

Pancreatic Adenocarcinoma: Epidemiology, Role of EUS in Diagnosis, Role of ERCP, Endoscopic Palliation

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ABSTRACT

Pancreatic cancer is the seventh leading cause of cancer deaths worldwide and is associated with a poor survival rate. The vast majority of pancreatic cancers are inoperable at the time of diagnosis. In the absence of metastatic disease, operability depends on the extent of local disease; in particular, the presence or absence of vascular and lymph node involvement. Adequate staging is vital in deciding an appropriate treatment plan. Cross sectional imaging including CT, MRI and PET-CT are commonly used for staging. However, EUS is a useful adjunct for accurate loco-regional staging in addition to allowing diagnostic tissue samples to be obtained. Emerging EUS-guided therapeutic techniques have opened up new horizons in the management of pancreatic malignancy. EUS guidance can be used for coeliac plexus neurolysis in patients with intractable pain and fiducial placement in directing stereotactic radiotherapy. The majority of patients with cancer of the pancreatic head present with biliary obstruction. ERCP can be used to drain the obstructed biliary system with plastic or metal stents and offers an opportunity to confirm the diagnosis by obtaining brush cytology and forceps biopsy specimens. EUS-guided choledochoduodenostomy or hepatico-gastrostomy is increasingly being employed for draining the biliary system if ERCP is unsuccessful.

KEYWORDS

pancreatic adenocarcinoma; endoscopic ultrasound; ERCP

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1. PANCREATIC CANCER EPIDEMIOLOGY

Pancreatic cancer is considered one of the deadliest cancers and is associated with a poor survival rate. Currently, it is the seventh leading cause of cancer deaths worldwide and the sixth leading cause in the UK (1). The incidence of pancreatic cancer is increasing, with 458,918 new cases diagnosed worldwide and 11,374 new cases in the UK according to the GLOBOCAN 2018 estimates (2).

While the causes of pancreatic cancer are still not completely known, non-modifiable and modifiable risk factors have been identified. Non-modifiable risk factors include familial cancer syndromes, increasing age, diabetes mellitus, and hereditary and other forms of chronic pancreatitis. Significant modifiable risk factors include smoking, obesity, toxins and dietary factors (such as non-vegetarian diet and alcohol) (3). There is also a higher incidence in developed countries and pancreatic cancer is slightly more common in men compared to women.

Pancreatic cancers can arise from the exocrine or neuroendocrine cells of the pancreas. Over 90% of pancreatic cancers are exocrine tumours, of which the vast majority are pancreatic ductal adenocarcinomas (PDACs). Pancreatic neuroendocrine tumours (NETs) make up less than 5% of all cases (4). All tumours are staged using various imaging modalities such as CT, MRI, PET-CT, endoscopic ultrasound (EUS) or laparoscopy. Adequate staging is vital in deciding appropriate treatment plan. The 8th edition of TNM (Tumour, Nodes, Metastases) staging system is currently used for pancreatic cancer, Table 1 (4).

Tab. 1 The 8th edition of TNM (Tumour, Nodes, Metastases) staging system of pancreatic cancer.

T1	Maximum tumor diameter ≤ 2 cm
T2	Maximum tumor diameter > 2 cm and ≤ 4 cm
T3	Maximum tumor diameter > 4 cm
T4	Tumor involves the celiac axis, common hepatic artery or the superior mesenteric artery
N0	No regional lymph node metastasis
N1	Metastasis in 1–3 regional lymph nodes
N2	Metastasis in ≥ 4 cm regional lymph nodes
M0	No distant metastasis
M1	Distant metastasis

The operability of pancreatic cancers depends on the extent of tumour spread locally and to distant organs as well as invasion of nearby vessels.

Patients with early-stage pancreatic cancer are usually clinically well. Symptoms of pancreatic cancer such as abdominal or mid-back pain, obstructive jaundice, and weight loss tend to occur after tumour invasion of surrounding tissue or metastatic spread (5). Consequently, patients tend to present late with advanced disease, with 79% of pancreatic cancer cases in England being diagnosed at Stage 3 or 4 and 21% diagnosed at Stage 1 or 2. This likely contributes to the high mortality rates associated with pancreatic cancer. An analysis of 182 pancreatic cancer patients by Shigehiro et al. found that only 12 (6.6%) patients survived more than 5 years after surgical resection (6).

According to 2010–11 data from Cancer Research UK, the 1-year, 5-year and 10-year survival rates of people diagnosed with pancreatic cancer in England and Wales are 21%, 3% and 1% respectively (1).

In England 2013–14, 10% of patients diagnosed with pancreatic cancer had surgery to remove their primary tumour as part of their primary cancer treatment, including those who had received chemotherapy or radiotherapy. There is a clear link between disease stage at time of diagnosis and whether the patient received surgery, as illustrated in Table 2. Other factors that affect whether a patient receives surgery include the patient's generally health (i.e. comorbidities), age, and patient's own treatment preference (1).

Tab. 2 Pancreatic cancer: Percentage of patients receiving surgery to remove the tumour in the 9 months after diagnosis, male and female, all ages, England 2013–2014 (1).

Stage at diagnosis	Percentage of patients
All stages combined	9.8%
Stage 1	21.2%
Stage 2	53.7%
Stage 3	7.6%
Stage 4	2.2%
Unknown stage	7.8%

2. ROLE OF ENDOSCOPIC ULTRASOUND IN PANCREATIC CANCER

Initial investigations in a patient with suspected pancreatic cancer include ultrasound scan of the abdomen, CT scan and MRI of the pancreas. However, over the last decade endoscopic ultrasound has emerged as an important modality in the diagnosis and management of pancreatic cancer. It not only augments the information obtained through other imaging modalities, but also offers the opportunity of obtaining tissue sample to confirm the diagnosis. In addition, it is emerging as a valuable therapeutic tool in the management of pancreatic cancer.

2.1 EUS AND DIAGNOSTIC STAGING OF PANCREATIC CANCER

Ultrasound is usually the initial diagnostic modality employed in patients who present with biliary obstruction. It can confirm the presence of biliary obstruction by detecting dilatation of intra-hepatic and extra-hepatic bile ducts, however, adequate visualisation of the lower common bile duct and pancreatic parenchyma is usually restricted due to the interference from gas in the gastrointestinal tract (7). A triple phase CT scan is the standard of care for the diagnosis of suspected pancreatic cancer. It is widely available and can detect the primary tumour, assess its local extent and relationship with the vasculature, identify spread to regional lymph nodes, and detect distant metastases (8). In patients who are unable to undergo contrast enhanced CT scanning due to allergy to iodine-based contrast agents, MRI provides an alternative

imaging modality. It has higher sensitivity in identifying small liver metastases compared to CT scans (9). Positron Emission tomography (PET scan) can provide additional information in assessment of metastatic disease in some patients but has limited value in the assessment of the primary lesion (10).

These cross sectional imaging techniques are useful for accurate staging of the tumour, which is important in determining the treatment approach to the disease. Unfortunately, the vast majority of pancreatic cancers (approximately 85%) are inoperable at the time of diagnosis due to the presence of metastatic disease or invasion of major vascular structures. In the absence of metastatic disease, operability depends on the extent of local disease; in particular, the presence or absence of vascular and lymph node involvement. The disease is frequently categorised into three groups, based on imaging results: operable, borderline operable, and locally advanced inoperable disease.

EUS provides high-resolution images of the pancreatic parenchyma as the EUS probe can be placed in close proximity of the lesion through the wall of the stomach or duodenum, depending on the location of the lesion. EUS can provide accurate loco-regional staging and can complement the findings of cross sectional imaging – especially in cases of borderline operable disease or in cases where CT and MRI are unable to detect a mass due to the enhancement pattern of the lesion (11).

EUS probes can either be radial or linear in configuration. Radial scopes provide images in a cross sectional plane similar to CT and MR imaging whereas linear probes provide imaging in a single plane parallel to tip of the scope (12). The endoscopist uses a ‘station approach’ to assess different parts of pancreas. The lesions in the pancreatic body and tail are evaluated from the gastric station whereas lesions in the pancreatic head and uncinate process are visualised through the duodenal stations. High-resolution images of the lesion are obtained using 7.5 MHz frequency (range 5–20 MHz). This enables accurate T-staging of the tumour with a sensitivity and specificity comparable to modern cross sectional imaging (13). Most pancreatic cancers on EUS image appear as hypoechoic heterogeneous lesions with irregular margins. The relationship of lesions to the coeliac trunk, portal vein confluence and superior mesenteric vein and artery is carefully assessed to determine operability of the lesion.

