayegh MH. Why do we reject a graft? Role of indirect allorecognition in graft rejection. Kidney Int 1999; 56(5):1967-79.

Case Report

Diagnosis, treatment and recall of a poorly managed submandibular cutaneous fistula of odontogenic origin: a case report utilizing CBCT

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ABSTRACT

Odontogenic cutaneous sinus tracts are often overlooked by medical and dental practitioners, leading to unnecessary therapeutic and surgical intervention. Chronic cutaneous fistula in the face and neck should elicit a suspicion about odontogenic origin since these lesions respond favorably to endodontic treatment. Here, we present a case of chronic cutaneous fistula from odontogenic origin in the right submandibular area. A 32-year-old Saudi male patient presented with a cutaneous fistula that was initially misdiagnosed and poorly managed. The patient had undergone multiple antibiotic courses systematically and locally before oral surgeon decided to extract the offending tooth. Luckily, the patient visited the college of dentistry clinic and a proper diagnosis was made with the help of cone beam computed tomography (CBCT), which confirmed that mandibular second molar is the culprit for the extra-oral cutaneous fistula. The treatment was done in the same visit that showed a quick resolution of the sinus tract and apical excellent healing was accomplished after one year. Cutaneous fistula from odontogenic origin can be easily misdiagnosed by medical and general dental practitioners. Our report emphasizes the role of endodontists and CBCT imaging in diagnosis and proper management of the case.

KEY WORDS: CBCT, cutaneous fistula, NSRCT, odontogenic, submandibular

INTRODUCTION

Odontogenic extra-oral cutaneous fistula is rare. However, it is well documented in dental and medical literature[1-10]. Although dental infection is top in the list in differential diagnosis of cutaneous fistula in the face and neck region[11], other causes include actinomycosis, foreign body, local skin infection, pyogenic granuloma, salivary gland and duct fistula, suppurative lymphadenitis and neoplasm[12]. Also, persistence of cutaneous fistulas is one of the complications of medication-related osteonecrosis of the jaw[13]. So, limited treatment of the skin lesion alone and not addressing the dental etiology inevitably results in recurrence or persistence of fistula[14].

Apical periodontitis from odontogenic origin arises as a sequela of pulpal disease that is left untreated or unsuccessfully treated[15,16]. Subsequently, the apical inflammation spreads, leading to bone resorption that dissects along the easiest path of resistance, erupting eventually as odontogenic cutaneous fistula located either intraorally or extra orally[17-20]. The direction of pus spread and accumulation is guided by muscle attachment of the jaw and fascial planes[21]. Extraoral odontogenic fistula presents as non-tender nodules with periodic puss discharge[18,22]; however, it can appear as ulcer, cyst, furuncle and sunken skin lesion, making the diagnosis a challenge[17,23,24].

In one study[5], the time needed for evolution of the fistula extra-orally was six months or less for 68% of the cases, while others reported a longer period of time[22,23,25]. The reason for this uneventful delay could be explained by lack of proper diagnosis of the main cause of the fistula, especially when there is no dental sign and symptoms, in addition to the large variety of clinical characteristics present in the skin[26].

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Figure 1: Composite image shows **(a)** photograph of the cutaneous fistula; **(b)** a snapshot from OPG focusing on mandibular second molar showed a small apical radiolucency; **(c)** CBCT sagittal view shows the bone destruction; **(d)** CBCT coronal section at the distal root shows the perforated lingual bone; **(e)** CBCT axial section at the apical part of the tooth; and **(f)** CBCT axial section 2mm under the apex of the tooth, both **e** and **f** show the boney destruction and the size of the lesion.

Literature showed that mandibular teeth are more associated with cutaneous sinus tract than maxillary teeth (80%-87%)[5,25], with infected mandibular molars being the most offending teeth[17]. In this report, we describe a case of misdiagnosed and managed submandibular swelling turned into a cutaneous fistula for more than one year.

CASE REPORT

A 32-year-old male Saudi patient presented to the dental clinic at College of Dentistry, Jazan University with a chief complaint of painless extra-oral cutaneous sinus tract in right submandibular area. The patient was not aware of any medical problems and had a history of hard submandibular swelling for about 8 months. During this time, the patient visited a physician first and as the problem was undiagnosed, the patient was referred to a dental practitioner who prescribed antibiotics and analgesics. After few months, the patient developed an extra-oral fistula with pus discharge which enlarged with time. The patient was worried and visited another dental office again and they also prescribed antibiotics (Mycoheal oral gel 40 gm and Dermofucin 2% cream as local antifungal and antibiotic, respectively). These local medications with systemic antibiotics did not work as well. Then the patient was referred to an oral surgeon who decided to extract the tooth. During the COVID-19 pandemic, the patient failed to get an appointment for extraction, and he decided to visit the dental clinic at

Figure 2: Composite image shows **(a)** post-operative PA x-ray; **(b)** photograph of the cutaneous fistula in a month showed the healing.

College of Dentistry, Jazan University. Upon examination, there was a nodule about 80x80 mm in size and dark in color (Figure 1a). The patient presented with a relatively recently taken orthopantomography (OPG) from the surgeon office which showed a radiolucency associated with mandibular right second molar (Figure 1b). Intra-oral examination revealed a carious tooth (right mandibular second molar) with necrotic pulp that did not respond to cold. The tooth was not tender to percussion and palpation, periodontium was within normal limit and no intra-oral swelling or fistula. The tooth seemed restorable, and the patient wanted to save it. A small field of few cone beam computed tomography (CBCT) (3D Accutomo 170, Osaka, Japan) was taken. Images showed extensive bone loss perforating the mandibular cortical bone lingually (Figure 1c, d, e, f). The diagnosis determined to be necrotic tooth with chronic apical abscess, associated with an extra-oral suppurating cutaneous fistula.

Treatment

Local anesthesia with epinephrine was administered and rubber dam isolation was achieved. Non-surgical root canal treatment (NSRCT) was performed in one visit with copious irrigation of 6% sodium hypochlorite. Post and core build up was placed as final restoration and the patient was advised to visit the clinic in a month (Figure 2a). No extra-oral intervention was performed, and no antibiotics were prescribed, only Ibuprofen 600mg was given to be used when needed. The patient was advised to finally restore the tooth if signs and symptoms disappear. In a month, the patient was asymptomatic, and the lesion subsided substantially (Figure 2b). He was given a recall appointment after 6 months, but he delayed and arrived after a year, with complete healing of the cutaneous fistula with minimum or no sign of a scar tissue. Intra-oral examinations were within normal limits, as well as extra-oral (Figure 3a). Periapical radiograph and CBCT (Orthophos XG 3D, Dentsply Sirona, USA) was taken to evaluate the healing of the bone (Figure 3b-g).

Interestingly, the healing of the bone around the apical area and the cortical bone lingually was almost complete. The patient was advised to place a full coverage crown as soon as possible to protect the tooth and an implant in the area of the missing first molar.

DISCUSSION

Several diseases have a clinical presentation as extraoral cutaneous fistula, such as bacterial infections, furuncles, deep fungal infections, osteomyelitis, pyogenic granulomas, squamous cell carcinomas and congenital fistulas[2,27]. Odontogenic origin should be highly suspected when a chronic draining fistula presents in the face and neck area[4,7]. Most cases are not associated with dental pain[4]. Hence, the proper diagnosis can be easily overlooked by physicians, while this is not the case for dentists[3]. However, in the present report, the case was misdiagnosed by both physician and dentists in spite of cutaneous fistula being well documented in dental literature[23]. Subsequently, improper diagnosis can lead to unnecessary treatment including abuse of antibiotics and surgical interventions[1,3]. Teeth in the area of the fistula should be carefully investigated by sensibility tests and radiographic examination for endodontic involvement. Conventional apical radiography and OPG should be performed initially for suspected teeth and once they are inconclusive or more details are needed, CBCT is indicated[1,2,7].

Conventional periapical radiography is used as standard for detecting apical lesions[28]. However, its inherited limitation as a 2-dimensional technology may hide early stages of apical bone destruction close to the offending teeth[29,30]. CBCT is a valid method in endodontics, where a precise detection of apical periodontitis and bone loss were demonstrated[31]. Many studies showed the superiority of CBCT compared to conventional radiography in detecting apical bone destruction[32-35]. The ability of the CBCT in accurately visualizing the lesion in 3-dimensions is important for proper diagnosis and consequently treatment planning.

In the present case report, CBCT was paramount, which showed great details about the size, extent of the lesion and where the cortical bone was perforated, while OPG failed to show these critical details.
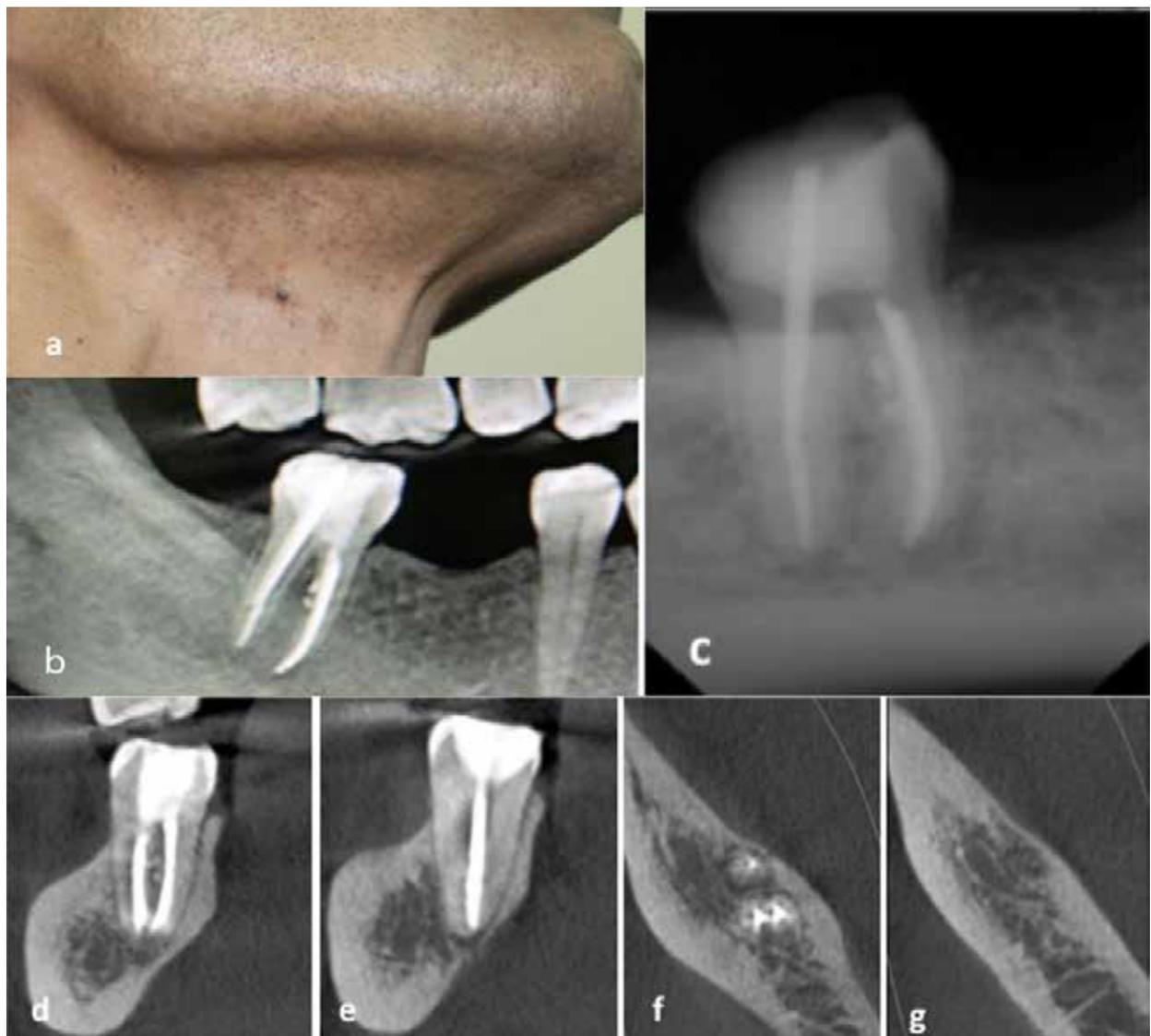
Figure 3: A one-year recall composite image shows **(a)** photograph of the patient shows a complete healing withs no or minimum scar tissue; **(b)** panoramic section from the CBCT focused on the mandibular molar tooth; **(c)** PA x-ray; **(d)** CBCT Coronal section at the mesial root; **(e)** CBCT coronal section at the distal root; **(f)** CBCT axial section at the apes, and **(g)** CBCT axial section 2 mm bellow the apex, all radiographs and clinical picture showed a tremendous almost complete healing of the cutaneous and bony lesion.

Mandibular teeth are more commonly associated with cutaneous fistula than maxillary teeth[17], with cutaneous fistula involving maxillary teeth might erupt on the cheek[1,3], while the most common locations in mandibular teeth are the jaw and chin[4].

Once the infection starts and no treatment is done, the inflammation finds its way through the easiest path of resistance until the cortical plate is penetrated, and the exit is identified based on the relationship between the location root apex to the muscle attachment of the jaw[23]. In the present case, the lingual cortical plate was perforated under mylohyoid muscle and the fistula erupted under the border of the mandible next to the offending tooth.

The treatment of choice once a diagnosis is made are either root canal (RCT) if a tooth is restorable, or extraction in case of a hopeless one[7]. The patient in our report was fortunate not to get an appointment to extract the tooth by the oral surgeon due to the COVID-19 pandemic, and he presented to the College of Dentistry clinics instead. Surgical intervention of the sinus tract and antibiotic regimen is not necessary once a proper RCT is done, however, other studies recommend otherwise[2,7]. In the present case, NSRCT was performed in one visit with final restoration and no other intervention was done. The fistula was dramatically healed in a month, and in one year, it was gone completely.

Our report demonstrated that cutaneous fistula diagnosis and treatment are a challenging task for physician and even for dental practitioners. Endodontists on the other hand, would be the least to misdiagnose cutaneous fistula, since the training they get at their residency program is sufficient enough.

Although cutaneous fistula is well documented in medical and dental literature[1-10], many cases are missed. Adding this topic to medical and dental curriculums and highlighting it is of a great need.

CONCLUSION

Cutaneous fistula from odontogenic origin can be easily misdiagnosed by medical and general dental practitioners. Our report emphasizes that endodontists should be the first line of referral since a diagnosis can be properly made and a non-invasive approach will be practiced to save the offending tooth and heal the cutaneous fistula. Also, as in this case, the use of small field of view CBCT imaging is encouraged, as it proved useful in identifying the offending tooth and detecting accurately the extension of the lesion.

REFERENCES

1. Al-Obaida MI, Al-Madi EM. Cutaneous draining sinus tract of odontogenic origin. A case of chronic misdiagnosis. Saudi Med J 2019; 40(3):292-7.
2. Bai J, Ji AP, Huang MW. Submental cutaneous sinus tract of mandibular second molar origin. Int Endod J 2014; 47(12):1185-91.
3. Chang LS. Common pitfall of plastic surgeon for diagnosing cutaneous odontogenic sinus. Arch Craniofac Surg 2018; 19(4):291-5.
4. Gimenez-Garcia R, Martinez-Vera F, Fuentes-Vera L. Cutaneous sinus tracts of odontogenic origin: Two case reports. J Am Board Fam Med 2015; 28(6):838-40.
5. Guevara-Gutierrez E, Riera-Leal L, Gomez-Martinez M, Amezcua-Rosas G, Chavez-Vaca CL, Tlacuilo-Parra A. Odontogenic cutaneous fistulas: clinical and epidemiologic characteristics of 75 cases. Int J Dermatol 2015; 54(1):50-5.
6. Sato T, Suenaga H, Igarashi M, Hoshi K, Takato T. Rare case of external dental fistula of the submental region misdiagnosed as inverted follicular keratosis and thyroglossal duct cyst. Int J Surg Case Rep 2015; 16:39-43.
7. Tian J, Liang G, Qi W, Jiang H. Odontogenic cutaneous sinus tract associated with a mandibular second molar having a rare distolingual root: a case report. Head Face Med 2015; 11:13.
8. Abuabara A, Schramm CA, Zielak JC, Baratoo-Filho F. Dental infection simulating skin lesion. An Bras Dermatol 2012; 87(4):619-21.
9. Sotiropoulos GG, Farmakis ET. Diagnosis and conservative treatment of extraoral submental sinus tract of endodontic origin: a case report. J Clin Diagn Res 2014; 8(10):ZD10-11.
10. Tavee W, Blair M, Graham B. An unusual presentation of a cutaneous odontogenic sinus. Arch Dermatol 2003; 139(12):1659-60.
11. Lewin-Epstein J, Taicher S, Azaz B. Cutaneous sinus tracts of dental origin. Arch Dermatol 1978; 114(8):1158-61.
12. Sammut S, Malden N, Lopes V. Facial cutaneous sinuses of dental origin - a diagnostic challenge. Br Dent J 2013; 215(11):555-8.
13. Mücke T, Krestan CR, Mitchell DA, Kirschke JS, Wutzl A. Bisphosphonate and medication-related osteonecrosis of the jaw: A review. Semin Musculoskelet Radiol 2016; 20(3):305-14.
14. Cohen PR, Eliezri YD. Cutaneous odontogenic sinus simulating a basal cell carcinoma: case report and literature review. Plast Reconstr Surg 1990; 86(1):123-7.
15. Braz-Silva PH, Bergamini ML, Mardegan AP, De Rosa CS, Hasseus B, Jonasson P. Inflammatory profile of chronic apical periodontitis: a literature review. Acta Odontol Scand 2019; 77(3):173-80.
16. Siqueira JF, Jr., Rôças IN, Ricucci D, Hulsmann M. Causes and management of post-treatment apical periodontitis. Br Dent J 2014; 216(6):305-12.
17. Cantatore JL, Klein PA, Lieblich LM. Cutaneous dental sinus tract, a common misdiagnosis: a case report and review of the literature. Cutis 2002; 70(5):264-7.
18. Mittal N, Gupta P. Management of extra oral sinus cases: a clinical dilemma. J Endod 2004; 30(7):541-7.
19. Qazi SS, Manzoor MA, Qureshi R, Arjumand B, Hussain SM, Afridi Z. Nonsurgical endodontic management of cutaneously draining odontogenic sinus. J Ayub Med Coll Abbottabad 2006; 18(2):88-9.
20. Sadeghi S, Dibaei M. Prevalence of odontogenic sinus tracts in 728 endodontically treated teeth. Med Oral Patol Oral Cir Bucal 2011; 16(2):e296-9.
21. Witherow H, Washan P, Blenkinsopp P. Midline odontogenic infections: a continuing diagnostic problem. Br J Plast Surg 2003; 56(2):173-5.
22. Spear KL, Sheridan PJ, Perry HO. Sinus tracts to the chin and jaw of dental origin. J Am Acad Dermatol 1983; 8(4):486-92.
23. Gupta M, Das D, Kapur R, Sibal N. A clinical predicament--diagnosis and differential diagnosis of cutaneous facial sinus tracts of dental origin: a series of case reports. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112(6):e132-6.
24. Samir N, Al-Mahrezi A, Al-Sudairy S. Odontogenic cutaneous fistula: Report of two cases. Sultan Qaboos Univ Med J 2011; 11(1):115-8.