Ancillary techniques can be used to improve characterisation of the lesion with EUS, including elastography and contrast enhanced imaging. Elastography involves assessment of firmness or elasticity of a target lesion compared to the surrounding normal tissue. The elastography data can be displayed qualitatively as a colour overlay on the standard B mode image. Pancreatic cancers are firm lesions appearing as blue while inflammatory lesions appear as green-yellow and soft lesions as red. Therefore, elastography could be a helpful tool in a patient with chronic pancreatitis where the difference between inflammatory and cancerous components can be really difficult. Alternatively, a quantitative scale called strain ratio can be obtained by selecting a target area compared to a normal tissue at the same level, which

is displayed as a numeric value. Contrast enhanced EUS involves using a specific intravenous ultrasound agent comprising of microbubbles, which can be detected flowing through the microvasculature of the lesion (14). The pancreatic cancers appear as hypochoic areas as they are usually hypovascular in nature (15). The role of these ancillary techniques in clinical practice remains investigational at present but experts believe that these can help in differentiating between different types of pancreatic lesions and enable targeted biopsies in difficult diagnostic situations.

Limited depth of penetration of the ultrasound waves prevents accurate assessment of structures that are located more than 5 or 6cm from the EUS probe. EUS therefore has limited role, if any, in the assessment of distant lymph nodes or metastatic disease. In cases where the anatomy is distorted or surgically altered (e.g. the presence of biliary stents), it may not be possible to obtain optimal imaging. Similarly, in patients with chronic pancreatitis, presence of calcification can significantly limit the image quality (15).

The information obtained from EUS should be considered complimentary to other imaging modalities in establishing accurate staging of pancreatic cancer.

2.2 EUS GUIDED TISSUE DIAGNOSIS

The main advantage of EUS lies in the ability to obtain tissue samples for establishing the diagnosis. This has become possible through linear EUS scopes with large accessory channels. Specifically, designed needles are passed through the scope channel, which exit near the tip of the scope along the plane of linear EUS probe. Thus, allowing passage of the sampling needle through the wall of the stomach or duodenum under direct ultrasound guidance into the target lesion while avoiding any vascular structure. The sample can be either a cytology specimen obtained through a hollow needle or a fine core of tissue acquired through specially designed needles. The former is called fine needle aspiration (FNA) and latter fine needle biopsy (FNB). Cytology specimens are either directly smeared on the slides or placed in saline or other preservative solution for spinning in order to obtain tissue blocks. Biopsy cores on the other hand are placed into formalin for fixation. Pro-core biopsy provides histological tissue assessment, including cellular and connective tissue analysis, to obtain unequivocal evidence of invasive disease. It also allows immuno-histochemical staining, which can provide important information in confirming diagnosis (11, 16, 17).

Tissue sampling is useful in cases where a diagnosis of pancreatic cancer is not clear on cross sectional imaging before embarking on major surgery. It is also considered mandatory by most institutions to obtain cytological or histological confirmation of malignancy in patients with borderline operable and inoperable tumours, prior to commencing neo-adjuvant or palliative chemotherapy respectively. In the era of targeted chemotherapy, it is envisaged that the need for tissue sampling is likely to increase and may be required at multiple points during the evolution of disease.

2.3 EUS GUIDED INTERVENTIONS IN PANCREATIC CANCER

Emerging EUS-guided therapeutic techniques have opened up new horizons in the management of pancreato-biliary diseases. Patients with advanced pancreatic carcinoma with intractable pain unresponsive to analgesics, caused by infiltration of the coeliac plexus, can benefit from EUS-guided coeliac ganglion or coeliac plexus neurolysis. This involves EUS guided injection of absolute alcohol diluted with local anaesthetic agent bupivacaine either directly into coeliac ganglia or around the coeliac plexus nerves using dedicated needles (18).

EUS FNA needles have been used to place fiducials in the pancreatic tumour or local lymph nodes to guide stereotactic radiotherapy (19). Dedicated needles with pre-loaded fiducials are now available from some manufacturers and allow placement of 3 or 4 fiducials without the need to remove the needle assembly for reloading (20), thus greatly reducing the time required for the procedure. EUS allows precise placement of the fiducials under EUS guidance even in very small lesions (21).

EUS can also be used to drain the biliary system in patients when ERCP is unsuccessful due to an impassable biliary stricture, altered anatomy due to previous surgery or development of duodenal obstruction by tumour infiltration. EUS guided choledocho-duodenostomy involves puncturing the CBD above the stricture from the duodenal bulb with the EUS needle and inserting a fully covered metal biliary stent over a guide-wire under fluoroscopy (22). Alternatively, the biliary system can be drained after performing hepatico-gastrostomy by puncturing the dilated radicles of the left system in segment 2 or 3 of the liver. A guide-wire is then advanced into the CBD and across the stricture into the duodenum, allowing the antegrade placement of a stent, in a fashion similar to percutaneous trans-hepatic cholangiography (23). If the wire cannot be passed through the stricture, a specially designed stent can be placed across the gastric puncture site. The distal end of stent, which is uncovered, extends into the bile duct above the level of obstruction while the proximal covered end is left in the gastric lumen, allowing the retrograde drainage of bile. The covered gastric end of the stent prevents bile leaking into the peritoneum whereas the distal uncovered end helps anchoring the stent and allows drainage of side branches of the biliary system. Recent design change has incorporated a 70% covered portion on the gastric end (24, 26). These techniques require dilatation of the tract with a 5–6 Fr catheter, which can deliver electrocautery through its tip such as a cystgastrostomy needle. New stent designs have incorporated electrocautery rings on the tip of stent delivery system, which allows entire procedure to be completed with a single puncture under image guidance. More recently, lumen-apposing stents (LAMS) have been specifically designed to appose the lumen of the GI tract with another organ or cavity, such as dilated bile duct, pancreatic fluid collection or distended gallbladder. It can also be used to create a gastro-jejunostomy to bypass duodenal obstruction in advanced pancreatic malignancy (25).

Radiofrequency probes have been designed to induce thermal necrosis of focal pancreatic lesions such as small

NETs and pancreatic cancers in patients who are not surgical candidates due to co-morbidities. Investigators have looked at the option of delivering EUS-guided chemotherapeutic agents, immunoreactive drugs or oncolytic viruses directly into pancreatic tumours or inducing vascular necrosis by occluding feeding vessels to the tumour as treatment options (27).

3. ERCP IN PANCREATIC CANCER

Symptoms of biliary obstruction such as jaundice, pruritis, pale stools and dark urine are the presenting symptom complex in the majority of patients with lesions arising in the head of pancreas. ERCP offers both a diagnostic and therapeutic role in such patients. In contrast, patients with lesions arising in the body or tail of the pancreas usually do not present with biliary obstruction until they metastasise to the liver hilum. In these cases, biliary obstruction is a late symptom usually caused by obstruction from hilar lymph node enlargement or central metastases to the liver (28).

3.1 THE DIAGNOSTIC ROLE OF ERCP IN PANCREATIC CANCER

ERCP drainage of the biliary system provides an opportunity to confirm the diagnosis by a combination of biliary brush cytology and forceps biopsy specimens. These techniques yield a positive diagnostic sample in about one third of the patients (30). Additional techniques such as aspiration of bile, balloon dilatation of stricture or needle aspiration do not increase the diagnostic yield in a significant way. If a plastic stent was previously placed, cytological sample can be obtained from it. While forceps biopsies have a greater yield, they are more invasive and are associated with a greater risk of complications (i.e. bleeding, pancreatitis, perforation) compared to biliary brush cytology. Despite its low yield, ERCP tissue sampling plays a useful diagnostic role in patients who require biliary drainage at presentation. If ERCP does not yield a positive sample, diagnosis can be confirmed through EUS guided tissue acquisition, which has a significantly superior sensitivity (31).

3.2 THE ROLE OF ERCP IN BILIARY DRAINAGE

Many centres advocate proceeding to surgery without draining the biliary system due to the concern that it can potentially delay definitive surgery, particularly if the patient develops a complication such as pancreatitis, perforation or sepsis secondary to instrumentation of a blocked biliary system. Other centres favour pre-operative biliary drainage to reduce the risk of postoperative complications such as coagulopathy, sepsis and renal failure (33). Most agree that patients should undergo biliary drainage in the setting of biliary sepsis, severe symptoms such as intractable pruritis or if delay in surgery is contemplated. Patients with borderline operable tumours or those with advanced disease invariably require biliary drainage prior to commencing chemotherapy (29).

The preferred method for biliary drainage is endoscopic stent placement as it is associated with fewer complications compared to percutaneous biliary drainage. The latter is generally reserved for cases where ERCP is unsuccessful or is not possible due to difficult anatomy (34). Biliary drainage can be achieved with either plastic or metal biliary stents. Although plastic stents are significantly cheaper, they tend to block within 10–12 weeks whereas metal biliary stents remain patent for longer (35, 36). Stent occlusion can cause sepsis requiring repeat procedures which can result in interruption of chemotherapy and delay in definitive surgical treatment (37).

In general, if the imaging favours a malignant process, a metal stent should be placed, as it does not interfere with

subsequent surgery, as long as the shortest possible stent is used. However, artefact from metal stents can impair visualisation during EUS and make it difficult to perform targeted needle aspiration especially in small lesions. In view of this, operators prefer performing EUS before stent insertion. However, this is not always possible due to limited availability of EUS as compared to ERCP, which is more widely practiced. Ideally, tumour staging and tissue acquisition by EUS should be followed immediately by either ERCP or EUS guided biliary drainage within the same session, if such a facility is available. If a diagnosis of malignancy is less certain, initial drainage of biliary obstruction with plastic stent is a reasonable option, which can be changed to a metal stent at a later date.

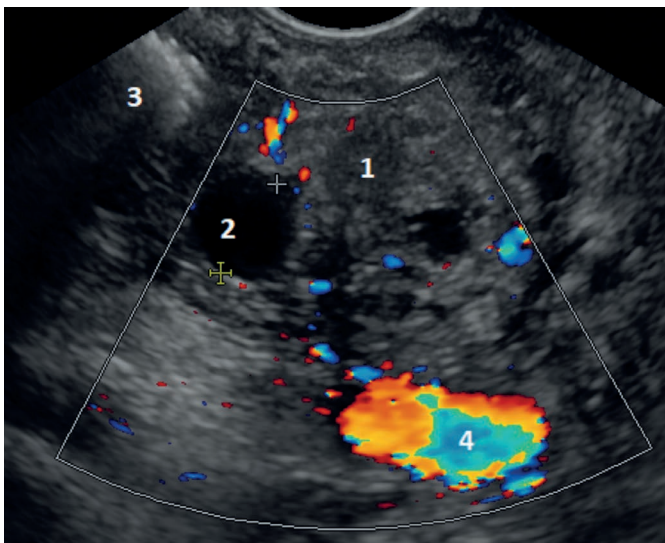


Fig. 1 Pancreatic adenocarcinoma of the uncinus process. 1 – carcinoma; 2 – dilated pancreatic duct; 3 – metal stent in the common bile duct (causing artefacts); 4 – superior mesenteric vein.

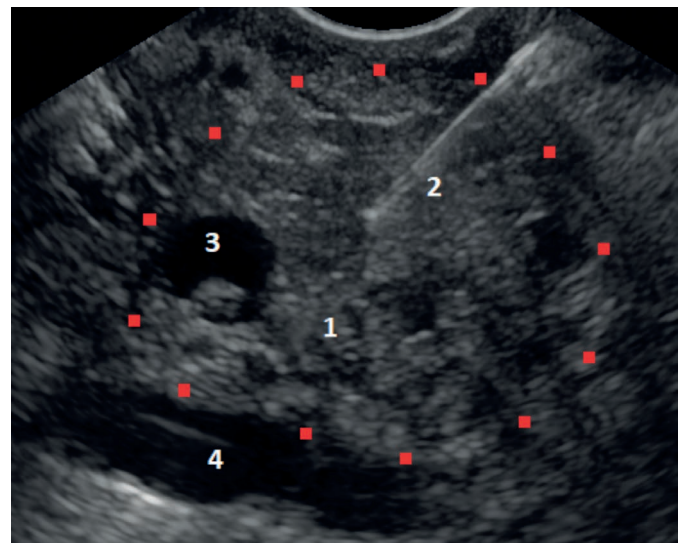


Fig. 2 Pancreatic adenocarcinoma of the uncinus process. 1 – carcinoma; 2 – FNB (fine needle biopsy) needle; 3 – dilated pancreatic duct with tissue inside; 4 – superior mesenteric vein.



Fig. 3 Pancreatic adenocarcinoma of the uncinus process causing dilatation of the pancreatic duct to 6.3 mm in the neck of the pancreas.

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Association of IL-6 -174 G>C Polymorphism with Susceptibility to Colorectal Cancer and Gastric Cancer: a Systematic Review and Meta-Analysis

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ABSTRACT

Background: The -174G>C (rs1800795) polymorphism at interleukin 6 (IL-6) gene has been reported to be related with the occurrence of colorectal (CRC) and gastric (GC) cancers. However, the results had been conflicting and controversial. In order to give a comprehensive and precise result, we summarized available data to analyze the association of this polymorphism with CRC and GC risk.

Methods: A comprehensive literature search on PubMed, Elsevier Science Direct, and CNKI database was performed to identify all eligible studies up to May 15, 2019. The strength of association was assessed by odds ratios (ORs) with 95% confidence intervals (CI).

Results: A total of 29 case-control studies including 16 studies with 7,560 cases and 9,574 controls on CRC and 13 studies with 1,445 cases and 2,918 controls on GC were selected. Overall, pooled data showed that the IL-6 -174G>C polymorphism was not significantly associated with increased risk of CRC and GC in overall. When stratified by ethnicity, we found a statistically significant association between the IL-6 -174 G>C polymorphism and CRC risk in Asians (CC vs. GG: OR = 1.860, 95% CI 1.061–3.258, $p = 0.030$; and CC vs. CG+GG: OR = 1.941, 95% CI 1.131–3.331, $p = 0.016$).

Conclusion: The meta-analysis suggests that IL-6 -174G>C polymorphism was not significantly associated with the increased risk of CRC and GC in overall population. However, the results showed that IL-6 -174G>C polymorphism may be associated with risk of GC in Asians. Further studies including a larger sample size will be necessary to clarify these results.

KEYWORDS

colorectal cancer; gastric cancer; interleukin 6; association; meta-analysis

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INTRODUCTION

Digestive system cancers especially colorectal cancer (CRC) and Gastric cancer (GC) are the most common causes of cancer-related death worldwide (1-3). CRC and GC were the fourth and second most common causes of cancer-related mortality worldwide in 2016, respectively (4, 5). The exact mechanism of CRC and GC is still not fully understood. However, CRC and GC are multifactorial and multistep diseases caused by complex interactions between environmental triggers and genetic factors (6, 7). To date, a wide range of gastrointestinal cancer susceptibility gene variations have been evaluated. Interleukin 6 (IL-6) gene promoter region polymorphisms have already been correlated to increased risks of developing CRC and GC (8, 9).

IL-6 is a pleiotropic cytokine with a wide range of biological activities in immune regulation, hematopoiesis, inflammation and oncogenesis. IL-6 is implicated in a wide variety of inflammation-associated disease states, such as diabetes mellitus, systemic juvenile rheumatoid arthritis and malignant diseases. The human IL-6 gene is mapped to chromosome 7p21-24, with an upstream promoter containing 303 bp, contains five exons and spans approximately 6.2 kb (10). Accumulating evidence indicates pathological roles for IL-6 in different malignancies, such as breast, vulvar, ovarian, hepatocellular, lung, gastric and colorectal cancer (11).

To date, several polymorphisms in the promoter region of the IL-6 gene including -598A>G, -597G>A, -572 C>G, and -174 G>C have been identified and are implicated in the increased level of IL-6. Of these polymorphisms, -174 G>C (rs1800795) is the most studied functional polymorphism in different malignancies. IL-6 -174 G>C is demonstrated to impact the adherence of the glucocorticoid receptor and then results in repressive transcriptional activation. Lots of studies have reported the role of IL-6 -174 polymorphism in the predisposition to CRC and GC. However, these studies results are inconclusive and also inconsistent. This may be because of inadequate sample sizes, patient selection, genotyping methods, and ethnicity of the populations studied. Moreover, an individual study may be insufficient to evaluate the potential small effect of the IL-6 -174 G>C polymorphism on risk of CRC and GC. Therefore, we performed a meta-analysis of all available eligible case-control studies to verify the precise association of the IL-6 -174 G>C (rs1800795) polymorphism with CRC and GC risk.

MATERIAL AND METHODS

LITERATURE COLLECTION AND SCREENING

A flow-diagram outlining the identification, screening, eligibility, and final datasets was constructed according to Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2009 guidelines. To identify all articles that evaluated the association of IL-6 -174 G>C polymorphism with CRC and GC risk, we performed a comprehensive literature search of the PubMed, EMBASE, Elsevier Science Direct, Google scholar, Chinese Bi-

omedical Literature database, China National Knowledge Infrastructure database (CNKI), and Wanfang database up to May 15, 2019. The following keywords and terms were used: ("colorectal cancer" OR "CRC" OR "bowel cancer" OR "colon cancer") AND ("gastric cancer" OR "GC" OR "stomach cancer") AND (Interleukin 6 OR IL-6 OR "-174G>C" OR "rs1800795") AND ("gene" OR "polymorphism" OR "mutation" OR "variation"). In addition, reference list of obtained literatures was reviewed to ensure that no relevant studies were missed.

INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria for the present study were as follows: 1) published case-control or cohort studies; 2) studies evaluated the association of IL-6 -174 G>C polymorphism with CRC and GC; 3) studies with sufficient data to calculate the odds ratio (OR) and 95% confidence interval (CI). Accordingly, the following exclusion criteria were used: 1) abstracts, posters, case reports, reviews, and letter to editors; 2) case only studies, sibling or linkage studies; 3) the study reported duplicated data or containing overlapping data.

DATA EXTRACTION

The data from the published studies were extracted independently by two of the authors, and the disagreement was resolved by a discussion involving a senior author. For each study, the following data were collected: first author's name, year of publication, country, ethnicity (Caucasian, Asian, African and others), sources of healthy controls, number of cases and controls, genotyping methods, allele numbers and genotype distributions in cases and controls, minor allele frequencies (MAFs) in control subjects, and the results of Hardy-Weinberg equilibrium (HWE) test.