25. Chan CP, Jeng JH, Chang SH, Chen CC, Lin CJ, Lin CP. Cutaneous sinus tracts of dental origin: clinical review of 37 cases. J Formos Med Assoc 1998; 97(9):633-7.

26. Ghodsi SZ, Ghiasi M. Two unusual cases of dental (periapical) infection. Dermatol Online J 2006; 12(4):15.

27. Yasui H, Yamaguchi M, Ichimiya M, Yoshikawa Y, Hamamoto Y, Muto M. A case of cutaneous odontogenic sinus. J Dermatol 2005; 32(10):852-5.

28. Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. Int Endod J 2006; 39(12):921-30.

29. Kanagasingam S, Lim CX, Yong CP, Mannocci F, Patel S. Diagnostic accuracy of periapical radiography and cone beam computed tomography in detecting apical periodontitis using histopathological findings as a reference standard. Int Endod J 2017; 50(5):417-26.

30. Tsai P, Torabinejad M, Rice D, Azevedo B. Accuracy of cone-beam computed tomography and periapical radiography in detecting small periapical lesions. J Endod 2012; 38(7):965-70.

31. Estrela C, Bueno MR, Leles CR, Azevedo B, Azevedo JR. Accuracy of cone beam computed tomography and panoramic and periapical radiography for detection of apical periodontitis. J Endod 2008; 34(3):273-9.

32. Torabinejad M, Rice DD, Maktabi O, Oyoyo U, Abramovitch K. Prevalence and size of periapical radiolucencies using cone-beam computed tomography in teeth without apparent intraoral radiographic lesions: A new periapical index with a clinical recommendation. J Endod 2018; 44(3):389-94.

33. Patel S, Dawood A, Whaites E, Ford TP. New dimensions in endodontic imaging: part 1. Conventional and alternative radiographic systems. Int Endod J 2009; 42(6):447-62.

34. Davies A, Mannocci F, Mitchell P, Andiappan M, Patel S. The detection of periapical pathoses in root filled teeth using single and parallax periapical radiographs versus cone beam computed tomography - a clinical study. Int Endod J 2015; 48(6):582-92.

35. Uraba S, Ebihara A, Komatsu K, Ohbayashi N, Okiji T. Ability of cone-beam computed tomography to detect periapical lesions that were not detected by periapical radiography: A retrospective assessment according to tooth group. J Endod 2016; 42(8):1186-90.

Case Report

An unanticipated case of gastrointestinal basidiobolomycosis, a rare and lethal cause of acute abdomen: A clinicopathological case report

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ABSTRACT

Gastrointestinal basidiobolomycosis (GIB) is a very rare fungal infection of the gastrointestinal tract and is therefore not commonly suspected in patients with signs and symptoms of gastrointestinal disease. If not diagnosed and treated on time, GIB can become aggressive and cause significant lifelong morbidities. Very few cases have been reported in literature. We, herein, describe one such case which was missed in spite of the patient's timely visit to the hospital. This is a case of a 15-year-old girl who on initial evaluation was mistreated as acute appendicitis.

Subsequently, she was suspected to have inflammatory bowel disease (IBD) or a gastrointestinal lymphoma. Her condition rapidly deteriorated and she went on to develop an intestinal perforation for which she had to undergo subtotal colectomy. Only upon histopathological evaluation, she was diagnosed to have GIB. Since it is so uncommon, its clinical presentation can be misinterpreted from the outset, as it was in our case, leading to initial mismanagement, delayed diagnosis and secondary complications.

KEY WORDS: Gastrointestinal basidiobolomycosis, gastrointestinal tuberculosis, inflammatory bowel disease and gastrointestinal lymphoma, necrotizing granulomatous inflammation

INTRODUCTION

Basidiobolomycosis is a rare fungal infection caused by *Basidiobolus ranarum*, which typically presents as a chronic infection of the subcutaneous tissue in immunocompetent individuals[1]. Very rarely, it infects the small intestine, colon and other parts of the gastrointestinal tract[1,2]. We report a case of gastrointestinal basidiobolomycosis (GIB) which was initially misdiagnosed and treated as acute appendicitis, and then later was thought to be either a case of fulminant inflammatory bowel disease (IBD) or a gastrointestinal lymphoma.

CASE REPORT

A 15-year-old girl from southern Saudi Arabia presented to our emergency department with acute abdominal pain. She gave a 3-month history of gradually progressive recurrent, sharp, colicky abdominal pain involving the right and left lower quadrants, radiating to the epigastric area. She also had persistent vomiting, non-bloody watery diarrhea, fever with night sweats, loss of appetite and weight loss of 15 kg in 3 months. There was no history of exposure to tuberculosis (TB), brucella, radiation and no family history of malignancies or IBD. Initially, she was misdiagnosed with acute appendicitis at another hospital where she underwent laparoscopic appendectomy. There is no other information available from that hospital except that post appendectomy, her symptoms got worse, for which she came to our center. On physical examination, she was pale and thin with a BMI of 12.5; the vital signs were reassuring; the abdomen was
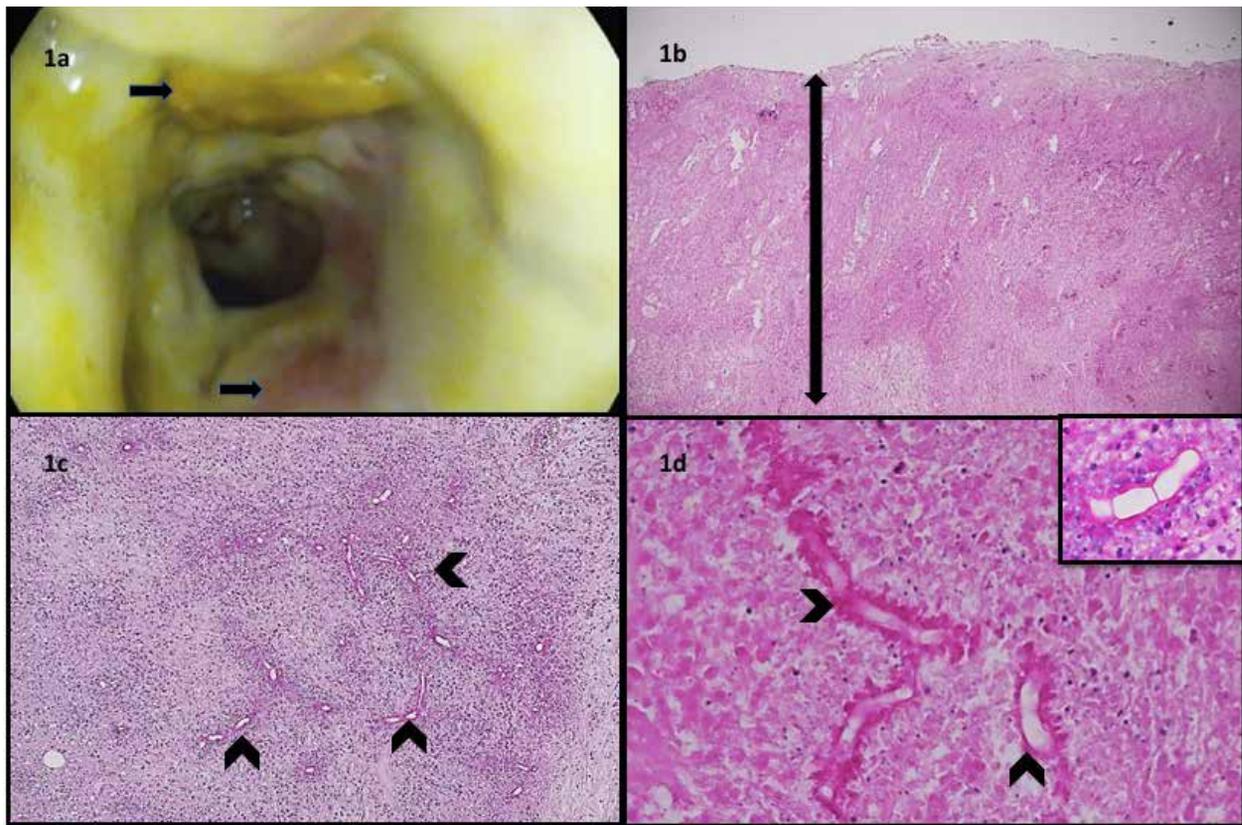
Figure 1: This is a case of necrotizing colitis secondary to intestinal Basidiobolomycosis fungal infection. **(1a)** Colonoscopic photograph shows multiple raised polypoidal mass like elevations and areas of hemorrhage and ulceration (arrows). **(1b to 1d)** Light microscopy photomicrograph of the colectomy shows extensive mucosal ulceration and full thickness necrosis of the colon wall (double headed arrow). The Basidiobolus ranarum fungal hyphae are seen infiltrating the necrotic colon wall (arrowheads) surrounded by sheath-like eosinophilic condensation called Splendore-Hoeppli phenomenon. Inset shows higher magnification of the broad fungal hyphae with septations (Hematoxylin and eosin stains, original magnifications: 1b, x20; 1c, x100; 1d, x400 and inset: x600).

soft and non-distended with mild left lower quadrant tenderness; there was no organomegaly and other system examinations were unremarkable. Laboratory testing showed leukocytosis with a white blood cell count (WBC) of 19.90×10^9/L; eosinophilia of 15.4% (absolute count 2.50×10^9/L); microcytic hypochromic anemia; hemoglobin of 69g/L; hypoalbuminemia of 12.77 g/L; and elevated erythrocyte sedimentation rate and C-reactive protein of 107 mm/h and 252.00 mg/L respectively. Hepatic profile revealed a mildly elevated alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). Her renal function tests, serology for hepatitis B and C, human immunodeficiency virus and for autoimmune disease were negative. Computerized tomography (CT) scan showed non-obstructive wall thickening with aneurysmal lumen dilatation of the bowel loops involving sigmoid colon, descending colon, cecum and terminal ileum along with retroperitoneal lymphadenopathy and moderate amount of free fluid in the abdomen and pelvis. Colonoscopy showed patchy non-obstructing mass-like thickening along the colon, especially at the splenic flexure with hemorrhage and focal necrosis

(Fig. 1a). Based on clinical, radiology and colonoscopic findings, the differential diagnosis included IBD, TB and lymphoma. The colonoscopic biopsy showed mucosal ulceration, granulation tissue and a focus suggestive necrotizing granulomatous inflammation. Quantiferon test for TB, culture and polymerase chain reaction (PCR), general blood, urine and stool cultures were all negative. Stool tests for parasites and ova were also negative. During her stay at the hospital, her condition rapidly deteriorated, her abdominal pain greatly increased in severity and she developed a spiking fever reaching 39.2 ºC and tachycardia. Abdominal examination revealed a distended, tympanic abdomen, with diffuse tenderness, rebound tenderness and rigidity. The WBC count increased to 27.80×10^9/L with predominantly neutrophils (77.9%) and abdominal X-ray showed multiple air fluid levels. An urgent exploratory laparotomy revealed a perforated transverse colon with peritonitis and markedly inflamed right colon and cecum. A subtotal colectomy with end ileostomy was performed. The excised colon was sent to the histopathology laboratory for evaluation and some tissue was also sent to the
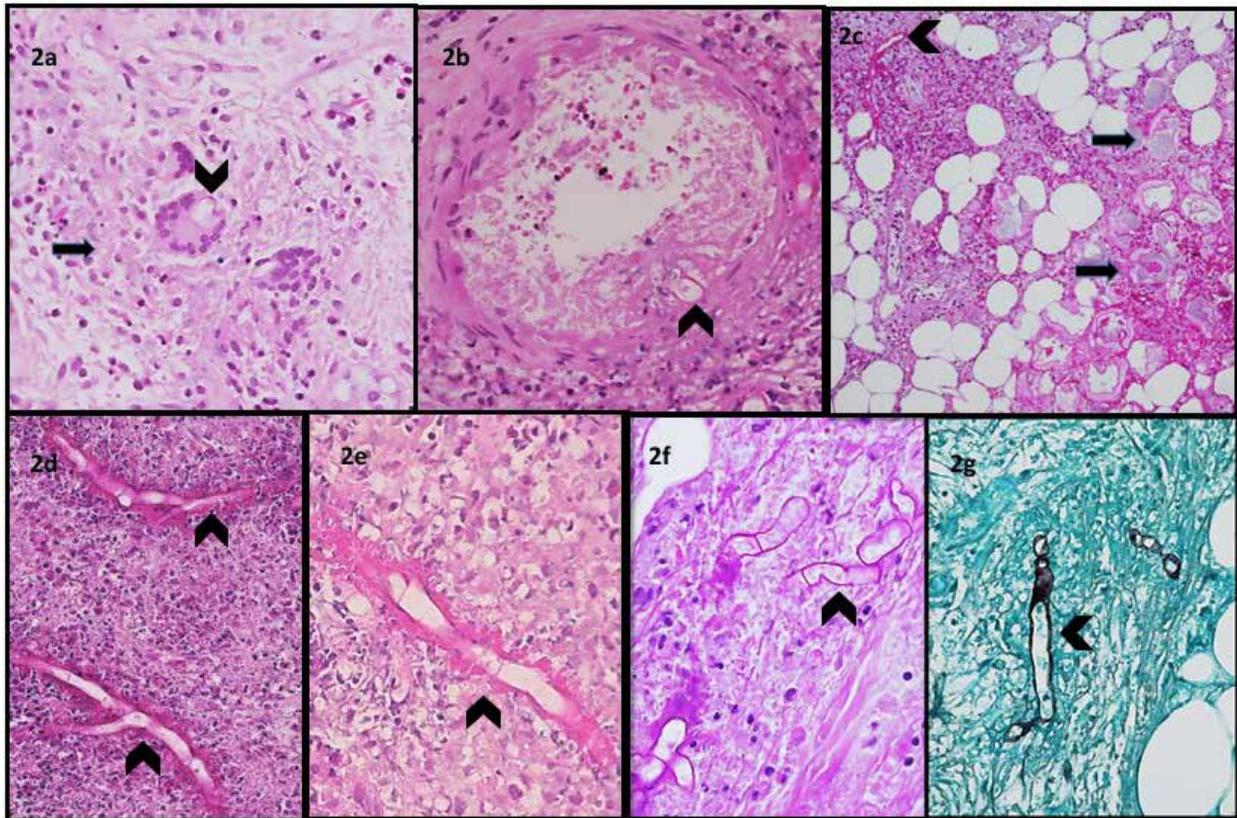
Figure 2: Light microscopy photomicrographs of the colon wall in a patient with intestinal Basidiobolomycosis infection shows **(2a)** granulomatous inflammation with multinucleated giant cells (arrow) and cross section of the fungal hyphae in the cytoplasm of the multinucleated giant cell (arrowheads). **(2b)** The fungal elements are seen infiltrating into the blood vessels (arrowhead) and **(2c)** fungal hyphae (arrowhead) and there is associated fat necrosis (arrows). **(2d and 2e)** The fungal hyphae are broad with septations and eosinophilic condensation of Splendore-Hoeppli phenomenon. (Hematoxylin and eosin stains, original magnifications: 2a, x40; 2b, x400; 2c, x100; 2d, x400 and 2e, x400). The fungal hyphae stain pink **(2f)** with the Periodic acid-Schiff stain (original magnifications: x400) and black **(2g)** with the Grimelius methenamine silver stain (original magnifications: x400.)

microbiology laboratory for TB-culture. On gross pathological examination, the wall of the entire colon was edematous, diffusely thickened with multiple foci of polypoidal elevations and extensive mucosal ulceration and there was a perforation in the region of the transverse colon. Microscopic examination revealed necrotizing granulomatous inflammation involving the cecum, ascending, transverse and descending colon. In the most affected areas, there was almost full-thickness necrosis. Embedded in the necrotic debris are numerous with broad septate fungal hyphae surrounded by eosinophilic condensation called as Splendore-Hoeppli phenomenon identified (Fig. 1b-d and Fig 2a-e). The fungi are surrounded by an acute neutrophilic and eosinophilic inflammatory infiltrate. The fungal elements are seen infiltrating the blood vessel wall and also the pericolic fat. The special stains Periodic acid-Schiff (PAS) and Gomori methenamine silver (GMS) are highlighting the fungal hyphae (Fig. 2f-g). The histomorphology of the fungal hyphae was consistent with basidiobolomycosis. The acid-fast bacilli stain was negative. A histopathological diagnosis of perforating intestinal fungal infection consistent with basidiobolomycosis with associated necrotizing granulomatous inflammation was made. The treating physician was informed immediately. Unfortunately, because a fungal infection was not suspected preoperatively, no tissue from the excision specimen was sent to mycology laboratory for fungal culture. Patient was immediately started on voriconazole and was continued for 12 months post-operatively. Laparoscopic ileostomy closure was performed 18 months post-colectomy. Currently, she is asymptomatic and is on regular outpatient follow-up with periodic abdominal CT scans.