STATISTICAL ANALYSIS

An ethical approval was not necessary as this study was a meta-analysis based on previous studies. The association of IL-6 -174 G>C polymorphism with CRC and GC risk was measured by ORs and its corresponding 95% CIs. The estimates of pooled ORs were obtained by calculating a weighted average of OR from each study and the significance of pooled ORs was determined by the Z-test. In this meta-analysis, the pooled ORs for IL-6 -174 G>C polymorphism was calculated under five genetic models, i.e., allele (C vs. G), homozygote (CC vs. GG), heterozygote (CG vs. GG), dominant (CC+CG vs. GG) and recessive (CC vs. CG+GG). Between-studies heterogeneity was assessed by a Chi-squared Q-test and I^2 statistics ($P < 0.05$). Additionally, the I^2 -value was applied to quantitatively evaluate the heterogeneity ($I^2 < 25\%$, low heterogeneity; $25\% \leq I^2 \leq 75\%$, moderate heterogeneity; $I^2 > 75\%$, high heterogeneity). The fixed-effects model (the Mantel-Haenszel method) and the random-effects model (the DerSimonian-Laird method) were utilized to pool ORs. Sensitivity analysis was used by omitting individual studies each time to assess the stability of the pooled results. The Hardy-Weinberg equi-

librium (HWE) test for each study was performed using chi-square test), and $P < 0.05$ was considered to indicate significant disequilibrium. We carried out subgroup analysis by cancer type, ethnicity, genotyping methods, source of controls and HWE (fall in HWE). Begg's funnel plot and Egger's test were used to evaluate the publication bias in the meta-analysis, in which $P < 0.05$ indicated that the result was statistically significant. All statistical analyses were performed using the comprehensive meta-analysis (CMA) software (version 2.0, Biostat, USA). Two-sided $P < 0.05$ was considered statistically significant.

RESULTS

LITERATURE SELECTION AND STUDY CHARACTERISTICS

A total of 118 articles were identified through the initial search in the database and by hand searching. As shown in Figure 1, after carefully screening the title and abstracts of the initial publications, 20 studies were promptly excluded. Consequently, 29 case-control studies were included in this meta-analysis. The characteristics of each study are summarized in Table 1. Of those studies, 16 studies with

Tab. 1 Main characteristics of studies included in this meta-analysis.

First Author	Country (Ethnicity)	Genotyping Method	SOC	Case/Control	Cases					Controls					MAFs	HWE
					Genotypes			Allele		Genotypes			Allele			
Colorectal Cancer					GG	GC	CC	G	C	GG	GC	CC	G	C		
Landi 2003 (12)	Spain (Caucasian)	TaqMan	PB	361/311	133	180	48	446	276	145	133	33	423	199	0.319	0.761
Theodoropoulos 2006 (13)	Greece (Caucasian)	PCR-RFLP	NS	222/200	111	76	35	298	146	64	86	50	214	186	0.465	0.054
Gunter 2006 (14)	USA (Caucasian)	TaqMan	HB	204/190	79	90	35	248	160	83	81	26	247	133	0.350	0.384
Gaustadnes 2006 (15)	Denmark (Caucasian)	CE	PB	230/540	64	115	51	243	217	184	263	93	631	449	0.415	0.952
Slattery 2007 (16)	USA (Caucasian)	TaqMan	HB	777/995	321	347	109	989	565	411	438	146	1260	730	0.366	0.098
Vogel 2007 (17)	Denmark (Caucasian)	Probe	HB	355/753	98	168	89	364	346	204	364	185	772	734	0.487	0.371
Wilkening 2008 (18)	Sweden (Caucasian)	TaqMan	HB	303/580	79	163	61	321	285	162	297	121	621	539	0.464	0.480
Kury 2008 (19)	France (Caucasian)	TaqMan	PB	1023/1121	363	489	171	1215	831	435	504	182	1374	868	0.387	0.078
Slattery 2009 (20)	USA (Caucasian)	TaqMan	HB	1573/1972	631	696	246	1958	1188	728	897	347	2353	1591	0.403	0.014
Tsilidis 2009 (21)	USA (Caucasian)	TaqMan	HB	200/354	68	93	39	229	171	113	170	71	396	312	0.440	0.626
Vasku 2009 (22)	Czech (Caucasian)	PCR-RFLP	HB	100/100	32	46	22	110	90	31	47	22	109	91	0.455	0.600
Ognjanovic 2010 (23)	USA (Caucasian)	TaqMan	PB	117/221	71	46	0	188	46	103	118	0	324	118	0.267	≤0.001
Cacev 2010 (24)	Croatia (Caucasian)	PCR-RFLP	HB	160/160	64	70	26	198	122	68	75	17	211	109	0.340	0.581
Abuli 2010 (25)	Spain (Caucasian)	TaqMan	HB	1405/1388	586	635	184	1807	1003	593	623	172	1809	967	0.348	0.672
Basavaraju 2015 (26)	Scotland (Caucasian)	TaqMan	HB	388/495	140	184	64	464	312	172	245	78	589	401	0.405	0.549
Banday 2017 (27)	Kashmiri (Asian)	PCR-RFLP	PHB	142/194	85	43	14	213	71	145	46	3	316	52	0.134	0.764
Gastric Cancer																
El-Omar 2003 (28)	USA (Caucasian)	TaqMan	PB	213/209	88	91	43	267	177	83	98	28	264	154	0.368	0.912
Hwang 2003 (29)	USA (Caucasian)	PCR-RFLP	HB	30/30	19	9	2	37	13	22	8	0	52	8	0.133	0.399
Hwang 2003	USA (Asian)	PCR-RFLP	HB	30/30	30	0	0	60	0	30	0	0	60	0	0.000	NA
Kamangar 2006 (30)	Finland (Caucasian)	TaqMan	PB	102/152	21	54	27	96	108	51	58	43	160	144	0.473	0.003
Xing 2006 (31)	China (Asian)	PCR-RFLP	PB	65/71	62	3	0	127	3	67	4	0	138	4	0.028	0.807

Deans 2007 (32)	UK (Caucasian)	TaqMan	HB	197/224	71	83	43	225	169	79	101	44	259	189	0.421	0.257
Gatti 2007 (33)	Brazil (Mixed)	PCR-RFLP	HB	56/112	42	13	1	97	15	48	53	11	73	39	0.334	0.509
Crusius 2008 (34)	France (Caucasian)	PCR-RFLP	PB	243/1138	78	122	43	278	208	415	517	206	1347	929	0.408	0.044
Zhao 2010 (35)	China (Asian)	PCR-RFLP	NS	142/200	105	37	0	247	37	198	2	0	398	2	0.005	0.943
Pohjanen 2013 (36)	Finland (Caucasian)	PCR-RFLP	PB	56/179	14	34	8	62	50	37	86	56	160	198	0.553	0.706
Cao 2014 (37)	China (Asian)	NS	NS	162/162	72	62	28	206	188	87	59	16	233	91	0.280	0.210
Sampaio 2015 (38)	Portugal (Caucasian)	SSP-PCR	NS	50/50	17	25	8	59	41	19	25	6	63	37	0.370	0.608
Attar 2017 (39)	Iran (Asian)	SSP-PCR	HB	100/361	60	30	7	150	47	161	187	13	509	213	0.295	≤0.001

PCR: Polymerase Chain Reaction Restriction; PCR-RFLP: Polymerase Chain Reaction Restriction Fragment Length Polymorphism; CE: Primer extension and capillary electrophoresis; HB: Hospital Based; PB: Population Based; NS: Not stated; MAFs: Minor Allele Frequencies; HWE: Hardy-Weinberg Equilibrium; NA: Not Applicable.

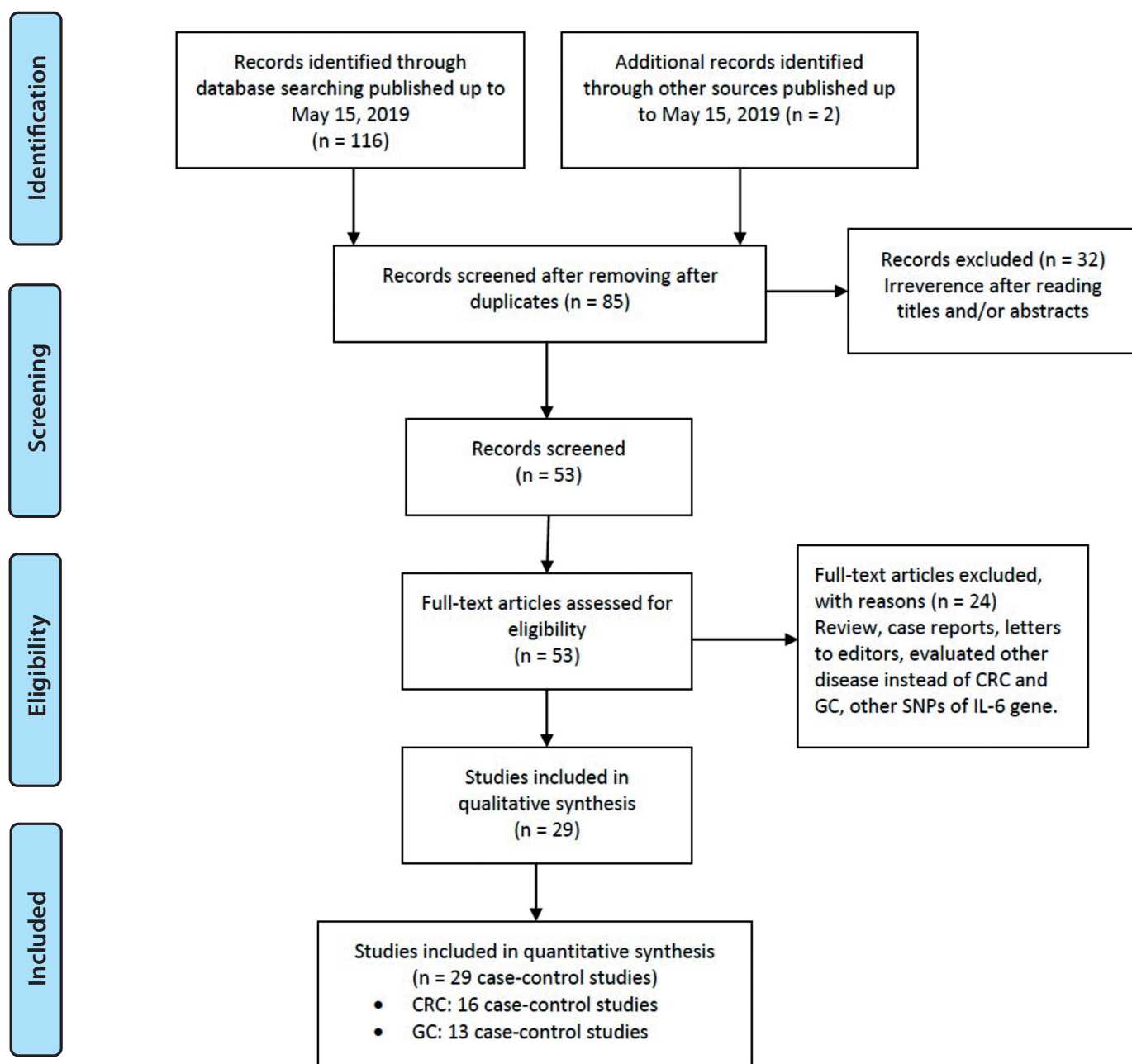


Fig. 1 The study selection and inclusion process.

7,560 cases and 9,574 controls were on CRC (12–27), and 13 studies with 1,445 cases and 2,918 controls were on GC (28–39). These included studies were published between 2003 and 2017. Twenty-two studies populations come from Caucasians, six studies were Asians, and only one from mixed populations. The genotype and allele distributions of the IL-6 -174 G>C polymorphism were shown in Table 1. Genotype distributions in the controls of all studies were in HWE except for five studies (Table 1).

QUANTITATIVE SYNTHESIS

IL-6 -174G>C Polymorphism and CRC

Table 2 shows the results of the association between IL-6 -174 G>C polymorphism and CRC risk. Overall, the pooled data showed that the IL-6 -174 G>C polymorphism was not significantly associated with an increased risk of CRC under all five genetic models, i.e., allele (C vs. G: OR = 1.028, 95% CI 0.936–1.128, p = 0.566), homozygote (CC vs.

GG: OR = 1.068, 95% CI 0.902–1.263, p = 0.447), heterozygote (CG vs. GG: OR = 1.007, 95% CI 0.902–1.124, p = 0.904), dominant (CC+CG vs. GG: OR = 1.021, 95% CI 0.903–1.155, p = 0.737, Figure 2A), and recessive (CC vs. CG+GG: OR = 1.037, 95% CI 0.917–1.171, p = 0.564). When further analyzed by genotyping methods, we have not found a significant association between IL-6 -174 G>C polymorphism and CRC. However, subgroup analysis by source of controls showed a significant association between IL-6 -174 G>C polymorphism and risk of CRC under the homozygote model (CC vs. GG: OR = 1.273, 95% CI 1.042–1.556, p = 0.018) in population-based (PB) group studies.

IL-6 -174G>C Polymorphism and GC

Table 3 shows the results of the association between IL-6 -174 G>C polymorphism and GC risk. Overall, the pooled data showed that there was no significant association between IL-6 -174 G>C polymorphism and increased risk of GC under all five genetic models, i.e., allele (C vs. G:

Tab. 2 Meta-analysis results of association of IL-6 -174 G>C polymorphism with CRC risk.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I ² (%)	P _H	OR	95% CI	Z _{test}	P _{OR}	P _{Begg}	P _{Egger}
Overall	C vs. G	Random	72.34	≤0.001	1.028	0.936–1.128	0.574	0.566	0.558	0.491
	CC vs. GG	Random	62.56	0.001	1.068	0.902–1.263	0.760	0.447	0.198	0.110
	CG vs. GG	Random	53.39	0.006	1.007	0.902–1.124	0.121	0.904	1.000	0.916
	CC+CG vs. GG	Random	67.49	≤0.001	1.021	0.903–1.155	0.336	0.737	0.964	0.781
	CC vs. CG+GG	Random	42.66	0.041	1.037	0.917–1.171	0.576	0.564	0.113	0.044
Genotyping Method										
TaqMan	C vs. G	Random	51.33	0.030	1.012	0.937–1.093	0.299	0.765	1.000	0.696
	CC vs. GG	Fixed	21.34	0.253	1.002	0.904–1.110	0.031	0.975	0.251	0.095
	CG vs. GG	Random	49.53	0.037	1.017	0.908–1.139	0.295	0.768	1.000	0.969
	CC+CG vs. GG	Random	56.96	0.013	1.018	0.907–1.143	0.303	0.762	0.858	0.840
	CC vs. CG+GG	Fixed	0.00	0.746	0.988	0.900–1.086	-0.243	0.808	0.175	0.055
PCR-RFLP	C vs. G	Random	90.52	≤0.001	1.081	0.619–1.886	0.273	0.785	0.308	0.196
	CC vs. GG	Random	86.82	≤0.001	1.345	0.472–3.829	0.555	0.579	0.308	0.072
	CG vs. GG	Random	74.52	0.008	0.919	0.558–1.512	-0.333	0.739	0.734	0.603
	CC+CG vs. GG	Random	86.40	≤0.001	0.991	0.525–1.871	-0.028	0.978	0.734	0.565
	CC vs. CG+GG	Random	81.96	0.001	1.374	0.602–3.137	0.754	0.451	0.308	0.055
Source of Controls										
HB	C vs. G	Fixed	0.00	0.548	0.983	0.934–1.036	-0.636	0.525	0.474	0.102
	CC vs. GG	Fixed	0.00	0.572	0.971	0.872–1.080	-0.548	0.584	0.210	0.053
	CG vs. GG	Fixed	0.00	0.934	0.975	0.902–1.055	-0.621	0.534	0.591	0.503
	CC+CG vs. GG	Fixed	0.00	0.774	0.975	0.906–1.050	-0.668	0.504	0.720	0.241
	CC vs. CG+GG	Fixed	0.00	0.738	0.984	0.893–1.085	-0.320	0.749	0.107	0.032
PB	C vs. G	Random	70.12	0.018	1.097	0.899–1.338	0.911	0.362	1.000	0.721
	CC vs. GG	Fixed	21.43	0.280	1.273	1.042–1.556	2.364	0.018	1.000	0.096
	CG vs. GG	Random	74.65	0.008	1.089	0.796–1.490	0.532	0.595	0.734	0.602
	CC+CG vs. GG	Random	76.71	0.005	1.111	0.812–1.519	0.657	0.511	0.734	0.666
	CC vs. CG+GG	Fixed	0.00	0.400	1.139	0.950–1.365	1.407	0.159	1.000	0.267

CRC: colorectal cancer; PCR-RFLP: Polymerase Chain Reaction Restriction Fragment Length Polymorphism; HB: Hospital Based; PB: Population Based.

OR = 1.282, 95% CI 0.927-1.774, p = 0.134), homozygote (CC vs. GG: OR = 1.209, 95% CI 0.967-1.512, p = 0.096), heterozygote (CG vs. GG: OR = 1.097, 95% CI 0.736-1.634, p = 0.649), dominant (CC+CG vs. GG: OR = 1.117, 95% CI 0.759-1.644, p = 0.573), and recessive (CC vs. CG+GG: OR = 1.115, 95% CI 0.803-1.548, p = 0.517, Figure 2B). When further analyz-

ed by ethnicity, we found a statistically significant association between IL-6 -174 G>C polymorphism and CRC risk under two genetic models i.e., homozygote (CC vs. GG: OR = 1.860, 95% CI 1.061-3.258, p = 0.030) and recessive (CC vs. CG+GG: OR = 1.941, 95% CI 1.131-3.331, p = 0.016) in Asians, but not in Caucasians.