DISCUSSION

Basidiobolomycosis is a rare type of fungal infection caused by a filamentous environmental saprophyte Basidiobolus ranarum, belonging to the fungal order Entomophthorales and class Zygomycetes, reported mainly in tropical and

subtropical regions[1]. Notably, it differs from other fungi, in that it is not opportunistic, rather it is seen in immunocompetent individuals. It primarily infects subcutaneous tissue of the thigh, buttock or trunk causing chronic subcutaneous zygomycosis[3]. Gastrointestinal tract (GIT) involvement is very rare[4,5]. The route by which the organism reaches the GIT remains elusive. It is presumably acquired via the ingestion of contaminated matter or possibly use of contaminated toilet paper[2]. The first case of GIB was reported in 1964, in a 6-year-old boy[6]. Recent years have witnessed an increased emergence of such cases and since 2001, there has been a 15-fold increase in the number of reported cases worldwide[4]. Out of the total number of reported cases, from 1964 to 2017, a significant 37.2% of the cases were from Saudi Arabia, followed by 22% from the United States of America and 21% from Iran[2,4]. Within Saudi Arabia specifically, most reported cases of GIB arose from the subtropical Southern region[5,7]. In GIB, the most common sites were colorectum (84.2%) and small bowel (31.7%), followed by other organs like stomach, liver, gallbladder and pancreas[2,4]. The most frequent symptoms were abdominal pain, a change in bowel habits, fever and weight loss[4].

The difficulty in diagnosing GIB lies in its rarity and also that it mainly affects immunocompetent individuals, so the index of suspicion is low. As a result, the physicians consider other more epidemiologically and demographically consistent differential diagnoses[7]. Clinico-radiological and colonoscopic findings of GIB overlap with various GIT diseases like IBD, acute appendicitis, lymphoma, gastrointestinal TB and even intestinal carcinomas in adults[4]. This low index of suspicion for GIB may lead to severe, sometimes fatal complications[2], as was the case in our patient. Delayed diagnosis and treatment led to perforation and emergency subtotal colectomy that will doubtlessly have a long-term effect on the quality of life of such a young patient. Due to the low index of suspicion, her diagnosis was so delayed. Had she been diagnosed sooner, then the perforation and subsequent colectomy could have been avoided. In an ideal scenario, the microscopic study of the colonoscopic biopsy should have shown the fungal elements. Unfortunately, it was not the case in our patient and even upon retrospective re-examination of the biopsy, there was no fungi identified; there was ulceration with necrotic debris and an area suspicious of a granuloma. Patients with GIB usually have leukocytosis with predominant eosinophilia and high erythrocyte sedimentation rate[8,9]. The elevated ALP and GGT may indicate possible hepatic involvement by the infection. Radiologically, the common abdominal CT-scan findings include

mass-like protrusions and thickening of the bowel wall, encompassing differential diagnoses like lymphoma, carcinoma and IBD[10,11]. Perforation and abscess formation have also been reported. These findings were present in our case. Despite being a great mimic of many gastrointestinal conditions, the histopathological findings of basidiobolomycosis tend to be classical and diagnostic, showing necrotizing granulomatous inflammation with multinucleated giant cells and a prominent eosinophilic and neutrophilic infiltration[2,12]. The diagnosis clencher is the identification of the characteristic broad thin-walled septate fungal hyphae surrounded by classical eosinophilic condensation termed Splendore-Hoeppli phenomenon on hematoxylin and eosin stained slides, further confirmed by special stains PAS and GMS. Histopathological differential diagnoses include eosinophilic gastroenteritis and other causes of granulomatous inflammation like tuberculosis. Once the classical fungal hyphae are identified, the diagnosis is evident. Culture and PCR testing are gold standard for diagnosis of GIB. However, most cases have been diagnosed based on histopathological appearance alone, the reason being that, in most cases including our case, a fungal infection was not suspected at the time of surgical excision and therefore sample is not sent for fungal culture[4,12-14]. New diagnostic modalities include PCR testing on fungal DNA from formalin-fixed, paraffin-embedded tissue and doing serological tests for cytokine responses[4,5,8,15].

Treatment involves a combination of surgery and antifungal drugs like itraconazole and voriconazole for a period of 6 months to 2 years[4,16]. Despite the invasiveness of this fungal infection and its ability to infiltrate different structures in the body, it responds well to antifungal treatment. Long-term prognosis, if diagnosed timely and treated optimally, is very good, as several cases have reportedly been cured completely with only antifungal medication, without any surgical intervention[13,14,17]. Any sequalae in our patient will be associated to the colectomy procedure and not the infection itself, which has completely resolved.

CONCLUSION

In summary, GIB is a rare but emerging infection being documented world over and due to its rarity, it is usually not in the general list of differential diagnoses in patients with GIT symptoms. We recommend that it should be considered as a possibility in patients, especially with irregular GIT presentations, as timely diagnosis may significantly affect the course and associated morbidity in such patients by reducing the need for surgical intervention, and hence its complications.

ACKNOWLEDGMENTS

REFERENCES

1. Prabhu RM, Patel R. Mucormycosis and entomophthoramycosis: a review of the clinical manifestations, diagnosis and treatment. Clin Microbiol Infect 2004; 10 Suppl 1:31-47.

2. Vikram HR, Smilack JD, Leighton JA, Crowell MD, De Petris G. Emergence of gastrointestinal basidiobolomycosis in the United States, with a review of worldwide cases. Clin Infect Dis 2012; 54(12):1685-91.

3. Choi HL, Shin YM, Lee KM, Choe KH, Jeon HJ, Sung RH, *et al.* Bowel infarction due to intestinal mucormycosis in an immunocompetent patient. J Korean Surg Soc 2012; 83(5):325-9.

4. Pezzani MD, Di Cristo V, Parravicini C, Sonzogni A, Tonello C, Franzetti M, *et al.* Gastrointestinal basidiobolomycosis: An emerging mycosis difficult to diagnose but curable. Case report and review of the literature. Travel Med Infect Dis 2019; 31:101378.

5. El-Shabrawi MHF, Kamal NM, Jouini R, Al-Harbi A, Voigt K, Al-Malki T. Gastrointestinal basidiobolomycosis: an emerging fungal infection causing bowel perforation in a child. J Med Microbiol 2011; 60(Pt 9):1395-402.

6. Edington GM. Phycomycosis in Ibadan, Western Nigeria. Two postmortem reports. Trans R Soc Trop Med Hyg 1964; 58:242-5.

7. Rabie ME, Qahtani AS, Jamil S, Mikhail NT, Hakeem I, Hummadi A, *et al.* Gastrointestinal basidiobolomycosis: An emerging potentially lethal fungal infection. Saudi Surg J 2019; 7(1):1.

8. El-Shabrawi MH, Kamal NM, Kaerger K, Voigt K. Diagnosis of gastrointestinal basidiobolomycosis: A mini-review. Mycoses 2014; 57 Suppl 3:138-43.

9. Al Qahtani S, Alangari A, Mohammed N, Albarrag A, Elzein F. Colonic basidiobolomycosis presenting with intestinal obstruction and a normal eosinophil count. IDCases 2019; 17:e00565.

10. Flicek KT, Vikram HR, De Petris GD, Johnson CD. Abdominal imaging findings in gastrointestinal basidiobolomycosis. Abdom Imaging 2015; 40(2):246-50.

11. Mandhan P, Hassan KO, Samaan SM, Ali MJ. Visceral basidiobolomycosis: An overlooked infection in immunocompetent children. African J Paediatr Surg 2015; 12(3):193-6.

12. Gugnani HC. A review of zygomycosis due to Basidiobolus ranarum. Eur J Epidemiol 1999; 15(10):923-9.

13. Albaradi BA, Babiker AMI, Al-Qahtani HS. Successful treatment of gastrointestinal basidiobolomycosis with voriconazole without surgical intervention. J Trop Pediatr 2014; 60(6):476-9.

14. Alsharidah A, Mahli Y, Alshabyli N, Alsuhaibani M. Invasive basidiobolomycosis presenting as retroperitoneal fibrosis: A case report. Int J Environ Res Public Health 2020; 17(2):535.

15. Khan ZU, Khoursheed M, Makar R, Al-Waheeb S, Al-Bader I, Al-Muzaini A, *et al.* Basidiobolus ranarum as an etiologic agent of gastrointestinal zygomycosis. J Clin Microbiol 2001; 39(6):2360-3.

16. Al-Shanafey S, Alrobean F, Hussain IB. Surgical management of gastrointestinal basidiobolomycosis in pediatric patients. J Pediatr Surg 2012; 47(5):949-51.

17. Almoosa Z, Alsuhaibani M, AlDandan S, Alshahrani D. Pediatric gastrointestinal basidiobolomycosis mimicking malignancy. Med Mycol Case Rep 2017; 18:31-3.

Case Report

Late manifestation of intracranial leptomeningeal carcinomatosis secondary to breast cancer: a case report

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ABSTRACT

Leptomeningeal carcinomatosis (LMC), also known as carcinomatous meningitis, is defined as the spread of malignant cells to pia and arachnoid maters. Most of the cases occur as an uncommon, catastrophic and late complication of metastatic carcinomas. Breast cancer is the most common cause, followed by lung cancer, melanoma and haematologic malignancies. Occurance of LMC without brain parenchymal involvement is rare. We present a 31-year-old female patient with history of breast cancer treatment 2 years ago, who was admitted to the emergency department with neurological deterioration and diagnosed with LMC without intraparenchymal metastasis.

KEY WORDS: breast cancer, carcinomatous meningitis, leptomeningeal carcinomatosis, MRI

INTRODUCTION

Leptomeningeal carcinomatosis (LMC), also known as carcinomatous meningitis, is defined as the spread of malignant cells to pia and arachnoid maters through the cerebrospinal fluid (CSF) spaces. LMC may be intracranial or spinal. These cells can be originated from primary central nervous system tumors as in drop metastases. However, most of the cases occur as an uncommon, catastrophic and late complication of metastatic carcinomas, likely via haematogenous spread with an approximate incidence of 5-8% in solid tumors and 5-15% in hematologic malignancies[1].

Over 50% of cases have concurrent brain parenchymal metastases[2]. Breast cancer is the most common cause, followed by lung cancer, melanoma and haematologic malignancies. Only about 5-8% of breast cancer patients develop leptomeningeal metastasis[3,4] and the prevalence of LMC without brain parenchymal involvement is around 3.5%[5]. We present a female patient with history of breast cancer treatment 2 years ago who was admitted to the emergency department with neurological deterioration and diagnosed with LMC without intraparenchymal metastasis.

CASE REPORT

A 31-year-old female patient presented to the emergency deparment with the complaints of headache, nausea, vomiting, diplopia, blurred speech, mental alteration and gait disturbance. She had a medical history of right breast lumpectomy and axillary dissection with the diagnosis of breast cancer 2 years ago. She had also received chemo-radiotherapy at that time. Head computed tomography (CT) performed at the day of admission showed moderate triventricular hydrocephalus and periventricular hypodensities suggesting transependymal CSF leakage. Supra and infratentorial sulcal effacement, loss of cerebral fissures and all basal cisterns were noted. Mesencephalon, pons and medulla oblongata were markedly compressed as the result of increased intracranial pressure (Fig. 1). Contrast enhanced brain magnetic resonance imaging (MRI) has been ordered and revealed diffuse hyperintensites of the tentorium and folia of cerebellum on pre-contrast T2W and FLAIR sequences. Faint, linear cerebral leptomeningeal hyperintensities were also observed on FLAIR images. The obliteration of basal cisterns, compression of brain

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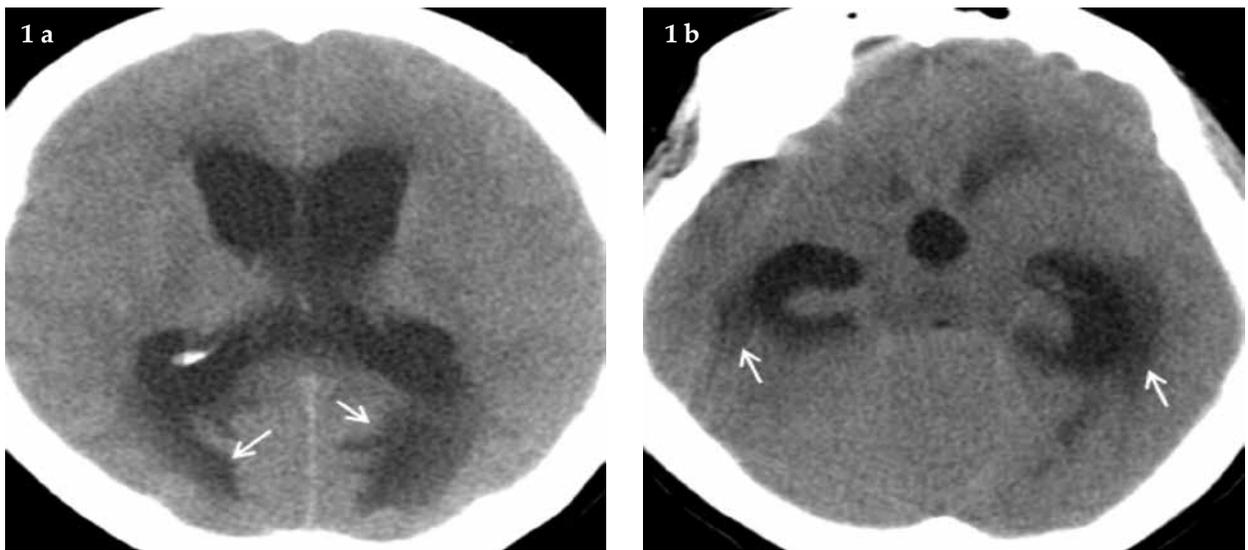
Figure 1: a, b) Head CT scan performed at the day of admission and showed howing moderate triventricular hydrocephalus and periventricular hypodensities suggesting transependymal CSF leakage **(a,b, arrows).** Note the effacemat of sulci, cerebral fissures and basal cisterns. The compressed brain stem is recognizable.
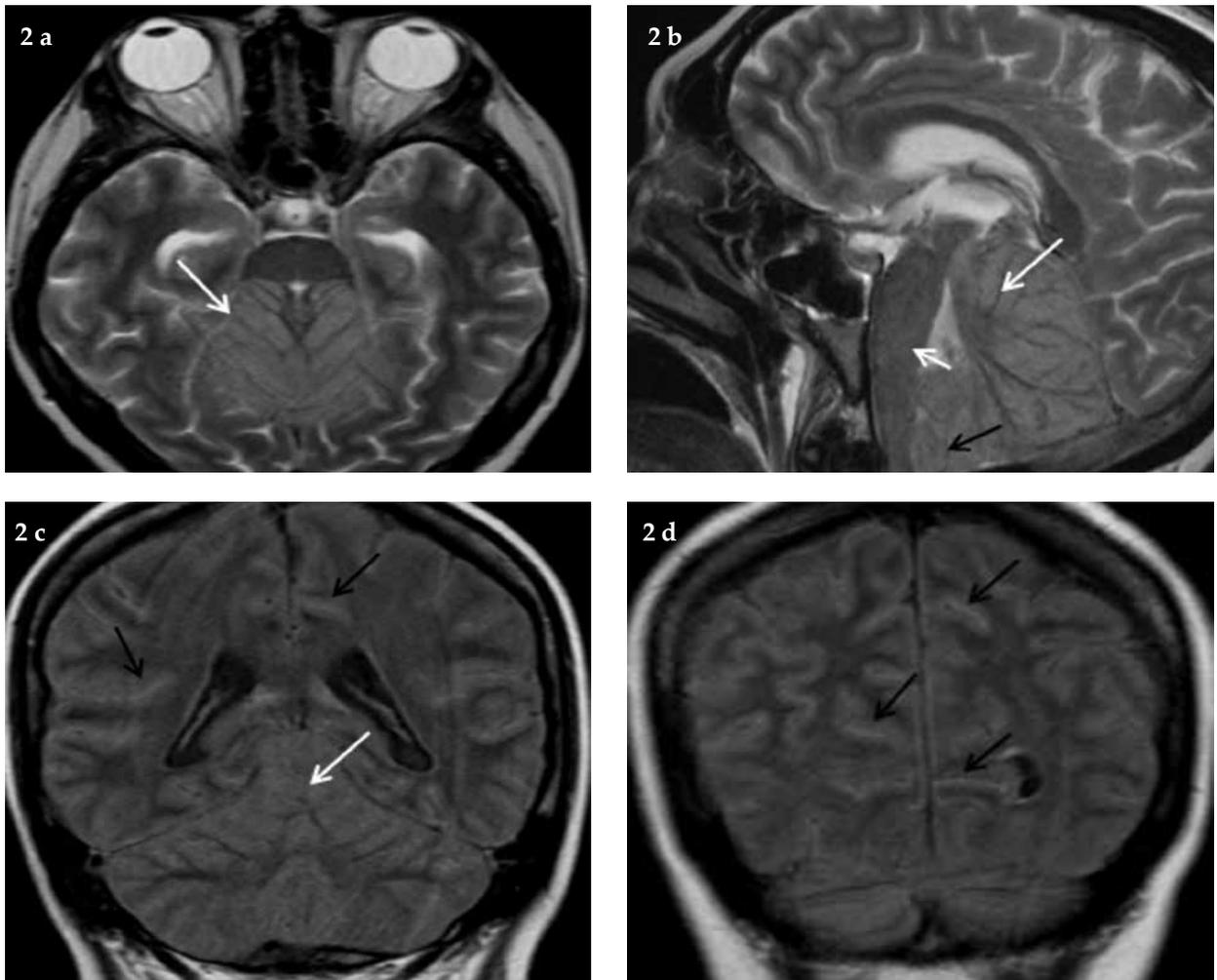

Figure 2: a,b,c,d) Precontrast brain MRI images showing diffuse hyperintensites of the tentorium and folia of cerebellum on T2W **(a,b, White arrows)** and FLAIR **(c, white arrow)** sequences. The lineer cerebral leptomeningeal hyperintensities are also seen on the FLAIR images **(c,d, black arrows).** Note the compression of the brain stem **(b, short white arrow)** and the cerebellar tonsillar herniation **(b, black arrow).**

stem and cerebellar tonsillar herniation were evident on sagittal images (Fig. 2). Diffuse linear leptomeningeal enhancement of both cerebral and cerebellar hemispheres, more prominently at posterior fossa, were seen on post-contrast T1W images (Fig. 3). No intraparenchymal lesion was detected. An external ventricular drainage (EVD) catheter was placed urgently for acute management of the hydrocephalus. Infectious etiologies were excluded by cytological analysis of CSF obtained from EVD rather than lumbar puncture, which is contraindicated in this case due to the patient's acute hydrocephalus and raised intracranial pressure. Atypical pleomorphic malignant cells were revealed. Based on both the cytological analysis of CSF (obtained from the EVD catheter) and on the MRI findings, the patient was diagnosed with LMC. MRI of entire spinal axis was recommended, but it could not be done because of the unstable status of the patient. Despite intrathecal methotrexate therapy and all other supportive treatments, the patient gradually deteriorated and deceased 30 days post admission.