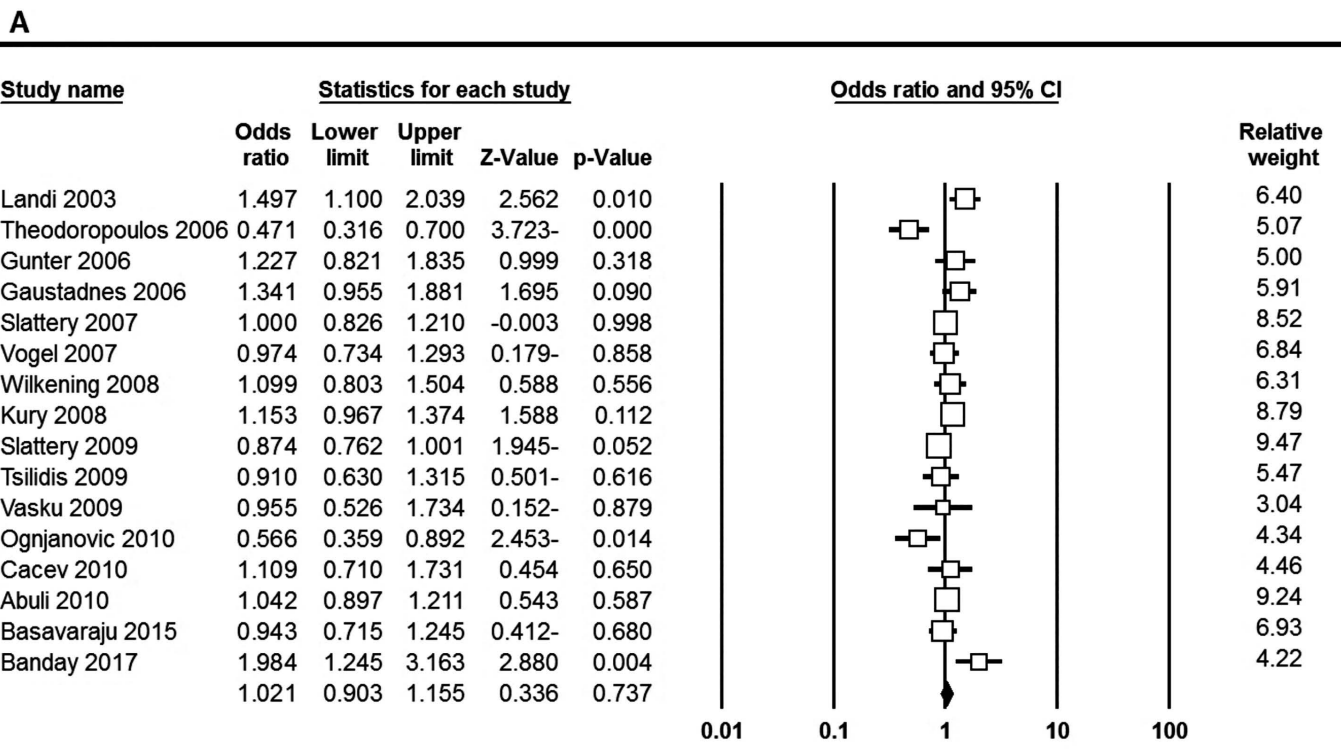


Fig. 2 Forest plot for association of IL-6 -174 G>C polymorphism with CRC and GC risk in random-effects model. A: CRC (dominant model: CC+CG vs. GG); B: GC (recessive model: CC vs. CG+GG).

Tab. 3 Meta-analysis results of association of IL-6 -174 G>C polymorphism with GC risk.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I ² (%)	P _H	OR	95% CI	Z _{test}	P _{OR}	P _{Begg}	P _{Egger}
Overall	C vs. G	Random	86.50	≤0.001	1.282	0.927-1.774	1.499	0.134	0.731	0.561
	CC vs. GG	Random	43.05	0.071	1.209	0.967-1.512	1.666	0.096	0.858	0.646
	CG vs. GG	Random	80.63	≤0.001	1.097	0.736-1.634	0.456	0.649	0.631	0.527
	CC+CG vs. GG	Random	81.53	≤0.001	1.117	0.759-1.644	0.563	0.573	1.000	0.653
	CC vs. CG+GG	Random	52.05	0.027	1.115	0.803-1.548	0.649	0.517	1.000	0.956
Ethnicity										
Caucasian	C vs. G	Fixed	25.45	0.235	1.084	0.961-1.222	1.319	0.187	0.548	0.862
	CC vs. GG	Fixed	21.99	0.262	1.152	0.901-1.472	1.131	0.258	0.548	0.816
	CG vs. GG	Fixed	20.91	0.270	1.137	0.941-1.373	1.328	0.184	0.548	0.688
	CC+CG vs. GG	Fixed	0.00	0.466	1.182	0.988-1.413	1.832	0.067	1.000	0.833
	CC vs. CG+GG	Fixed	45.88	0.086	1.040	0.837-1.291	0.352	0.881	0.763	0.859
Asian	C vs. G	Random	94.58	≤0.001	2.618	0.739-9.277	1.491	0.136	0.734	0.797
	CC vs. GG	Fixed	0.00	0.529	1.860	1.061-3.258	2.168	0.030	NA	NA
	CG vs. GG	Random	91.58	≤0.001	1.752	0.471-6.517	0.837	0.403	1.000	0.421
	CC+CG vs. GG	Random	91.78	≤0.001	1.845	0.522-6.525	0.951	0.342	1.000	0.468
	CC vs. CG+GG	Fixed	0.00	0.925	1.941	1.131-3.331	2.407	0.016	NA	NA
Genotyping Method										
TaqMan	C vs. G	Fixed	0.00	0.604	1.148	0.968-1.362	1.584	0.113	1.000	0.616

	CC vs. GG	Fixed	0.00	0.677	1.304	0.931-1.828	1.542	0.123	0.296	0.475
	CG vs. GG	Random	70.75	0.033	1.159	0.692-1.940	0.559	0.576	0.296	0.032
	CC+CG vs. GG	Fixed	47.78	0.147	1.169	0.908-1.505	1.213	0.225	1.000	0.194
	CC vs. CG+GG	Fixed	13.27	0.316	1.207	0.896-1.624	1.239	0.215	1.000	0.821
PCR-RFLP	C vs. G	Random	82.35	≤0.001	1.327	0.728-2.420	0.925	0.355	0.452	0.517
	CC vs. GG	Random	68.86	0.022	0.616	0.206-1.844	-0.866	0.386	0.734	0.572
	CG vs. GG	Random	86.24	≤0.001	1.374	0.566-3.337	0.701	0.483	1.000	0.766
	CC+CG vs. GG	Random	87.81	≤0.001	1.321	0.531-3.289	0.598	0.550	1.000	0.817
	CC vs. CG+GG	Random	63.96	0.040	0.618	0.250-1.531	-1.039	0.299	1.000	0.657
Source of Controls										
HB	C vs. G	Fixed	32.59	0.217	0.919	0.750-1.125	-0.820	0.412	0.734	0.837
	CC vs. GG	Fixed	52.39	0.098	1.075	0.687-1.682	0.315	0.753	0.734	0.825
	CG vs. GG	Random	73.58	0.010	0.585	0.317-1.080	-1.714	0.086	0.734	0.925
	CC+CG vs. GG	Random	80.19	0.002	0.620	0.318-1.208	-1.405	0.160	0.734	0.898
	CC vs. CG+GG	Fixed	46.16	0.134	1.211	0.804-1.826	0.916	0.360	1.000	0.956
PB	C vs. G	Fixed	41.99	0.142	1.079	0.940-1.239	1.079	0.281	0.462	0.548
	CC vs. GG	Fixed	53.38	0.092	1.140	0.857-1.517	0.901	0.368	0.734	0.515
	CG vs. GG	Fixed	39.23	0.160	1.189	0.955-1.479	1.550	0.121	0.806	0.966
	CC+CG vs. GG	Fixed	10.90	0.344	1.255	0.995-1.508	1.917	0.055	0.806	0.697
	CC vs. CG+GG	Random	68.17	0.024	0.919	0.571-1.481	-0.346	0.730	0.308	0.496

GC: gastric cancer; PCR-RFLP: Polymerase Chain Reaction Restriction Fragment Length Polymorphism; HB: Hospital Based; PB: Population Based; NA: Not Applicable.

HETEROGENEITY ANALYSIS AND SENSITIVITY ANALYSIS

There was a significant heterogeneity under all five genetic models for both CRC and GC. As shown in tables 2 and 3, the I^2 decreased obviously and p-value exceeded 0.05 after excluding by source of controls for CRC and by ethnicity and source of controls for GC, indicating that ethnicity and source of controls are the major source of heterogeneity in this meta-analysis. Sensitivity analysis was performed by sequentially removing each study to examine the influence of the removed data to the overall ORs. No individ-

ual study significantly altered the pooled ORs. Moreover, by limiting the meta-analysis to those studies in accordance with HWE, the sensitivity analysis was performed in another way. However, the corresponding ORs were not substantially altered in comparisons, indicating that our results were relatively robust.