DISCUSSION

LMC is one of the late complications of metastastic cancers. Clinical presentations vary with the site of the nervous system infiltrated by tumor cells. Cerebral involvement manifests as headaches, nausea, seizures and communicating hydrocephalus. Cranial nerve involvement may cause diplopia, decreased acuity of vision, hearing loss or facial numbness. Spinal involvement may result in extremity weakness, paresthesias and/or pain[5]. Our case had all kinds of symptoms associated with cerebral, cerebellar and cranial nerve involvement. Rarely, LMC may be the first presentation of the patient with no history of primary malignancy. Shin *et al* reported three cases of LMC who presented with meningitis symptoms to the emergency department without previous diagnosis of malignancy[6]. Although CSF cytology has a high specificity, it is not sensitive with high false-negative rates as reported in many studies[7,8]. Therefore, LMC is a diagnosis, primarily based on MRI findings. The primary finding is diffuse or focal, linear-nodular gyriform leptomeningeal enhancements often scattered over the brain in a sugar coated manner and possible nerve root enhancements[9]. However, leptomeningeal enhancement is not specific for LMC and can also be observed in other conditions resulting in leptomeningeal irritations, such as infectious and inflamatory meningitis, chemical meningitis, vasculitis and neurosarcoidosis[10]. Additionally, false positive leptomeningeal enhancement may be seen in patients under immunotherapy[11], intrathecal therapy and patients with intracranial hypotension[12]. On
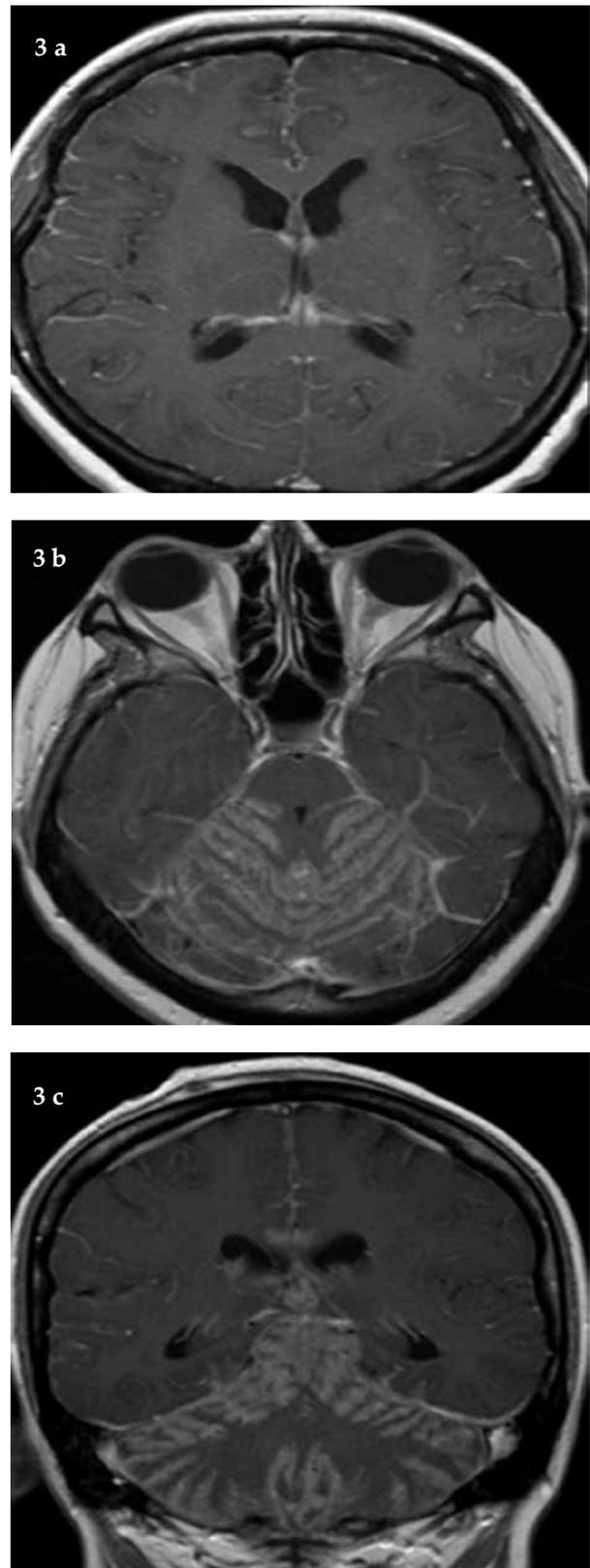
Figure 3: a,b,c) Postcontrast brain MRI images showing diffuse linear leptomeningeal enhancement of both cerebral **(a)** and cerebeller hemispheres **(c,d)**, more prominently at posterior fossa on post-contrast T1W images.

precontrast images, conventional T1W and T2W sequences may be normal, but abnormally elevated signals within the sulci on FLAIR images may often be seen[13].

CONCLUSION

In patients with previous history of malignancy and new onset or worsening headache and/or other neurologic manifestations contrast enhanced MRI of the brain and whole spinal axis should be performed. Early recognition of acute hydrocephalus secondary to LMC, together with timely placement of EVD catheter are pivotal steps in the initial management. In these patients, EVD is therapeutic and also diagnostic in that it allows for CSF sample collection safely (in contrast to LP, which should be avoided). In cases without malignant cells on CSF cytology, other possible causes of leptomeningeal enhancement on brain MRI should also be ruled out.

ACKNOWLEDGMENT

REFERENCES

1. Beauchesne P. Intrathecal chemotherapy for treatment of leptomeningeal dissemination of metastatic tumours. Lancet Oncol 2010; 11(9):871-9.
2. Clarke JL, Perez HR, Jacks LM, Panageas KS, DeAngelis LM. Leptomeningeal metastases in the MRI era. Neurology 2010; 74(18):1449-54.
3. Le Rhun E, Taillibert S, Chamberlain MC. Carcinomatous meningitis: Leptomeningeal metastases in solid tumors. Surg Neurol Int 2013; 4(Suppl 4):S265-S288.
4. Nayar G, Ejikeme T, Chongsathidkiet P, Elsamadicy AA, Blackwell KL, Clarke JM, et al. Leptomeningeal disease: current diagnostic and therapeutic strategies. Oncotarget 2017; 8(42):73312-28.
5. Posner JB. Neurologic complications of cancer. Philadelphia, PA: F A Davis Company, 1995; 143-68.
6. Ahn S, Lim KS. Three cases of neoplastic meningitis initially diagnosed with infectious meningitis in Emergency Department. Case Rep Emerg Med 2013; 2013:561475.
7. Wasserstrom WR, Glass JP, Posner JB. Diagnosis and treatment of leptomeningeal metastases from solid tumors: Experience with 90 patients. Cancer 1982; 49(4):759-72.
8. Kak M, Nanda R, Ramsdale EE, Lukas RV. Treatment of leptomeningeal carcinomatosis: current challenges and future opportunities. J Clin Neurosci 2015; 22(4):632-7.
9. Chamberlain MC. Comprehensive neuraxis imaging in leptomeningeal metastasis: A retrospective case series. CNS Oncol 2013; 2(2):121-28.
10. Aiken AH. Central nervous system infection. Neuroimaging Clin N Am 2010; 20(4):557-80.
11. Bier G, Klumpp B, Roder C, Garbe C, Preibsch H, Ernemann U, et al. Meningeal enhancement depicted by magnetic resonance imaging in tumor patients: neoplastic meningitis or therapy-related enhancement? Neuroradiology 2019; 61(7):775-82.
12. Wang N, Bertalan MS, Brastianos PK. Leptomeningeal metastasis from systemic cancer: Review and update on management. Cancer 2018; 124(1):21-35.
13. Singh SK, Agris JM, Leeds NE, Ginsberg LE. Intracranial leptomeningeal metastases: comparison of depiction at FLAIR and contrast-enhanced MR imaging. Radiology 2000; 217(1): 50-3.

Brief Communication

Is it time to consider retinal imaging as a game-changer in the health care?

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ABSTRACT

Increased life expectancy and an aging population with exponentially growing cases of age-related ocular and systemic diseases, including multimorbidity, represent a public health challenge followed by medico-social challenge with an economic burden. Artificial intelligence (AI) was implemented to overcome the burden, demonstrating promising results. With ongoing advancements, the study of biomarkers in the eye powered by cutting-edge machine learning techniques could redefine the way ocular and general health conditions are categorized and diagnosed and lead to more effective preventive measures, and personalized treatments capable to provide the patients with the best care possible.

KEY WORDS: AI-based retinal image analysis, multimorbidity, retinal imaging, systemic diseases

INTRODUCTION

The history of retinal imaging starts from the invention of the ophthalmoscope by Hermann Von Hemholtz in 1851[1]. "In whole history of medicine, there is no more beautiful episode than the invention of the ophthalmoscope, and physiology has few greater triumphs"[2], wrote American physician Edward Loring in his Textbook of Ophthalmology in 1892.

The ophthalmoscope opened a new era in clinical ophthalmology for accurate diagnosis of a myriad of posterior segment diseases, and ocular manifestations of systemic diseases (diabetes mellitus, systemic hypertension). However, it is worth noting that the main disadvantage of this device, besides the exam requiring pupil dilation, it is non-recordable and non-comparable monocular view with lack of stereopsis, and valueless for patient management and monitoring. These findings underscored the need for addressing new approaches to retinal exam. Currently, the ophthalmoscope is replaced by digital retinal imaging devices, as a phone invented by Bell is replaced by cellular phones. The first commercially available fundus camera was invented and introduced by Carl Zeiss Company in 1926[3] providing a 20-degree view of the retina. A widefield (a 130-degree) view of the retina became a reality in 1997. The new millennium brought cutting-edge technologies with ultra-widefield imaging[3]. At present, the non-mydriatic automatic fundus camera is available, which gives an opportunity to view the fundus on an electronic screen, keep the records for follow-up and send the image on-line for remote consultation[3].

Increased life expectancy and an aging population with exponentially growing cases of age-related ocular and systemic diseases, including multimorbidity, represent a public health challenge followed by medico-social challenge with an economic burden. Artificial Intelligence (AI) was implemented to overcome this burden. The term "Artificial intelligence" was introduced by John McCarthy in 1956. A lot has changed since then.

Recent reviews[4,5] cover a significant amount of works that apply AI to retinal image analysis in order to diagnose such ocular disorders as diabetic retinopathy, retinopathy of prematurity, age-related macular degeneration, glaucoma and retinal vein occlusion.

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The general consensus is that AI has demonstrated promising results, however challenges still exist.

It must be taken into consideration that the eye is a window to the body, which is why it is impossible to underestimate a significance of retinal imaging for a general health assessment. It should be noted that the retina is a unique place in the whole human organism, where the vessels of an alive person are visible. The retina works as a "mirror" reflecting changes in the rest of the body. Alterations in the retinal microvasculature can be used as non-invasive biomarkers not only for systemic disease detection, but also for its prediction, specifically for cardiovascular diseases, which makes retinal imaging extremely valuable[6-11]. It must be taken into consideration that cardiovascular disease as a leading cause of global morbidity and mortality will induce a medical and social challenge with an economic burden, which underscores the significance of population screening, providing a window of opportunity for the prevention of heart disorders. Asymptomatic retinal emboli as a subtle, incidental finding on routine fundus examination, uncovered asymptomatic carotid artery stenosis, giving practitioners the opportunity to prevent a potentially life-threatening event[12].

Recent systematic review and meta-analysis by Zhang and colleagues[11] cover a significant amount of works that apply retinal image analysis to microvascular changes in order to identify the risk of coronary heart disease. The authors highlighted that for those with narrower retinal arterioles and wider retinal venules, vessel occlusion have been associated with increased risk of coronary heart disease, particularly in females and younger adults. Earlier, Guo et al[13] evidenced that similar findings on retinal vessel caliber may be markers for heart failure.

Recently, many studies have been conducted to evaluate the suitability of deep learning (DL), a subfield of machine learning, as a subset of AI, in the prediction of cardiovascular disease (CVD)[10,14-16]. Tseng et al[10] validated a DL-based retinal biomarker (Reti-CVD) and concluded that "Reti-CVD has the potential to identify individuals with ≥ 10% 10-year CVD risk who are likely to benefit from preventative CVD interventions". Positive outcomes were obtained by Yi et al[14] in capability of Reti-CVD to identify persons with intermediate- and high-risk for CVD.

A study initiated by Zhang et al[15] was focused on developing another DL algorithm (termed Reti-WHO). Despite promising results, a high false-negative rate in individuals at high risk of CVD indicates a need for ongoing research. Oyewola et al[16] have validated classification and prediction model based on the set of DL algorithms, which have

evidenced a high accuracy (98.45%) "to diagnose whether people have CVD or not and to provide awareness or diagnosis on that". However, despite presented findings, researchers have stated that future developments of the model with various DL algorithms are required.

Recent systematic review by Prem Senthil et al[17] uncovered a capability of retinal imaging in evaluation of peripheral artery disease (PAD) associated with atherosclerosis and deteriorated bloodstream to the limbs. Early microvascular alterations, such as microaneurysms, retinal hemorrhages and hard exudates, could serve as a retinal biomarker for PAD.

Another major public health problem worldwide is a chronic kidney disease (CKD) with silent progression, low awareness and high mortality, indicating a need for early detection. The review by Wen et al[18] describes the studies proving the capability of different retinal image-based DL models for CKD prediction or early diagnosis. However, further developments are required for early recognition, referral and prompt treatment monitoring, like in the case of haemodialysis, in order to achieve the best outcome in patients with CKD.

Retinal imaging was also used in such neurodegenerative disorders such as Alzheimer's disease (AD)[19] and Parkinson disease[20]. For the first time, Ashraf et al[19] conducted a systematic review and meta-analysis evaluating retinal biomarkers, selecting the studies using brain amyloid beta status for AD definition. The limitation of this review is the lack of comparability between included reports, which makes a clear conclusion on diagnostic accuracy of some retinal parameters unfeasible. The authors highlighted the importance of adequately powered future studies. DL capability to assess neurologic dysfunction in Parkinson disease by retinal imaging was tested at a single tertiary-care hospital based on developed algorithm[20]. Study results indicate a starting point, summarizing that "further research is needed to expand the clinical implication of this algorithm"[20].

All the reports described above underscore the potential utility of AI based system in general health screening. There is still room for improvement.

Foreseen future research should be aimed at the development of versatile retinal imaging system capable of diagnosing different eye diseases (diabetic retinopathy, glaucoma, age-related macular degeneration, cataract, etc,) and predicting cardiovascular, kidney and neurodegenerative diseases. In an effort to achieve thoughtful management in health care, each person should have permanently annually updating retinal "ID Card".

With ongoing advancements, the study of biomarkers in the eye powered by cutting-edge machine learning techniques could redefine the way ocular and general health conditions are categorized and diagnosed and lead to more effective preventive measures, and personalized treatments capable to provide the patients with the best care possible.

REFERENCES

1. Ivanišević M. First look into the eye. Eur J Ophthalmol 2019; 29(6):685-8.
2. Loring EG. Text-Book of Ophthalmology, Part 1. London, England: Henry Kimpton; 1892.
3. Panwar N, Huang P, Lee J, Keane PA, Chuan TS, Ricchariya A, *et al*. Fundus photography in the 21st century--A review of recent technological advances and their implications for worldwide healthcare. Telemed J E Health 2016; 22(3):198-208.
4. Wawer Matos PA, Reimer RP, Rokohl AC, Caldeira L, Heindl LM, Große Hokamp N. Artificial intelligence in ophthalmology - status quo and future perspectives. Semin Ophthalmol 2023; 38(3):226-37.
5. Than J, Sim PY, Muttuvelu D, Ferraz D, Koh V, Kang S, *et al*. Teleophthalmology and retina: a review of current tools, pathways and services. Int J Retina Vitreous 2023; 9(1):76.
6. Li H, Cao J, Grzybowski A, Jin K, Lou L, Ye J. Diagnosing systemic disorders with AI algorithms based on ocular images. Healthcare (Basel) 2023; 11(12):1739.
7. Al-Halafi AM. Applications of artificial intelligence-assisted retinal imaging in systemic diseases: A literature review. Saudi J Ophthalmol 2023; 37(3):185-92.
8. Colcombe J, Mundae R, Kaiser A, Bijon J, Modi Y. Retinal findings and cardiovascular risk: Prognostic conditions, novel biomarkers, and emerging image analysis techniques. J Pers Med 2023; 13(11):1564.
9. Huang Y, Cheung CY, Li D, Tham YC, Sheng B, Cheng CY, *et al*. AI-integrated ocular imaging for predicting cardiovascular disease: advancements and future outlook. Eye (Lond) 2023; 38(3).
10. Tseng RMWW, Rim TH, Shantsila E, Yi JK, Park S, Kim SS, *et al*. Validation of a deep-learning-based retinal biomarker (Reti-CVD) in the prediction of cardiovascular disease: data from UK Biobank. BMC Med 2023; 21(1):28.
11. Zhang W, Guo X, Jiang X, Liu J, Han X, Guo C. Retinal microvascular changes and risk for coronary heart disease: A systematic review and meta-analysis. Retina 2024; 44(2):333-44.
12. Riese N, Smart Y, Bailey M. Asymptomatic retinal emboli and current practice guidelines: a review. Clin Exp Optom 2023; 106(1):4-9.
13. Guo S, Yin S, Song W, Tse G, Liu J, Hei K, *et al*. Retinal microvascular abnormalities predict clinical outcomes in patients with heart failure. Diagnostics (Basel) 2022; 12(9):2078.
14. Yi JK, Rim TH, Park S, Kim SS, Kim HC, Lee CJ, *et al*. Cardiovascular disease risk assessment using a deep-learning-based retinal biomarker: a comparison with existing risk scores. Eur Heart J Digit Health 2023; 4(3):236-44.
15. Zhang W, Tian Z, Song F, Xu P, Shi D, He M. Enhancing stability in cardiovascular disease risk prediction: A deep learning approach leveraging retinal images. Informatics in Medicine Unlocked 2023; 42:101366.
16. Oyewola DO, Dada EG, Misra S. Diagnosis of cardiovascular diseases by ensemble optimization deep learning techniques. International Journal of Healthcare Information Systems and Informatics (IJHISI) 2024; 19(1):1-21.
17. Senthil MP, Kurban C, Nguyen NT, Nguyen AP, Chakraborty R, Delaney C, *et al*. Role of noninvasive ocular imaging as a biomarker in peripheral artery disease (PAD): A systematic review. Vasc Med 2024; 29(2):215-22.
18. Wen J, Liu D, Wu Q, Zhao L, Iao WC, Lin H. Retinal image-based artificial intelligence in detecting and predicting kidney diseases: Current advances and future perspectives. View 2023; 4:20220070.
19. Ashraf G, McGuinness M, Khan MA, Obtinalla C, Hadoux X, van Wijngaarden P. Retinal imaging biomarkers of Alzheimer's disease: A systematic review and meta-analysis of studies using brain amyloid beta status for case definition. Alzheimers Dement (Amst) 2023; 15(2):e12421.
20. Ahn S, Shin J, Song SJ, Yoon WT, Sagong M, Jeong A, *et al*. Neurologic dysfunction assessment in Parkinson disease based on fundus photographs using deep learning. JAMA Ophthalmol 2023; 141(3):234-40.

Brief Communication

The role of molecular genetic testing in the standard management of retinoblastoma

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ABSTRACT

Cancer is a multifaceted process that has been envisaged as an epigenetic disease, a systematic disease and also as a developmental disorder. With every distinct category, various forms of aberrant growth can be seen with it. Among the youngster population, retinoblastoma (Rb) remains to be the leading type of intraocular cancer, primarily manifesting in the first three years of life, with an estimated global incidence of 1 in 15,000-20,000 live births annually. With the bulk of cases resulting from Rb tumour suppressor gene mutation, distinguishing the status of RB1 germline status is crucial for ascertaining the type of mutation. This report will look into the diagnosis, prognosis and current treatment modalities available for retinoblastoma. It will also delve into the role of personal medicine in management, along with long-term effects of treatment modalities. Finally, it will review the impact counselling holds on long-term outcome.

KEY WORDS: enucleation, RB2 germline, retinoblastoma, personal medicine

INTRODUCTION

Retinoblastoma (Rb) remains to be the leading type of intraocular cancers among children, primarily manifesting in the first three years of life, with an estimated global incidence of 1 in 15,000-20,000 live births annually[1]. The vast majority of cases result from Rb tumour suppressor gene mutation, located on chromosome 13, and distinguishing status of RB1 germline is crucial to ascertain whether mutation is heritable or sporadic[2]. Despite being recessive at the cellular level, Rb portrays an autosomal dominant pattern of transmission[3]. Early identification and management are fundamental for minimizing morbidity and expanding longevity, and the last twenty years have witnessed remarkable advances in the management of this condition[3-5]. This report will review the diagnosis, prognosis and current treatment trends for Rb. It will also discuss the role of personal medicine, long-term effects of treatment, and the impact counselling has on long-term outcomes.

DISCUSSION

To begin with, appropriate diagnosis is a crucial initial step for guiding future management. This involves an in-depth look at the human genome through molecular genetic testing, to identify any duplications or deletions, and perform direct gene analysis[6-7]. When markers linked to the disease are identified but the causative gene is not known, linkage analysis technique is implemented instead[6]. Patients with Rb show two patterns of genetic mutation: germinal or somatic. In either case, both alleles need to be non-functional to meet the two-hit hypothesis and initiate tumour growth (Table 1)[2,4].

Somatic mutations account for 40% of cases. It shows familial presence, and Rb1 mutations are found within all body cells. Germinal, on the other hand, accounts for the remaining 60%, and mutations are found only within the malignant cells in the body.

Certain families demonstrate increased tendencies for inheriting retinoblastoma (Figure 1)[3,4]. Despite having a limited role in forecasting sequela on the long run, screening for mutations remains crucial for risk stratifying family members[8]. The two main essential prognostic indicators of outcome and survival are severity of the condition once identified, and access to therapeutic modalities[9]. The definitive way of diagnosing and ascertaining the chance of passing

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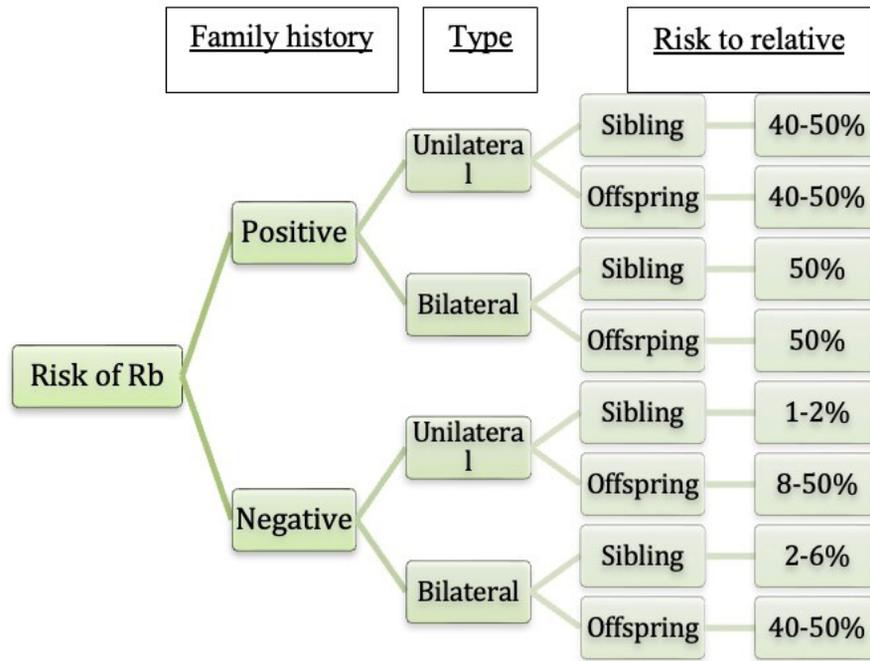
Figure 1: Hierarchy of the risk of retinoblastoma among siblings and offspring of affected patients.

altered genes is via molecular genetic screening, benefits of which include[10-11];

1. Screening to identify asymptomatic carriers.
2. Early identification of germline mutation carriers, who show preponderance towards harbouring secondary malignancies. These entail both tumours of the eyes and the rest of the body.
3. Early identification of carriers prone to developing malignancies from radiation exposure.
4. Planned parenthood, through prenatal diagnosis or pre-implantation genetic diagnosis.
5. Planned treatment and prevention of enucleation with localized treatment.
6. Assessing risk of recurrence and prognosis.
7. Analysing financial implications.

Risk of unilateral Rb depends on degree of penetrance, and percentage quoted encompasses that of both unifocal and multifocal disease. For patients

Table 1: The two-hit hypothesis of initiation of retinoblastoma.

Germinal	Somatic
60%	40%
Sporadic	Familial
No cases in relatives	Cases present in relatives
Child inherits 2 Rb+ alleles	Child inherits one RB- allele
Somatic mutation ensues independently in both alleles	Somatic mutation follows in the other allele
Baby Rb+ Rb+	Baby Rb+ Rb-
Tumour Rb- Rb-	Tumour Rb- Rb-

with no family history, unilateral Rb risk is wide-ranging, depending on the presence of germline mutation or not.

At the molecular level, mutations can be of four categories: substitution (60%), small deletion/insertion (20%), large rearrangement (10-15%) and hypermethylation (10-15%)[4,6]. The majority of RB1 gene mutations (>90%) are measurable at the molecular level, and only a minority of cases with unilateral Rb have gene changes not detected by the genetic blood tests[5]. Understanding the particular molecular genetics for each case is vital for guiding the course of management. This entails the full process, beginning with appropriate diagnosis of the case, then onto the suitable management plan, and finally with the prospective prognosis and outcomes of it.

The road towards healing begins with an in-depth analysis of the disease stage (Table 2), which is done via a thorough examination under anaesthesia. This is accompanied by looking for extraocular manifestations as well as results of genetic testing[12].

Retinoblastoma is a spectrum of five grades, group A being the mildest (small, confined, not visually threatening) & group E the most severe (extensive, unsalvageable with risk of metastases). Treatment modalities encompass chemotherapy, cryotherapy and enucleation (Figure 2)[3,5]. Since 1990's, chemotherapy became the most favorable therapy[9]. Methods of administration depend on both the clinical picture and expected results (Table 3)[13].
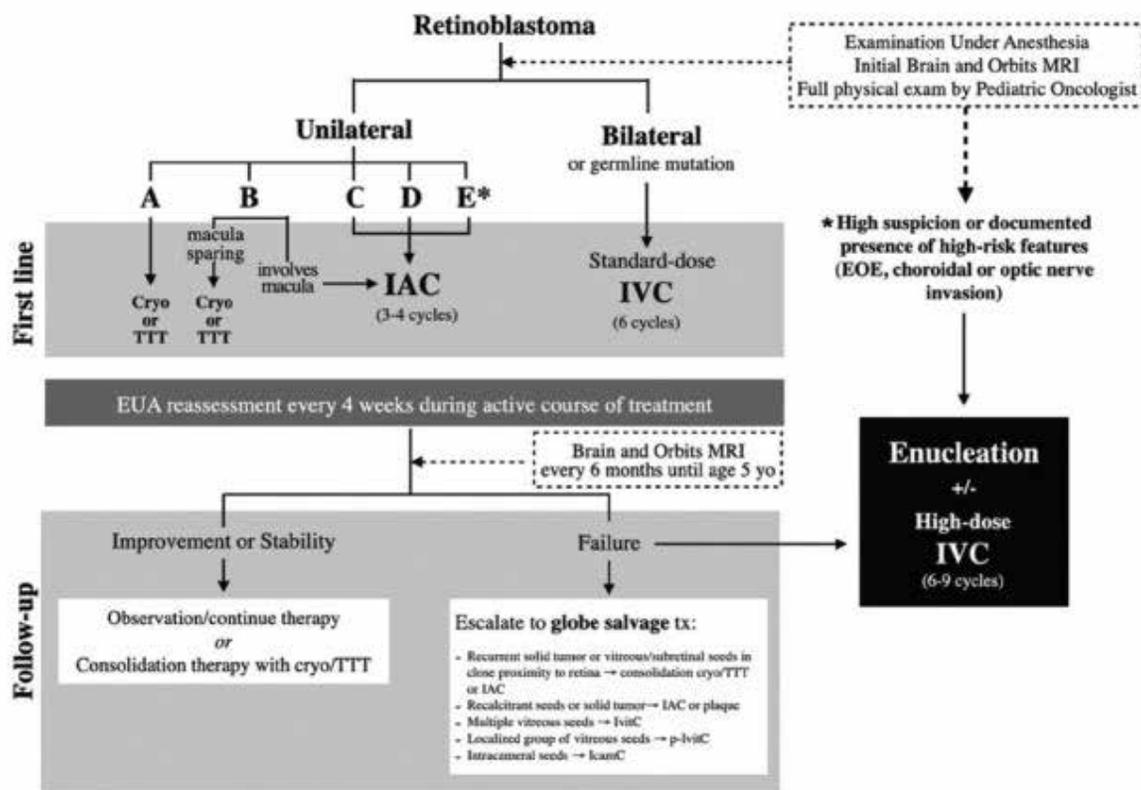
Table 2: International Classification of Retinoblastoma (ICRB) staging.

Group	Mnemonic	Features
A	Small tumour	Rb <3mm in basal diameter or thickness
B	Bigger tumour beside the macular or optic nerve	Rb >3mm in basal diameter or thickness OR tumour location <3mm from foveola tumour location <1.5mm from optic disc tumour-associated subretinal fluid <3mm from tumour margin.
C	Contiguous seeds	Rb with subretinal seeds <3mm from tumour vitreous seeds <3mm from tumour subretinal & vitreous seeds <3mm from tumour.
D	Diffuse seeds	Rb with subretinal seeds >3mm from tumour vitreous seeds >3 mm from tumour subretinal & vitreous seeds >3mm from tumour.
E	Extensive tumour	Rb occupying >50% of the globe OR neovascular glaucoma opaque media from hemorrhage in subretinal space, vitreous, or anterior chamber invasion of post-laminar optic nerve, choroid (>2mm), sclera, orbit, anterior chamber.

A study looking into the salvage rate following targeted therapy with intraarterial chemotherapy (IAC) showed a statistically significant overall salvage rate in patients receiving this mode of therapy as a primary treatment modality, as compared to those receiving it as a second line therapy (Figure 3)[14]. Complementing intravenous chemotherapy with intraarterial chemotherapy or plaque radiotherapy within two years of commencing treatment also showed improved overall globe salvage rates ($P<0.05$)[15]. The response patients show initially is important in directing future-plan, as studies have shown that the response patients show within the first two years after treatment is most likely the response they will be exhibiting a decade or two down the line[15].

Figure 2: Treatment algorithm for retinoblastoma based on laterality and ICRB stage. [Cryo: cryotherapy; EOE: extra ocular extension; EUA: examination under anaesthesia; IAC: intraarterial chemotherapy; IcamC: Intracameral chemotherapy; IVC: intravenous chemotherapy; IvitC: intravitreal chemotherapy; MRI: magnetic resonance imaging; p-IvitC: precision intravitreal chemotherapy; TTT: transpupillary thermotherapy; Tx: treatment, yo: years old]
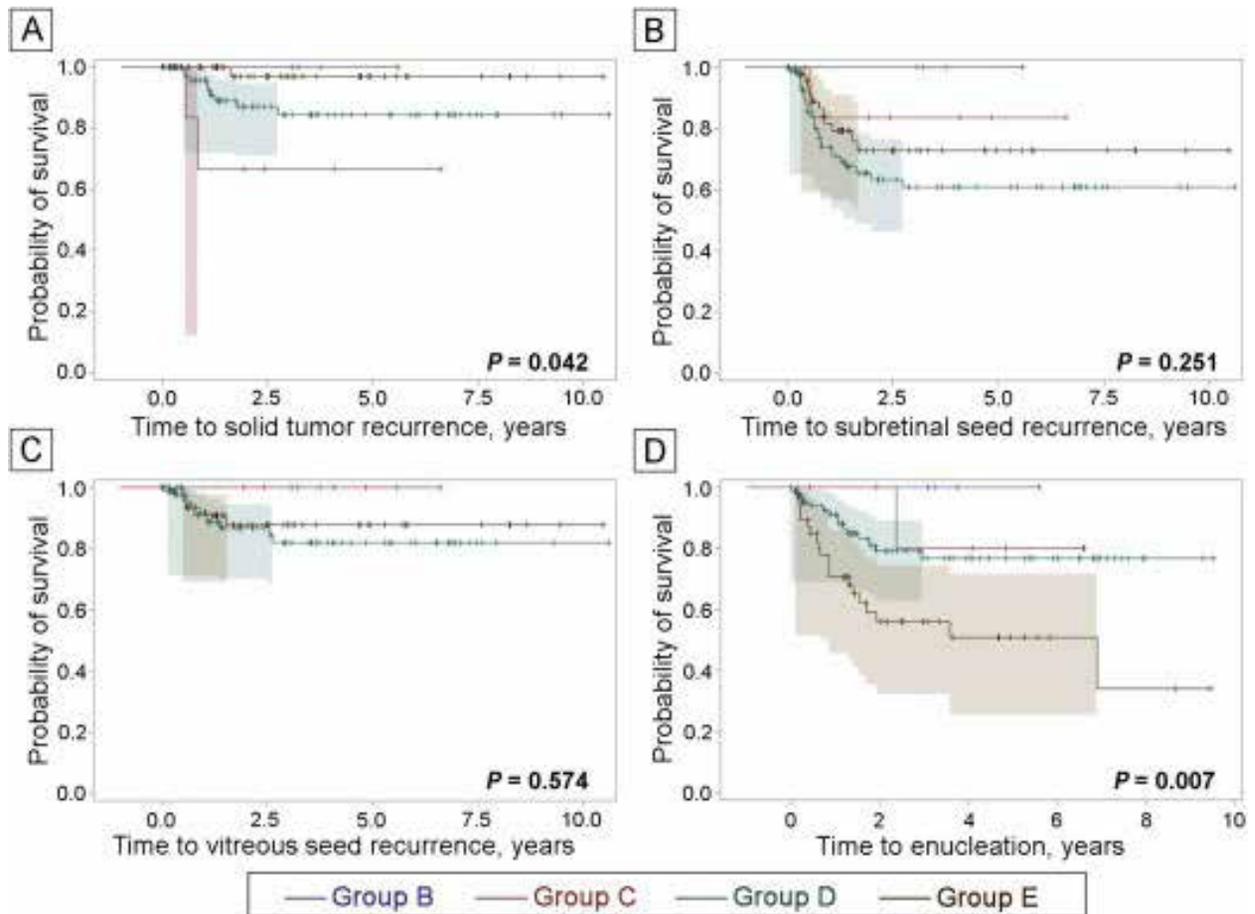
Figure 3: Kaplan-Meir curve for the global salvage rate with IAC for RB patients.