PUBLICATION BIAS

Begg's test and Egger's test were calculated to assess the publication bias of literatures. The shapes of the funnel

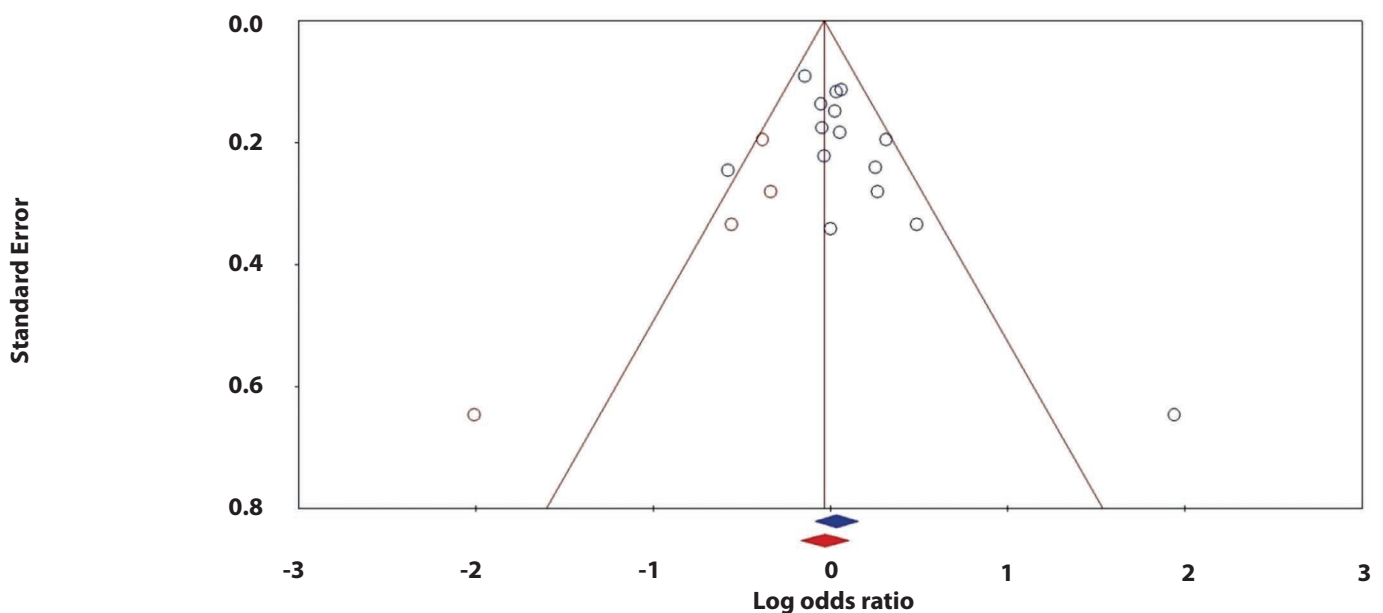


Fig. 3 Funnel plot for publication bias in the meta-analysis of IL-6 -174 G>C polymorphism with CRC under recessive model (CC vs. CG+GG). Blue line without and red line with trim and fill test.

plot did not reveal any publication bias for IL-6 polymorphism and GC risk under all five genetic models. In addition, No statistically significant difference was found in the Egger's test. However, there was a possible publication bias between IL-6 polymorphism and CRC risk under recessive model ($P_{\text{Begg's}} = 0.113$ and $P_{\text{Eggers}} = 0.044$). Therefore, we have used The Duval and Tweedie non-parametric "trim and fill" method to adjust publication bias. As shown in figure 3, meta-analysis with and without "trim and fill" did not draw different conclusion, suggesting that the results of synthetic analysis were robust.

DISCUSSION

To date, several case-control studies and meta-analyses have explored the association of IL-6 -174 G>C polymorphism on the susceptibility to CRC and GC. However, the small size, different genotyping methods and ethnicity, and the minor statistical power of the single epidemiological studies caused to the lack in consistency of those studies results. Thus, we did this meta-analysis to study the association of IL-6 -174 G>C polymorphism with susceptibility to CRC and GC. Our results suggested that the IL-6 -174G>C polymorphism was not significantly associated with increased risk of CRC and GC in overall population. In this meta-analysis, we found that similar mechanisms adapted by GC and CRC to development.

In the current meta-analysis based on 16 case-control available studies with 7,560 cases and 9,574 controls up to December 2018, our results indicated that there was no significant association between IL-6 -174G>C polymorphism and CRC risk. In 2013, Hu et al. performed a meta-analysis of eleven individual studies with 6,481 cases and 7,935 controls to evaluate the association of IL-6 -174G>C polymorphism with risk of CRC. Similarly, they have not found a significant association between IL-6 -174G>C polymorphism and CRC (9). In 2016, Wang et al., conducted a meta-analysis to explore the association of polymorphisms at IL-6/JAK/STAT3 pathway genes with CRC risk. Their results indicated that IL-6 -174G>C polymorphism (allele model: OR = 1.05, 95% CI = 1.00, 1.09) and JAK2 (recessive model: OR = 1.40, 95% CI = 1.15, 1.65) were significantly associated with increased risk of CRC. However, their results showed that the IL-6 -174G>C polymorphism was significantly associated with increased risk of CRC in Caucasians (40). Inconsistent with their results, our pooled data showed that the IL-6 -174G>C polymorphism did not significantly associated with increased risk of CRC in Caucasians.

Previously, the relationship between IL-6 -174 G>C polymorphism and GC risk has been systematically evaluated, but their results had been conflicting and controversial. In the current meta-analysis we found that the IL-6 -174G>C polymorphism was not significantly associated with increased risk of GC in overall. Recently, Wang et al., performed a meta-analysis to evaluate the association of IL-6 -174 G>C, -572 G>C and -597 G>A with GC risk (8). Their results showed that IL-6 polymorphisms were not associated with increased risk of GC. However, their results should be interpreted with caution due to the limited number of se-

lected studies. Compared with their meta-analysis, we only focused on the association of IL-6 -174G>C polymorphism with GC, while they analyzed different polymorphisms at other interleukin genes, including IL-6 rs1800796, IL-8 rs4073, IL-10 rs1800871, IL-10 rs1800872 and IL-10 rs1800896 polymorphisms with GC risk up to May 2018. Moreover, we performed subgroup analysis by genotyping methods and source of controls. In the same year, Liu et al., have performed a met-analysis of 13 studies (1,446 cases and 2,918 controls) to explore the roles of polymorphisms at IL-2, IL-4, IL-6 and IL-8 genes with GC risk (41). Their results revealed that IL-6 -572C>G polymorphism was significantly associated with the risk of GC, but not IL-6 -174G>C polymorphism. Inconsistent with their results, we found a statistically significant association between IL-6 -174G>C polymorphism and GC risk in Asians under two genetic models i.e., homozygote (CC vs. GG: OR = 1.860, 95% CI 1.061-3.258, $p = 0.030$) and recessive (CC vs. CG+GG: OR = 1.941, 95% CI 1.131-3.331, $p = 0.016$).

Between-studies heterogeneity was demonstrated under all five genetic models for both CRC and GC, and we then conducted a subgroup analysis to explore the potential sources of heterogeneity, including ethnicity, source of controls, and genotyping methods. The results manifested that the heterogeneity could be mainly attributed by source of controls for CRC and by ethnicity and source of controls for GC. However, CRC and GC have a complex etiology and pathophysiology generated by the interaction of several genes and environmental factors (7, 42). Thus, besides the indeterminate number of characteristics that vary among studies, other confounding factors such as age, gender, lifestyle further contribute to between-study heterogeneity (43, 44). In addition, there was a publication bias across the selected studies in this meta-analysis. We suggested that the detected publication bias in a few studies could be attributed to relative small sample sizes in certain studies.

Though we included the latest data, several potential limitations must also be noticed in our meta-analysis. First, most of the selected studies were performed among Caucasians and Asians. Moreover, subgroup analysis based on ethnicity could not be assessed on CRC. Therefore, there was a lack of statistical power to better evaluate the association of IL-6 -174 G>C polymorphism with CRC and GC risk, especially in subgroup analysis. In addition, this bias may exist because the individuals may not be a full representative of the whole population. Thus, future studies with large sample sizes in different ethnicities are needed to determine the potential effects of ethnic variation on CRC and GC susceptibility. Second, our search results were restricted to publications in Chinese and English, other relevant published and unpublished studies, which are likely to have null results, were not included. Third, there was a significant publication bias in this meta-analysis under recessive model for CRC, which may be due to the small number of studies in the meta-analysis. Fourth, our meta-analysis was largely performed by unadjusted estimates, because of the limitations in selected studies that presented adjusted estimates. Finally, gene-gene and gene-environment interactions were not analyzed due to the lack of original data. It is possible that specific envi-

ronmental and lifestyle factors may alter the association of IL-6 -174 G>C polymorphism with CRC and GC risk.

In conclusion, our meta-analysis suggested that the IL-6 -174 G>C polymorphism was not significantly associated with increased risk of CRC and GC in overall population. However, subgroup analysis by ethnicity showed that IL-6 -174 G>C polymorphism might be associated with an increased risk of GC in Asians. Due to the limitations, studies with larger sample size and in different ethnicities are needed to confirm our results.