Patients receiving IAC as primary therapy had an overall salvage rate of 76% (group B 100%, group C 80%, group D 78% & group E 55%). On the other hand, those receiving IAC as a secondary therapy had an overall global salvage rate of 71% ($P<0.05$)

Enucleation remains to be an acceptable treatment modality for certain categories; however, rates have lately witnessed a notable descent[4-5]. Delaying enucleation to deliver chemotherapy does not carry any survival benefit, and could in fact increase the risk of death from metastatic spread[4]. This modality is presently used for:

1. Advanced group E growths with neovascularization.
2. Growth dominating the vitreous volume (>75%).
3. Growth necrotic with secondary inflammation of the orbit.
4. Penetration of the choroid or optic nerve.
5. Presence of invasion of anterior chamber, glaucoma or spread outside the globe.
6. Poor imagining of the tumour characteristics (i.e., from vitreous haemorrage/ hyphema).
7. Refractory tumours unresponsive to alternative means of therapy.
8. Unavailability of chemotherapy.

Enucleation impacts the patient's life from multiple aspects, and implementing a targeted treatment approach that is unique for each case can have a significant impact on the patients' psychiatric, physical and functional wellbeing[5,16]. Collaboration between the multidisciplinary team members is vital for a successful individualized management plan, which entails a meticulously tailored schedule for each presentation, locally available resources and modes of treatment, along with local traditions and accepted

Table 3: Routes of administering chemotherapeutic agent for retinoblastoma, along with the indications for each route.

	Route	Indications
1	Intravenous	1.Germline mutation (familial, BL)
		2.High risk of metastases
2	Intra-arterial	1st line → non-germinal mutation (UL)
		2nd line → recurrent solid retinoblastoma
		Seeding (subretinal, vitreous)
3	Periocular	1.Advanced group D & E tumours (BL)
		2.Localised recurrence
4	Intravitreal	Recurrent vitreous seeding

beliefs[5]. Though the majority of recurrences occur within the first three years after treatment, reappearance at a later stage is possible. This, therefore, necessitates a life-long monitoring plan for survivors of this condition, to look for both recurrences and long-term effects of the treatment regimes[17].

It is essential to maintain a balance between the mode of treatment selected and the probable long-term sequela, as though children are likely to have a normal lifespan with the current advances in management, certain treatment modalities can impact their health on the long-term. This depends on a number of factors, including age at diagnosis, genetic preponderance (heritable vs. nonheritable), age of commencing therapy, type and dose of therapy[18]. Long-term effects can be grouped into ocular and extra-ocular. The former encompasses amblyopia, glaucoma, cataract, vitreous hemorrhage and retinal detachment[5,9]. The latter, on the other hand, includes the following:

1. Impaired cardiac function
2. Impaired renal function
3. Impaired development of reproductive organs
4. Delayed growth
5. Bone deformities
6. Increased likelihood of second cancers

Another hurdle that could hinder the course of recovery is the development of drug resistance. This could arise from low infiltration levels of the tumour by the chemotherapeutic agent, or from displaying drug-resistant proteins, such as enhanced p-glycoprotein expression that accompanies MDR1 gene upregulation[19]. Modalities used to counteract these effects entail using cyclosporine A to evade the impact of high levels of p-glycoprotein, as well as determining level of p-glycoprotein early on in the course of treatment to optimize chances of success[19]. This emphasizes the importance of long-term follow-up as a means of promptly identifying those untoward side effects and consequently managing them on time.

Counseling patients, their relatives and care-takers is essential for optimizing care delivered and ensuring follow-up on the long run. Educating parents about the nature of this condition and its heritable preponderance translates into favorable long-term results. It also enables selection of the most appropriate and favored treatment modality, delivery of psychotherapy, family support and early family planning [5]. Early screening programs have been shown to correlate with higher levels of globe salvage, lower levels of cancer and therapy burden, and ultimately better outcomes for the patient's eyesight ($P<0.05$)[20]. Age at diagnosis should not be used to stratify the risk for the presence of a germline mutation in the setting of unilateral Rb. Considering the ease and accessibility of testing of the RB1 gene, all children with unilateral Rb should undergo genetic testing as a means of risk stratifying future ocular and non-ocular diseases for both them and their families[2].

CONCLUSION

To sum up, it can be seen that retinoblastoma is a multifaceted and overwhelming condition that poses medical, emotional and financial implications. Weighing every individual's risk is vital for determining future decisions regarding screening and consequent plans for reproduction. Screening for familial Rb has a crucial role in saving lives and saving eyes, whereby it enables early identification, use of less exhaustive medical treatment and achieving far better outcomes.

Despite management preferences varying among centers, saving the patients' life, preventing spread of disease, preserving the patient's eye and finally optimizing vision remain to be the universal goals of treatment. As the management is heading more towards personalized therapy, it is vital to delve further into the field of gene therapy and its role in advancing care for this complex, yet highly treatable form of cancer.

ACKNOWLEDGMENT

REFERENCES

1. Broaddus E, Topham A, Singh AD. Incidence of retinoblastoma in the USA: 1975-2004. Br J Ophthalmol 2009; 93(1):21-3.
2. Mallipatna A, Marino M, Singh AD. Genetics of retinoblastoma. Asia Pac J Ophthalmol 2016; 5(4):260-4.
3. Lohmann D, Gallie B. Gene Review: Retinoblastoma, University of Washington, Seattle. 2018 https://www.ncbi.nlm.nih.gov/books/NBK1452/#:~:text=Molecular%20genetic%20testing%20approaches%20to,and%20chromosomal%20microarray%20(CMA).&text=Individuals%20with%20bilateral%2C%20unilateral%20familial%2C%20or%20unilateral%20multifocal%20retinoblastoma [Accessed 23.02.2023]

4. Pandey AN. Retinoblastoma: An overview. Saudi J Ophthalmol 2014; 28(4):310-5.
5. Ancona-Lezama D, Dalvin LA, Shields CL. Modern treatment of retinoblastoma: A 2020 review. Indian J Ophthalmol 2020; 68(11):2356-65.
6. Harada S, Korf BR. Overview of molecular genetic diagnosis. Curr Protoc Hum Genet 2013; Chapter 9:Unit 9.1.
7. Shields JA, Shields CL. Intraocular tumors – a text and atlas. WB Saunders Company; Philadelphia, PA, USA: 1992.
8. Manukonda R, Pujar A, Ramappa G, Vemuganti GK, Kaliki S. Identification of novel RB1 genetic variants in retinoblastoma patients and their impact on clinical outcomes. Ophthalmic Genet 2022; 43(1):64-72.
9. Gallie BL, Zhao J, Vandezande K, White A, Chan HSL. Global issues & opportunities for optimized retinoblastoma care. Pediatr Blood Cancer 2007; 49(7 Suppl):1083-90.
10. Berry JL, Lewis L, Zolfaghari E, Green S, Le BHA, Lee TC, et al. Lack of correlation between age at diagnosis and RB1 mutations for unilateral retinoblastoma: the importance of genetic testing. Ophthalmic Genet 2018; 39(3):407-9.
11. Dommering CJ, Garvelink MM, Moll AC, van Dijk J, Imhof SM, Meijers-Heijboer H, et al. Reproductive behavior of individuals with increased risk of having a child with retinoblastoma. Clin Genet 2012; 81(3):216-23.
12. Shields CL, Mashayekhi A, Au AK, Czyz C, Leahey A, Meadows AT, et al. The International Classification of Retinoblastoma predicts chemoreduction success. Ophthalmology 2006; 113(12):2276-80.
13. Shields CL, Lally SE, Leahey AM, Jabbour PM, Caywood EH, Schwendeman R, et al. Targeted retinoblastoma management: when to use intravenous, intra-arterial, periocular, and intravitreal chemotherapy. Curr Opin Ophthalmol 2014; 25(5):374-85.
14. Shields CL, Dockery PW, Yaghy A, Duffner ER, Levin HJ, Taylor OS, et al. Intraarterial chemotherapy for retinoblastoma in 341 consecutive eyes (1292 infusions): comparative analysis of outcomes based on patient age, race, and sex. J AAPOS 2021; 25(3):150e1-150.e9.
15. Shields CL, Bas Z, Tadepalli S, Dalvin LA, Rao R, Schwendeman R, et al. Long-term (20-year) real-world outcomes of intravenous chemotherapy (chemo-reduction) for retinoblastoma in 964 eyes of 554 patients at a single center. Br J Ophthalmol 2020; 104(11):1548-55.
16. Shah V, Yadav L, Singh M, Kharbanda S. Custom ocular prosthesis in rehabilitation of a child operated for retinoblastoma. Natl J Maxillofac Surg 2015; 6(2):232-6.
17. Shah SU, Sheilds CL, Lally SE, Shields JA. Hydroxyapatite orbital implant in children following enucleation: analysis of 531 sockets. Ophthalmic Plast Reconstr Surg 2015; 31(2):108-14.
18. American Cancer Society medical & editorial content team. After Treatment for Retinoblastoma. 2018. https://www.cancer.org/content/dam/CRC/PDF/Public/8802.00.pdf [Accessed 03.03.2023]
19. Gerlach JH, Kartner N, Bell DR, Ling V. Multidrug resistance. Cancer Surv 1986; 5(1):25-46.
20. Soliman SE, El Manhaly M, Dimaras H. Knowledge of genetics in familial retinoblastoma. Ophthalmic Genet 2017; 38(3):226-32.

Selected Abstracts of Articles Published Elsewhere by Authors in Kuwait

Expert Consensus on Oral Corticosteroids Stewardship for the Treatment of Severe Asthma in the Middle East and Africa

Mona Al-Ahmad [1], Ashraf Al Zaabi [2], Ashraf Madkour [3], Haider Abdulhameed Alqaraghuli [4], Hajar Al Hayaan [5], Hassan Mobayed [6], Majdy Idrees [7], Nasser Al Busaidi [8], Salah Zeineldine [9]

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In the Middle East and Africa (MEA) region, overuse of oral corticosteroids (OCS) for asthma management, both as burst and maintenance therapy, poses a significant challenge. Gaps in knowledge regarding the need to taper OCS in patients with severe asthma and the use of OCS in comorbid conditions have been noted. OCS stewardship can help attain optimal and effective OCS tapering along with reducing OCS overuse and over-reliance. In this paper, we discuss current practices regarding the use of OCS in asthma, globally and in the MEA region. Expert recommendations for achieving OCS stewardship in the MEA region have also been presented. Regional experts increasing awareness among patients about the consequences of OCS overuse, engaging community pharmacists, and educating primary healthcare professionals about the benefits of prompt appropriate referral. Innovative local referral tools like ReferID can be utilized to refer patients with asthma to specialist care. The experts also endorse a multidisciplinary team approach and accelerating access to newer medicines like biologics to implement OCS stewardship and optimize asthma care in the MEA region.

Polyarteritis nodosa presenting as cholecystitis—a case report

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General Surgery, Al Salam Al Assima Hospital, Bneid AL Gar, Port Said street, 35151, Kuwait

Medium and small arteries are mainly affected by polyarteritis nodosa. Lungs are spared but any other organ can be involved. Gallbladder can be part of this systemic disease. Isolated gallbladder disease is not common. The presentation of the systemic polyarteritis nodosa as acute cholecystitis is described in this case report. Management of the disease depends on the involved organs and usually consists of systemic steroids. The diagnosis of polyarteritis nodosa should be considered in patients with previous systemic symptoms who develop picture of acute cholecystitis.

Analysis of potential microRNA biomarkers for multiple sclerosis

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Multiple sclerosis (MS) is a chronic demyelinating autoimmune neurodegenerative disorder for which no specific blood biomarker is available. MicroRNAs (miRNAs) have been investigated for their diagnostic potential in MS. However, MS-associated miRNAs are rarely replicated in different MS populations, thus impeding their use in clinical testing. Here, we evaluated the fold expression of seven reported MS miRNAs associated with MS incidence and clinical characteristics in 76 MS patients and 75 healthy control plasma samples. We found miR-23a-3p to be upregulated in relapsing-remitting MS (RRMS), while miR-326 was downregulated. MiR-150-5p and -320a-3p were significantly downregulated in secondary progressive MS (SPMS) patients compared to RRMS. High disability was associated with low miR-320a-3p, whereas low BDNF levels were associated with upregulation of miR-150-5p and downregulation of miR-326 expression in the total cohort. MiR-23a-3p and miR-326 showed significant diagnostic sensitivity, specificity, and accuracy for RRMS diagnosis. In addition, miR-150-5p and miR-320a-3p had comparable significant diagnostic test performance metrics distinguishing SPMS from RRMS. Therefore, there is potential for including miR-23a-3p and miR-326 in an RRMS diagnostic miRNA panel. Moreover, we have shown that miR-150-5p and miR-320a-3p could be novel RRMS conversion to SPMS biomarkers. The use of these miRNAs in MS diagnosis and prognosis warrants further investigation.

Pemphigus Herpetiformis: A Report of an Unusual Type of Pemphigus in a Three-Year-Old Female

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Pemphigus herpetiformis (PH) is a rare autoimmune blistering disorder that typically presents in adults. However, its occurrence in paediatric patients, especially in very young children, is exceedingly rare. It presents with clinical features resembling dermatitis herpetiformis (DH) and immunologic characteristics similar to pemphigus, belonging to the group of intraepidermal autoimmune bullous diseases. We present the case of a three-year-old female with a history of annular and vesicular lesions on both forearms and legs. A skin biopsy revealed epidermal acanthosis, marked spongiosis, numerous intra-epidermal blisters, and exocytosis of eosinophils and neutrophils. A superficial perivascular lymphocytic infiltrate, accompanied by eosinophils and neutrophils, was also observed in the dermis. The diagnosis was also supported by direct and indirect immunofluorescence. The patient was treated with clobetasol ointment and dapsone, which showed significant improvement in the skin lesions. This case underscores the importance of considering PH in the differential diagnosis of vesicobullous diseases in children and the need for further research to elucidate its pathogenesis and optimal management.

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Vineetha Elizabeth Mammen

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WHO-Facts Sheet

1. Anaemia
2. Cardiovascular diseases
3. Food safety
4. Osteoarthritis
5. Scabies

Compiled and edited by
Vineetha E Mammen

1. Anaemia

KEY FACTS
- Anemia is major public health concern, mainly affecting young children, pregnant and postpartum women, and menstruating adolescent girls and women.
- Low- and lower-middle income countries bear the greatest burden of anaemia, particularly affecting populations living in rural settings, in poorer households and who have received no formal education.
- Globally, it is estimated that 40% of all children aged 6–59 months, 37% of pregnant women and 30% of women 15–49 years of age are affected by anaemia.
- Anaemia caused 50 million years of healthy life lost due to disability in 2019. The largest causes were dietary iron deficiency, thalassaemia and sickle cell trait, and malaria (1).

Overview
Anaemia is a condition in which the number of red blood cells or the haemoglobin concentration within them is lower than normal. It mainly affects women and children.

Anaemia occurs when there isn't enough haemoglobin in the body to carry oxygen to the organs and tissues. In severe cases, anaemia can cause poor cognitive and motor development in children. It can also cause problems for pregnant women and their babies.

Anaemia can be caused by poor nutrition, infections, chronic diseases, heavy menstruation, pregnancy issues and family history. It is often caused by a lack of iron in the blood. Anaemia is preventable and treatable. In many low- and lower-middle income settings, the most commonly- recognized causes of anaemia are iron deficiency and malaria.

Scope of the problem
The population groups most vulnerable to anaemia include children under 5 years of age, particularly infants and children under 2 years of age, menstruating adolescent girls and women, and pregnant and postpartum women.

Anaemia is estimated to affect half a billion women 15–49 years of age and 269 million children 6–59 months of age worldwide. In 2019, 30% (539 million) of non-pregnant women and 37% (32 million) of pregnant women aged 15–49 years were affected by anaemia.

The WHO Regions of Africa and South-East Asia are most affected with an estimated 106 million women and 103 million children affected by anaemia in Africa and 244 million women and 83 million children affected in South-East Asia.

Signs and symptoms
Anaemia causes symptoms such as fatigue, reduced physical work capacity, and shortness of breath. Anaemia is an indicator of poor nutrition and other health problems.

Common and non-specific symptoms of anaemia include:
- tiredness
- dizziness or feeling light-headed
- cold hands and feet
- headache
- shortness of breath, especially upon exertion.

Severe anaemia can cause more serious symptoms including:
- pale mucous membranes (in the mouth, nose etc.)
- pale skin and under the fingernails
- rapid breathing and heart rate

- dizziness when standing up
- bruising more easily.

Causes

Anaemia is diagnosed based on blood haemoglobin concentrations falling below specified thresholds established based on age, sex, and physiological status. It is considered a symptom of an underlying condition(s).

Anaemia may be caused by several factors: nutrient deficiencies, inadequate diet (or the inadequate absorption of nutrients), infections, inflammation, chronic diseases, gynaecological and obstetric conditions, and inherited red blood cell disorders.

Iron deficiency, primarily due to inadequate dietary iron intake, is considered the most common nutritional deficiency leading to anaemia. Deficiencies in vitamin A, folate, vitamin B12 and riboflavin can also result in anaemia due to their specific roles in the synthesis of haemoglobin and/or erythrocyte production. Additional mechanisms include nutrient losses (e.g. blood loss from parasitic infections, haemorrhage associated with childbirth, or menstrual loss), impaired absorption, low iron stores at birth, and nutrient interactions affecting iron bioavailability.

Infections can be another important cause of anaemia, depending on the local burden of infectious diseases, such as malaria, tuberculosis, HIV and parasitic infections. Infections can impair nutrient absorption and metabolism (e.g. malaria, ascariasis) or can cause nutrient loss (e.g. schistosomiasis, hookworm infection). Many different chronic conditions can cause inflammation and lead to anaemia of inflammation or anaemia of chronic disease. HIV infection causes anaemia through a wide range of mechanisms including ineffective production or excessive destruction of red blood cells, blood loss, and side effects of the drug treatment.

Consistent heavy menstrual losses, maternal blood volume expansion during pregnancy, and blood loss during and after childbirth, particularly in cases of postpartum haemorrhage, commonly lead to anaemia.

Additionally, in some regions, inherited red blood cell disorders are a common cause of anaemia. These include conditions such as α- and β-thalassemia due to abnormalities of haemoglobin synthesis, sickle cell disorders due to changes in the haemoglobin structure, other haemoglobinopathies due to haemoglobin gene variants, abnormalities of red cell enzymes, or abnormalities of the red blood cell membrane.

Treatment and prevention

The treatment and prevention of anaemia depend on the underlying cause of the condition. There are many effective ways to treat and prevent anaemia.

Changes in diet can help reduce anaemia in some cases, including:
- eating foods that are rich in iron, folate, vitamin B12, vitamin A, and other nutrients
- eating a healthy diet with a variety of foods
- taking supplements if a qualified health-care provider recommends them.

Other health conditions can cause anaemia. Actions include:
- prevent and treat malaria
- prevent and treat schistosomiasis and other infections caused by soil-transmitted helminths (parasitic worms)
- get vaccinated and practice good hygiene to prevent infections
- manage chronic diseases like obesity and digestive problems
- wait at least 24 months between pregnancies and use birth control to prevent unintended pregnancies
- prevent and treat heavy menstrual bleeding and haemorrhage before or after birth
- delay umbilical cord clamping after childbirth (not earlier than 1 minute)
- treat inherited red blood cell disorders like sickle-cell disease and thalassemia.

Self-care

There are several ways to help prevent and manage anaemia in daily life, including eating a healthy and diverse diet and speaking to a health-care provider early if you have symptoms of anaemia.

To keep a healthy and diverse diet:
- eat iron-rich foods, including lean red meats, fish and poultry, legumes (e.g. lentils and beans), fortified cereals and dark green leafy vegetables;
- eat foods rich in vitamin C (such as fruits and vegetables) which help the body absorb iron; and
- avoid foods that slow down iron absorption when consuming iron-rich foods, such as bran in cereals (wholewheat flour, oats), tea, coffee, cocoa and calcium.

If you take calcium and iron supplements, take them at different times during the day. People with heavy menstrual bleeding should see their doctor for treatment. Doctors may recommend iron supplements or hormonal contraceptives. Some infections can cause anaemia. Wash your hands with soap and water and use clean toilets to reduce the risk of infection.

Malaria can also cause anaemia. People living in places where malaria is common should follow prevention advice from local health authorities. Seek prompt treatment if you suspect you have malaria.

Global impact

The consequences of anaemia can vary. It can affect school performance (through developmental delays and behavioural disturbances such as decreased motor activity, social interaction and attention to tasks), productivity in adult life and overall quality of life in general. During pregnancy, anaemia has been associated with poor maternal and birth outcomes, including premature birth, low birth weight and maternal mortality. In addition to the health consequences, anaemia can have important financial impacts for individuals, families, communities and countries. It is estimated that for every US$ 1 invested in reducing anaemia in women, US$ 12 in economic returns could potentially be produced *(2)*.

WHO response

Anaemia reduction is included as one of six World Health Assembly Global Nutrition Targets within the Comprehensive implementation plan on maternal, infant and young child nutrition. Additionally, anaemia in women 15–49 years of age is one of the targets for the United Nations 2030 Agenda for Sustainable Development.

WHO has committed to supporting countries to reduce anaemia. At the Nutrition for Growth Summit in 2021, WHO committed to develop a comprehensive framework for action to prevent, diagnose and manage anaemia through a multisectoral approach. WHO, together with UNICEF, is also establishing an Anaemia Action Alliance, bringing partners across sectors together to support implementation of the framework at the country level.

REFERENCES

1. Global Health Metrics. Anaemia–Level 1 impairment. Lancet. 2019; 393 https://www.healthdata.org/results/gbd_summaries/2019/anemia-level-1-impairment
2. Walters D, Kakietek J, Eberwein JD, Shekar M. An investment framework for meeting the global nutrition target for anemia. Washington DC: World Bank; 2017.

2. Cardiovascular diseases

KEY FACTS

- Cardiovascular diseases (CVDs) are the leading cause of death globally.
- An estimated 17.9 million people died from CVDs in 2019, representing 32% of all global deaths. Of these deaths, 85% were due to heart attack and stroke.
- Over three quarters of CVD deaths take place in low- and middle-income countries.
- Out of the 17 million premature deaths (under the age of 70) due to noncommunicable diseases in 2019, 38% were caused by CVDs.
- Most cardiovascular diseases can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol.
- It is important to detect cardiovascular disease as early as possible so that management with counselling and medicines can begin.

What are cardiovascular diseases?

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. They include:
- coronary heart disease – a disease of the blood vessels supplying the heart muscle;
- cerebrovascular disease – a disease of the blood vessels supplying the brain;
- peripheral arterial disease – a disease of blood vessels supplying the arms and legs;
- rheumatic heart disease – damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria;
- congenital heart disease – birth defects that affect the normal development and functioning of the heart caused by malformations of the heart structure from birth; and
- deep vein thrombosis and pulmonary embolism – blood clots in the leg veins, which can dislodge and move to the heart and lungs.

Heart attacks and strokes are usually acute events and are mainly caused by a blockage that prevents blood from flowing to the heart or brain. The most common reason for this is a build-up of fatty deposits on the inner walls of the blood vessels that supply the heart or brain. Strokes can be caused by bleeding from a blood vessel in the brain or from blood clots.

What are the risk factors for cardiovascular disease?

The most important behavioural risk factors of heart disease and stroke are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol. The effects of behavioural risk factors may show up in individuals as raised blood pressure, raised blood glucose, raised blood lipids, and overweight and obesity. These "intermediate risks factors" can be measured in primary care facilities and indicate an increased risk of heart attack, stroke, heart failure and other complications.

Cessation of tobacco use, reduction of salt in the diet, eating more fruit and vegetables, regular physical activity and avoiding harmful use of alcohol have been shown to reduce the risk of cardiovascular disease. Health policies that create conducive environments for making healthy choices affordable and available are

essential for motivating people to adopt and sustain healthy behaviours.

There are also a number of underlying determinants of CVDs. These are a reflection of the major forces driving social, economic and cultural change – globalization, urbanization and population ageing. Other determinants of CVDs include poverty, stress and hereditary factors.

In addition, drug treatment of hypertension, diabetes and high blood lipids are necessary to reduce cardiovascular risk and prevent heart attacks and strokes among people with these conditions.

What are common symptoms of cardiovascular diseases?

Symptoms of heart attacks and strokes

Often, there are no symptoms of the underlying disease of the blood vessels. A heart attack or stroke may be the first sign of underlying disease. Symptoms of a heart attack include:

- pain or discomfort in the centre of the chest; and/or
- pain or discomfort in the arms, the left shoulder, elbows, jaw, or back.

In addition, the person may experience difficulty in breathing or shortness of breath; nausea or vomiting; light-headedness or faintness; a cold sweat; and turning pale. Women are more likely than men to have shortness of breath, nausea, vomiting, and back or jaw pain.

The most common symptom of a stroke is sudden weakness of the face, arm, or leg, most often on one side of the body. Other symptoms include sudden onset of:

- numbness of the face, arm, or leg, especially on one side of the body;
- confusion, difficulty speaking or understanding speech;
- difficulty seeing with one or both eyes;
- difficulty walking, dizziness and/or loss of balance or coordination;
- severe headache with no known cause; and/or
- fainting or unconsciousness.

People experiencing these symptoms should seek medical care immediately.

What is rheumatic heart disease?

Rheumatic heart disease is caused by damage to the heart valves and heart muscle from the inflammation and scarring caused by rheumatic fever. Rheumatic fever is caused by an abnormal response of the body to infection with streptococcal bacteria, which usually begins as a sore throat or tonsillitis in children.

Rheumatic fever mostly affects children in developing countries, especially where poverty is widespread. Globally, about 2% of deaths from cardiovascular diseases are related to rheumatic heart disease.

Symptoms of rheumatic heart disease

Symptoms of rheumatic heart disease include: shortness of breath, fatigue, irregular heartbeats, chest pain and fainting. Symptoms of rheumatic fever include: fever, pain and swelling of the joints, nausea, stomach cramps and vomiting.

Why are cardiovascular diseases a development issue in low- and middle-income countries?

At least three-quarters of the world's deaths from CVDs occur in low- and middle-income countries. People living in low- and middle-income countries often do not have the benefit of primary health care programmes for early detection and treatment of people with risk factors for CVDs. People in low- and middle-income countries who suffer from CVDs and other noncommunicable diseases have less access to effective and equitable health care services which respond to their needs. As a result, for many people in these countries detection is often late in the course of the disease and people die at a younger age from CVDs and other noncommunicable diseases, often in their most productive years.

The poorest people in low- and middle-income countries are most affected. At the household level, evidence is emerging that CVDs and other noncommunicable diseases contribute to poverty due to catastrophic health spending and high out-of-pocket expenditure. At the macro-economic level, CVDs place a heavy burden on the economies of low- and middle-income countries.

How can the burden of cardiovascular diseases be reduced?

The key to cardiovascular disease reduction lies in the inclusion of cardiovascular disease management interventions in universal health coverage packages, although in a high number of countries health systems require significant investment and reorientation to effectively manage CVDs.

Evidence from 18 countries has shown that hypertension programmes can be implemented efficiently and cost-effectively at the primary care level which will ultimately result in reduced coronary heart disease and stroke. Patients with cardiovascular disease should have access to appropriate technology and medication. Basic medicines that should be available include:

- aspirin;
- beta-blockers;
- angiotensin-converting enzyme inhibitors; and
- statins.

An acute event such as a heart attack or stroke should be promptly managed. Sometimes, surgical operations are required to treat CVDs. They include:
- coronary artery bypass;
- balloon angioplasty (where a small balloon-like device is threaded through an artery to open the blockage);
- valve repair and replacement;
- heart transplantation; and
- artificial heart operations.

Medical devices are required to treat some CVDs. Such devices include pacemakers, prosthetic valves, and patches for closing holes in the heart.

WHO response

In 2013, WHO Member States agreed on global mechanisms to reduce the avoidable NCD burden including a "Global action plan for the prevention and control of NCDs 2013-2020". This Plan aims to reduce the number of premature deaths from NCDs by 25% by 2025 through nine voluntary global targets. Two of the targets directly focus on preventing and controlling CVDs.

Target 6: Reduce global prevalence of raised blood pressure by 25% between 2010 and 2025.

Target 8: At least 50% of eligible people should receive drug therapy and counselling (including glycaemic control) to prevent heart attacks and strokes by 2025.

In addition, target 9 states that there should be 80% availability of the affordable basic technologies and essential medicines, including generics, required to treat major NCDs in both public and private facilities.

Achieving these targets will require significant investment in and strengthening of health systems.

WHO is currently working on increasing the normative guidance available for the management of acute coronary syndrome and stroke which will provide guidance in these important areas.

3. Food safety

KEY FACTS
- Food safety, nutrition and food security are inextricably linked.
- An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years (DALYs).
- US$ 110 billion is lost each year in productivity and medical expenses resulting from unsafe food in low- and middle-income countries.
- Children under 5 years of age carry 40% of the foodborne disease burden, with 125 000 deaths every year.
- Foodborne diseases impede socioeconomic development by straining health care systems and harming national economies, tourism and trade.

Overview

Access to sufficient amounts of safe and nutritious food is key to sustaining life and promoting good health. Unsafe food containing harmful bacteria, viruses, parasites or chemical substances causes more than 200 diseases, ranging from diarrhoea to cancers. It also creates a vicious cycle of disease and malnutrition, particularly affecting infants, young children, elderly and the sick. Good collaboration between governments, producers and consumers is needed to help ensure food safety and stronger food systems.

Major foodborne illnesses and causes

Foodborne illnesses are usually infectious or toxic in nature and caused by bacteria, viruses, parasites or chemical substances entering the body through contaminated food. Chemical contamination can lead to acute poisoning or long-term diseases, such as cancer. Many foodborne diseases may lead to long-lasting disability and death. Some examples of food hazards are listed below.

Bacteria
- *Salmonella, Campylobacter* and enterohaemorrhagic *Escherichia coli* are some of the most common foodborne pathogens that affect millions of people annually, sometimes with severe and fatal outcomes. Symptoms can be fever, headache, nausea, vomiting, abdominal pain and diarrhoea. Foods involved in outbreaks of salmonellosis include eggs, poultry and other products of animal origin. Foodborne cases with *Campylobacter* are mainly caused by raw milk, raw or undercooked poultry and drinking water. Enterohaemorrhagic *Escherichia coli* is associated with unpasteurized milk, undercooked meat and contaminated fresh fruits and vegetables.
- *Listeria* infections can lead to miscarriage in pregnant women or death of newborn babies. Although disease occurrence is relatively low, *Listeria*'s severe and sometimes fatal health consequences, particularly among infants, children and the elderly, count them among the most serious foodborne infections. *Listeria* is found in unpasteurised dairy products and various ready-to-eat foods and can grow at refrigeration temperatures.
- *Vibrio cholerae* can infect people through contaminated water or food. Symptoms may

include abdominal pain, vomiting and profuse watery diarrhoea, which quickly lead to severe dehydration and possibly death. Rice, vegetables, millet gruel and various types of seafood have been implicated in cholera outbreaks.

Antimicrobials, such as antibiotics, are essential to treat infections caused by bacteria, including foodborne pathogens. However, their overuse and misuse in veterinary and human medicine has been linked to the emergence and spread of resistant bacteria, rendering the treatment of infectious diseases ineffective in animals and humans.

Viruses

Some viruses can be transmitted by food consumption. Norovirus is a common cause of foodborne infections that is characterized by nausea, explosive vomiting, watery diarrhoea and abdominal pain. Hepatitis A virus can also be transmitted by food and can cause long-lasting liver disease and spreads typically through raw or undercooked seafood or contaminated raw produce.

Parasites

Some parasites, such as fish-borne trematodes, are only transmitted through food. Others, for example tapeworms like *Echinococcus* spp, or *Taenia* spp, may infect people through food or direct contact with animals. Other parasites, such as *Ascaris, Cryptosporidium, Entamoeba histolytica* or *Giardia*, enter the food chain via water or soil and can contaminate fresh produce.

Prions

Prions, infectious agents composed of protein, are unique in that they are associated with specific forms of neurodegenerative disease. Bovine spongiform encephalopathy (BSE, or so-called mad cow disease) is a prion disease in cattle, associated with the variant Creutzfeldt-Jakob disease (vCJD) in humans. Consuming meat products containing specified risk material, such as brain tissue, is the most likely route of transmission of the prion agent to humans.

Chemicals

Of most concern for health are naturally occurring toxins and environmental pollutants.

- **Naturally occurring toxins** include mycotoxins, marine biotoxins, cyanogenic glycosides and toxins occurring in poisonous mushrooms. Staple foods like corn or cereals can contain high levels of mycotoxins, such as aflatoxin and ochratoxin, produced by mould on grain. A long-term exposure can affect the immune system and normal development, or cause cancer.

- **Persistent organic pollutants (POPs)** are compounds that accumulate in the environment and human body. Known examples are dioxins and polychlorinated biphenyls (PCBs), which are unwanted by-products of industrial processes and waste incineration. They are found worldwide in the environment and accumulate in animal food chains. Dioxins are highly toxic and can cause reproductive and developmental problems, damage the immune system, interfere with hormones and cause cancer.

- **Heavy metals** such as lead, cadmium and mercury cause neurological and kidney damage. Contamination by heavy metal in food occurs mainly through pollution of water and soil.

- **Other chemical hazards** in food can include radioactive nucleotides that can be discharged into the environment from industries and from civil or military nuclear operations, food allergens, residues of drugs and other contaminants incorporated in the food during the process.

The burden of foodborne diseases

The burden of foodborne diseases to public health and to economies has often been underestimated due to underreporting and difficulty to establish causal relationships between food contamination and resulting illness or death.

The 2015 WHO report on the estimates of the global burden of foodborne diseases presented the first-ever estimates of disease burden caused by 31 foodborne agents (bacteria, viruses, parasites, toxins and chemicals) at global and sub-regional level, highlighting that more than 600 million cases of foodborne illnesses and 420 000 deaths could occur in a year. The burden of foodborne diseases falls disproportionately on groups in vulnerable situations and especially on children under 5, with the highest burden in low- and middle-income countries.

The 2019 World Bank report on the economic burden of the foodborne diseases indicated that the total productivity loss associated with foodborne disease in low- and middle-income countries was estimated at US$ 95.2 billion per year, and the annual cost of treating foodborne illnesses is estimated at US$ 15 billion.

The evolving world and food safety

Safe food supplies support national economies, trade and tourism, contribute to food and nutrition security, and underpin sustainable development. Urbanization and changes in consumer habits have increased the number of people buying and eating food prepared in public places. Globalization has triggered growing consumer demand for a wider

variety of foods, resulting in an increasingly complex and longer global food chain. Climate change is also predicted to impact food safety.

These challenges put greater responsibility on food producers and handlers to ensure food safety. Local incidents can quickly evolve into international emergencies due to the speed and range of product distribution.

A public health priority

Governments should make food safety a public health priority, as they play a pivotal role in developing policies and regulatory frameworks and establishing and implementing effective food safety systems. Food handlers and consumers need to understand how to safely handle food and practicing the WHO Five keys to safer food at home, or when selling at restaurants or at local markets. Food producers can safely grow fruits and vegetables using the WHO Five keys to growing safer fruits and vegetables.

WHO response

WHO aims to strengthen national food control systems to facilitate global prevention, detection and response to public health threats associated with unsafe food. To do this, WHO supports Member States by:

- providing independent scientific assessments on microbiological and chemical hazards that form the basis for international food standards, guidelines, and recommendations, known as the Codex Alimentarius;
- assessing the performance of national food control systems throughout the entire food chain, identifying priority areas for further development, and measuring and evaluating progress over time through the FAO/WHO food control system assessment tool;
- assessing the safety of new technologies used in food production, such as genetic modification, cultivated food products and nanotechnology;
- helping implement adequate infrastructure to manage food safety risks and respond to food safety emergencies through the International Food Safety Authorities Network (INFOSAN);
- promoting safe food handling through systematic disease prevention and awareness programmes, through the WHO Five keys to safer food message and training materials;
- advocating for food safety as an important component of health security and for integrating food safety into national policies and programmes in line with the International Health Regulations (IHR 2005);

- monitoring regularly the global burden of foodborne and zoonotic diseases at national, regional and international levels, and supporting countries to estimate the national burden of foodborne diseases; and
- updating the WHO Global Strategy for Food Safety (2022–2030) to support Member States to strengthen their national food control systems and reduce the burden of foodborne diseases.

WHO works closely with Food and Agriculture Organization (FAO), the World Organization for Animal Health (OIE), The UN Environment Programme (UNEP) and other international organizations to ensure food safety along the entire food chain from production to consumption.

4. Osteoarthritis

KEY FACTS

- In 2019, about 528 million people worldwide were living with osteoarthritis; an increase of 113% since 1990 (1).
- About 73% of people living with osteoarthritis are older than 55 years, and 60% are female (1).
- With a prevalence of 365 million, the knee is the most frequently affected joint, followed by the hip and the hand (2).
- 344 million people living with osteoarthritis experience severity levels (moderate or severe) that could benefit from rehabilitation (3).
- With ageing populations and increasing rates of obesity and injury, the prevalence of osteoarthritis is expected to continue to increase globally.
- Osteoarthritis is not an evitable consequence of ageing.

Overview

Osteoarthritis is a degenerative joint condition. It causes pain, swelling and stiffness, affecting a person's ability to move freely. Osteoarthritis affects the entire joint, including the tissues around it. It is most common in the knees, hips, spine and hands.

Many factors can contribute to developing osteoarthritis. Some include a history of joint injury or overuse, older age and being overweight. It affects women more than men.

Exercise and healthy eating to build strong muscles and keep a healthy weight can reduce symptoms. Surgery to replace joints is used in severe cases to reduce pain and regain mobility.

Once pain and loss of movement function become chronic, people with osteoarthritis often experience restrictions in participating in meaningful activities, decreased well-being, and psychological distress.

Scope of the problem

Osteoarthritis is one of the significant contributors to years lived with disability among the musculoskeletal conditions. As osteoarthritis is more prevalent in older people (about 70% are older than 55), global prevalence is expected to increase with the ageing of populations. The typical onset is in the late 40s to mid-50s, although osteoarthritis may also affect younger people, including athletes and people who sustain joint injury or trauma. About 60% of people living with osteoarthritis are women.

Signs and symptoms

Symptoms of osteoarthritis include pain, swelling, stiffness and trouble moving the affected joint. As a consequence of reduced movement, muscles often lose strength and people become less able to perform physical activities. Osteoarthritis can affect any joint but is most common in the knees, hips, spine and small joints in the hands. Muscles and tissue around the joint are often affected.

Symptoms can develop slowly or start quickly after an injury or strain. Osteoarthritis is chronic and often progressive, so changes happen gradually over time. In severe cases, it can make the joint unusable and cause long-term pain. Some people feel pain even when resting.

Being less physically active can lead to other conditions, including cardiovascular diseases, obesity and diabetes. Osteoarthritis can greatly reduce the quality of life. It makes movement painful and difficult, which can stop people from participating in home, work or social activities. This can lead to mental health impacts, trouble sleeping and problems in relationships.

Cause and risk factors

Several risk factors are known to increase the risk of developing osteoarthritis:
- injury to the joint, e.g. fractures, strains, repeated stress in sport or at work;
- pre-existing joint diseases, such as rheumatoid arthritis or gout;
- specific metabolic diseases, such as diabetes;
- obesity – specifically for hip and knee osteoarthritis – as characterized by metabolic abnormalities, systemic inflammation, and contributing to excessive load on the joints;
- genetics;
- sociodemographic factors (age, female sex).

Prevention and control

Several key prevention strategies have been proposed to prevent osteoarthritis and control the disease progression. In particular, reducing overuse of joints (e.g. related to workload), and promoting healthy lifestyles (e.g. regular physical activity, maintaining a normal body weight) play an important role.

Treatment and management

Management of osteoarthritis often involves different health workers, who contribute to a rehabilitative strategy tailored to a person's needs and preferences. Being diagnosed early and following a treatment plan is the best way to slow the disease and optimize function.

Exercise can strengthen the affected muscles and help mobility. Other therapeutic approaches can help the joint to move properly and allow people to continue their daily activities. Braces and other assistive technologies can help people to stay independent when movement becomes more difficult.

Medicines like non-steroidal anti-inflammatory drugs (NSAIDs) may be prescribed to control pain. Joint replacement surgery can reduce pain, restore movement and improve quality of life for most people with severely affected joints. These surgeries are most commonly performed at the hip and knee.

It is important to stay at a healthy weight. Education and counselling are important to help people manage their symptoms and work-related tasks. Most guidelines suggest that opioid analgesics, glucosamine and visco-supplementation therapies are not effective for osteoarthritis and there is insufficient evidence to suggest stem cell therapy is beneficial.

Self-care

Self-care is an important part of managing osteoarthritis. Education and support can help people learn to cope with the physical and mental effects of osteoarthritis. People with osteoarthritis should speak to a health worker to build a tailored care plan. Staying active and maintaining a healthy weight can help reduce symptoms and the risk of their progression.

WHO response

WHO is taking action to extend access to care for people with osteoarthritis in different ways:

WHO Rehabilitation 2030 Initiative

The Package of Interventions for Rehabilitation provides information on essential interventions for rehabilitation (including assistive products), and human and material resources for 20 health conditions, including osteoarthritis.

UN Decade of Healthy Ageing

WHO recommends a reorientation of health and care systems to promote healthy ageing and address the diverse needs of older persons.

The Integrated Care for Older People (ICOPE) approach promotes the person-centred assessment of the older person to guide the design of personalized, health and social care, including long-term care interventions. Specific recommendations are provided to prevent the loss of locomotor and psychological capacity because of pain.

REFERENCES

1. GBD 2019: Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. https://vizhub.healthdata.org/gbd-results/.
2. Long H, Liu Q, Yin H, Diao N, Zhang Y, Lin J et al. Prevalence trends of site-specific osteoarthritis from 1990 to 2019: Findings from the global burden of disease study 2019. Arthritis Rheumatol 2022; 74(7): 1172-1183.
3. Cieza A, Causey K, Kamenow K, Wulf Hansen S, Chatterji S, Vos T. Global estimates of the need for rehabilitation based on the Global Burden of Disease study 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020 Dec 19; 396(10267): 2006–2017.

5. Scabies

KEY FACTS
- Human scabies is a parasitic infestation caused by Sarcoptes scabiei var hominis
- At least 200 million people worldwide suffer from scabies at any one time
- An estimated 5–50% of children in resource-poor areas are affected by scabies.
- Scabies occurs worldwide but is most common in hot, tropical countries and in areas of high population density.

Overview

Scabies is a parasitic infestation caused by tiny mites that burrow into the skin and lay eggs, causing intense itching and a rash. Scabies can lead to skin sores and serious complications like septicaemia (a bloodstream infection), heart disease and kidney problems. It is treated using creams or oral medications.

Scabies is contagious and spreads through skin-to-skin contact. It occurs worldwide but is most common in low-income tropical areas. Children and older people in resource-poor areas are at higher risk.

Scope of the problem

Scabies is one of the commonest dermatological conditions, accounting for a substantial proportion of skin disease in developing countries. Globally, it is estimated to affect more than 200 million people at any time and more than 400 million people cumulatively every year.

Scabies is found in every country but is particularly common in many resource-poor tropical settings, particularly in children and older people. Prevalence among children in these settings may vary from 5% up to 50%. Recurrent infestations are common. The sheer burden of scabies infestation and its complications imposes a major cost on health care systems. Cases are sporadic in high-income countries, yet outbreaks in health institutions and vulnerable communities contribute to significant economic cost in national health services.

Several studies have shown that outbreaks of scabies are a major risk factor for kidney disease in the form of acute post-streptococcal glomerulonephritis. A growing body of evidence also implicates impetigo caused by *Streptococcus pyogenes* in the pathogenesis of rheumatic fever and rheumatic heart disease.

Symptoms

Symptoms of scabies usually begin 4–6 weeks after infestation. Sometimes there are visible signs before symptoms begin.

Symptoms of scabies include:
- severe itch, often worse at night;
- itchy lines (linear burrows) and bumps (papules) on the fingers, wrists, arms, legs and belt area;
- enflamed bumps on male genitalia and female breasts; and
- larger rash in infants and small children, including on the palms, soles of the feet, ankles and scalp.

Most individuals are infected with 10–15 mites.

People with suppressed immune systems, including people living with HIV, may develop crusted (Norwegian) scabies. This severe infection can have thousands or millions or mites and causes dry, scaley areas on the skin. It often does not cause itch. Crusted scabies spreads very easily and can cause secondary infections. It is life threatening.

Scabies mites burrow into the top layer of skin, where the adult female lays eggs. The eggs hatch in 3–4 days and develop into adult mites in 1–2 weeks. After 4–6 weeks the patient develops an allergic reaction to the presence of mite proteins and faeces in the scabies burrow, causing intense itch and rash.

Mite effects on immunity, as well as the direct effects of scratching, can lead to inoculation of the skin with bacteria, leading to the development of impetigo (skin sores), especially in the tropics. Impetigo may become complicated by deeper skin infection such as abscesses or serious invasive disease, including septicaemia. In tropical settings, scabies-associated skin infection is a common risk factor for kidney disease and possibly rheumatic heart disease. Evidence of acute renal damage can be found in up to 10% of children with scabies infestation in resource-poor settings and,

in many, this persists for years following infection contributing to permanent kidney damage.

Prevention

Treating scabies as soon as possible is the best way to prevent outbreaks. The mites that cause scabies usually die after 2–3 days away from human skin.

Prevent scabies from spreading with these steps:

- avoid skin-to-skin contact with an infested person, especially if they have an itchy rash;
- treat all members of the household if someone has scabies to prevent the mites from spreading to others;
- wash and dry bedding and clothing that has been in contact with the infested person, using hot water and drying in direct sunlight, a hot dryer cycle or dry cleaning;
- seal items that can't be washed in a plastic bag for a week to help eliminate the mites; and
- clean and vacuum or sweep rooms after an infested person has been treated, especially for people with crusted scabies.

Transmission

Scabies is transmitted person-to-person through close skin contact (e.g. living in the same residence) with an infested individual. The risk of transmission increases with the level of infestation, with highest risk due to contact with individuals with crusted scabies. Transmission due to contact with infested personal items (e.g., clothes and bed linens) is unlikely with common scabies but may be important for individuals with crusted scabies. As there is an asymptomatic period of infestation, transmission may occur before the initially infested person develops symptoms.

Diagnosis

Diagnosis of scabies is based on clinical recognition of the typical features of infestation. The diagnosis of scabies can be supported by visual imaging techniques such as dermatoscopy or microscopy of skin scrapings from burrows, but this is generally not necessary, especially in highly endemic areas. Patients typically present with severe itch, linear burrows and papules around the finger webs, wrists, upper and lower limbs, and belt area. Infants and small children may have a more widespread rash, including involvement of the palms, soles of the feet, ankles, and sometimes the scalp. Inflammatory scabies nodules may be seen, particularly on the penis and scrotum of adult males and around the breasts of females. Because of the delay between initial infection and development of symptoms, scabies lesions may be seen in close contacts that have not yet developed itch.

Treatment

Scabies can be treated with topical creams or oral medication in more severe cases. Itchiness often gets worse for 1–2 weeks after treatment starts.

Topical treatments that are applied to the whole body include:

- 5% permethrin cream
- 0.5% malathion in aqueous base
- 10–25% benzyl benzoate emulsion
- 5–10% sulphur ointment.

Ivermectin taken orally is also highly effective, but it should not be taken by pregnant women or children who weigh less than 15 kg.

Treatments do not kill the parasite's eggs, and treatment should be repeated to kill newly hatched mites. People do not experience symptoms in the early stages of infestation. To reduce spread, all people in the household should be treated, even if they do not have symptoms.

Other treatments may be needed to treat the complications of scabies. Antiseptics or antibiotics are used to treat bacterial skin infections or impetigo. Patients with crusted scabies are highly infectious and a source of reinfection to the rest of the community. They need intense treatment with both topical and oral medications.

Disease control

Population control of scabies and its complications has been identified by a number of countries as a public health priority, and several studies have shown that mass drug administration (MDA) strategies have the potential to substantially reduce prevalence of scabies, with concomitant reductions in impetigo. In 2019 WHO convened an informal consultation of global experts to review available data and develop recommendations on strategies for global and country level control. The experts agreed that there is convincing evidence that MDA can be highly effective in places where the prevalence is %10 or greater, but the evidence for its effectiveness in places with lower prevalence is less clear. The current recommendation is that two doses of ivermectin (dose 200μg/kg) should be administered and a topical agent such as permethrin %5 cream should be given when ivermectin is contraindicated or not available. There is ongoing research to determine if one dose of treatment is sufficient for MDA, however currently the evidence is inconclusive. Further research is required to define the strategies to be used when the prevalence is low, either at baseline or when achieved by MDA.

Outbreaks of scabies can occur in either closed, institutional settings (such as hospitals, boarding schools or long-term care facilities) or open community

settings. Refugee or internally displaced person camps are at particularly high risk due to overcrowding which increases skin to skin contact. Outbreaks can be extended and difficult to control. The general principles include surveillance in high-risk settings, early confirmation of an outbreak, and involvement of public health experts.

The WHO informal consultation on a Framework for Scabies Control Meeting Report outlines the key operational research that is still required to develop guidelines for control and surveillance strategy for all contexts. Large scale scabies MDAs are ongoing in PNG, Vanuatu, Fiji and Solomon Islands.

WHO response

In 2017, scabies and other ectoparasites were included as Neglected Tropical Diseases (NTDs) by the WHO, in response to requests from Member States and the recommendations of the WHO Strategic and Technical Advisory Group for NTDs.

WHO 2030 global targets for scabies include:
- countries to incorporate scabies management in the universal health coverage package of care; and
- countries to conduct MDA intervention in endemic areas (areas where prevalence is 10% or greater).

WHO works with Member States and partners to develop control strategies and scabies outbreak response plans. WHO recommends that control strategies for scabies should be part of an integrated skin NTDs approach adapted to the diseases present in a particular country in order to facilitate rapid, cost-effective uptake of the strategy. Ivermectin is now included on the WHO essential medicines list for scabies and a number of suppliers have been WHO prequalified.

REFERENCES

1. WHO informal consultation on a framework for scabies control, meeting report, WHO Regional Office for the Western Pacific, Manila, Philippines, 19–21 February 2019. https://www.who.int/publications/i/item/9789240008069
2. The global burden of scabies: a cross-sectional analysis from the Global Burden of Disease Study 2015. Karimkhani C, Colombara DV, Drucker AM, Norton SA, Hay R, Engelman D, et al. The global burden of scabies: a cross-sectional analysis from the Global Burden of Disease Study 2015. Lancet Infect Dis. 2017;17:1247–54. https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(17)30483-8/fulltext
3. Tsoi SK, Lake SJ, Thean LJ, Matthews A, Sokana O, Kama M, Amaral S, Romani L, Whitfeld M, Francis JR, Vaz Nery S, Marks M, Kaldor JM, Steer AC, Engelman D. Estimation of scabies prevalence using simplified criteria and mapping procedures in three Pacific and southeast Asian countries. BMC Public Health. 2021 Nov 10;21(1):2060. https://pubmed.ncbi.nlm.nih.gov/34758806/
4. Romani L, Whitfeld MJ, Koroivueta J, Kama M, Wand H, Tikoduadua L, et al. Mass Drug Administration for Scabies Control in a Population with Endemic Disease. The New England journal of medicine. 2015;373(24):2305-13. https://www.nejm.org/doi/full/10.1056/nejmoa1500987