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Robotic Management of Endometriosis: Discussion of Use, Criteria and Advantages: a Review of the Literature

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ABSTRACT

Endometriosis is a very common benign condition affecting fertility and quality of life. Different methods, either definitive or fertility sparing are used for its management by using open, laparoscopic, and robotic techniques. This is a literature review presenting the role and the advantages of robotic surgery in endometriosis. Such a management is effective, safe, and feasible in hands of well-trained multidisciplinary teams even for severe cases of endometriosis.

KEYWORDS

da Vinci[®] robot; endometriosis; treatment; advantages; criteria; quality of life

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INTRODUCTION

Endometriosis occurs in 5–15% of the general population and although a benign condition, sometimes it might require difficult surgical dissections as it could be locally infiltrative, invasive, and widely disseminated. The typical patient is nulliparous, infertile, and around 30-years-old. The most common sites of endometriosis intra-abdominally include adnexae (two out of three cases), pouch of Douglas, uterosacral and broad ligaments, uterovesical fold, ureters, bladder, appendix, rectosigmoid colon or caecum, and small bowel loops (1, 2).

The treatment options of endometriosis include medical (e.g. progestins, danazol, GnRH- analogues) or surgical options which could be classified as most definitive (including hysterectomy and bilateral salpingo-oophorectomy) or fertility-sparing with the aim to excise all peritoneal endometriotic implants and adhesions but preserve fertility (3–6). Laparoscopic or open techniques, depending on each surgeon's preference and experience, are offered as treatment options, while recently robotic procedures are also suggested.

The da Vinci® surgical system received FDA approval in 2005. Robotic procedures have been introduced in order to improve surgical performance. Increased dexterity, greater range of motion, and better depth perception are the main advantages of robotic-assisted techniques (7). Its limitations include lack of tactile feedback and increased cost (8). Robotic procedures combine the advantages of open and laparoscopic procedures and are another alternative in the management of endometriosis.

The aim of this narrative review is to present the use, the criteria, and the advantages of robotic surgery in the treatment of endometriosis.

DISCUSSION

Different techniques including open, laparoscopic or robotic procedures can equally be used in the management of endometriosis (9–11). The patient is preoperatively assessed with imaging scans including ultrasound and MRI and she signs the informed consent when she is informed about the type of planned procedure and possible risks of it including infection, bleeding, and injury of adjacent organs. Multidisciplinary experts, including gynaecologists, urologists, and general surgeons, should cooperate in order to achieve the optimal outcome in the most severe cases.

Fertility-sparing techniques are used in order to destroy all endometriotic implants and remove all the possible adhesions. Removal and not lysis of them is preferred. Endometriomas larger than 3 cm are also excised either with cystectomy, or if that is not technically possible, with oophorectomy. If tubes are affected, salpingectomy is performed and IVF procedures are used for pregnancy achievement. If both adnexae are affected and bilateral salpingo-oophorectomy is essential, the uterus is preserved and donor eggs could be used for pregnancy. In robotic cases, all endometriotic implants are either excised or destroyed with scissors or diathermy. Segmental bowel

resections, rectal shaving, and partial bladder resection are described in the literature (12). Ureteral endometriosis could be treated with partial ureterectomy and ureteroneocystostomy (13). Special care should be taken during the excision from small or large bowel and/or urinary tract to avoid any injuries. However, deep infiltrative endometriosis of the rectovaginal septum is one of the most severe types of endometriosis (14). For this reason, any hidden endometriosis should be completely excised to avoid developing deeper nodular lesions in the future (14). It was recently shown that infiltrating colorectal endometriosis could be safely and effectively treated robotically even by performing a rectosigmoidectomy if that is essential (1). In case of most definitive techniques, total or even modified radical hysterectomies plus bilateral salpingo-oophorectomies might be essential to treat the disease. Of course, as in open and laparoscopic techniques, preoperative use of GnRH analogues for three to six months can improve surgical success.

Robotic system preserves the advantages of conventional laparoscopy while it offers the possibility to the gynaecologist to dissect down and into the narrow pelvic floor. It is suggested that a diagnostic laparoscopy should be used in order to clarify the range of the disease in the upper abdomen before docking the robot in order to know exactly where the disease is. In robotic systems, the CO₂ pressure required for exposure is often lower in correlation with traditional laparoscopy as result of the mechanical lift of the robot (15). Robotic procedures can be safely performed after taking into account the physiological changes of pneumoperitoneum and steep Trendelenburg position during a preoperative anaesthetic review (16). Robotics, also shares similar benefits of laparoscopy including smaller incisions (at most 10 to 12 mm) (15). The three-dimensional stereoscopic vision by the use of binocular optics, the filtration of the tremor, and the less operator fatigue are some of the obvious advantages of such operations. The articulated instruments permit a wide range of motions while they increase the ability of the surgeon to work efficiently. All the above mentioned advantages can lead to more anatomical procedures. In addition, the 360° motion of the robotic wrist permits the fine adhesiolysis and removal of any suspicious nodule, even if it is quite deep. More specifically, Patzkowsky et al., comparing robotic to laparoscopic treatment in over 500 patients, showed that age, body mass index, operative time, and estimated blood loss were not statistically different between the two procedures. Furthermore, robotic techniques could be easier used in larger uterus, cases with more severe adhesions, and stage III-IV endometriosis (17). However, the rates of urinary tract infections were higher in the robotic group (17). According to another study, including women treated with robotic-assisted laparoscopy for stage III and IV endometriosis, the median actual surgical time was 145 minutes (ranging from 67 to 325 minutes), while the median blood loss was 100 ml (ranging from 20 to 400 ml) depending on the severity of the case and the experience of the surgeon (18). Another study group, showed that uterine weight higher than 250 grams and older age predispose to longer surgical time (19). In all those studies, the rates of conversion

to open surgery and blood transfusion are minimal. So, the robotic assisted surgery also permits the realization of a key hole operation which can be interpreted into significantly less blood loss, less pain, shorter recovery time, as well as shorter hospital stay and better aesthetic result. Additionally, a shorter hospital stay and a quicker return to normal activity may mean less postoperative problems such as infection or pulmonary embolism. Last but not least, the use of robotic systems gives the opportunity of rapid acquisition of surgical skills required in order to perform laparoscopic surgery, while at the same time enable gynaecologists to reach at least as good clinical outcomes as conventional laparoscopy and within shorter operating times once they exceed the initial stage of the learning curve.

On the other hand, the high costs of use, the bulky machinery, and the need for staff training are the most important drawbacks in the utilization of robot in such operations. Of course, entry of new robotic systems in the market, as well as the use of the robot by different surgical teams, and in a high volume of patients could decrease the cost disadvantage. Short term complications include vaginal cuff abscess (18), ureterovaginal fistulas (20), and higher rates of urinary tract infection caused by extended use of Foley catheter for urinary retention (21). A rare case of rhabdomyolysis and compartment syndrome, after a 12-hour duration robotic operation, is also presented in the literature (22), showing the need for training and time managing of such operations. Although larger prospective studies as well as longer follow-up periods are necessary to clarify the long term outcomes including fertility results, pain, and quality of life, it seems that robotic management of even severe cases of endometriosis is an effective, feasible, and safe alternative in well-trained hands as it was also shown in a recent systematic review (23) and can be used without compromising the principles of open or laparoscopic operations.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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Genotype C/C 13910 of the Lactase Gene as a Risk Factor for the Formation of Insulin-Resistant Obesity in Children

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ABSTRACT

Introduction: To reduce the risk of insulin resistance in obesity in children with lactase gene genotypes, we studied the factors that stimulate the chronic inflammatory process. **Material and methods:** 109 children 6–18 years of age were investigated. The main group (n = 56) was presented by children with signs of insulin-resistant obesity according to the criteria of the European Society of Endocrinology and the Pediatric Endocrine Society. The control group (n = 53) included obese children without insulin resistance. A comprehensive clinical examination, food diary analysis, genotyping of the lactase gene by means of the polymerase chain reaction, the Immunochemical Test Method with Electrochemiluminescent Detection of basal insulinemia, Hydrogen breath test with lactose load, sequential analysis, ROC analysis were carried out. **Results:** Clinical manifestations of lactose maldigestion in a child increased the risk of possible insulin resistance (prognostic coefficient (PC +2.6), as well as the presence of the lactase C/C 13910 gene genotype (PC +5.8) did. The genotype C/T 13910 in children had a protective effect on the risk of obesity (PC -2.9). The lowest risk of insulin-resistant obesity in observed among children with the genotype T/T 13910 (PC -12). **Conclusion:** The presence of the C/C 13910 genotype of the lactase gene is the main factor formation of insulin resistance in children's obesity.

What is known? The genotype C/C 13910 of the lactase gene as a risk factor for the chronic inflammatory process in the body.

What is New? Genotype C/C 13910 of the lactase gene as a risk factor for insulin-resistant obesity in children.

KEYWORDS

genotype C/C 13910; children; lactose maldigestion; obesity; insulin resistance

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INTRODUCTION

The discrepancy between the amount of lactose being consumed in the diet and the ability to secrete the lactase enzyme underlies the persistence of the chronic inflammatory process and the formation of obesity and insulin resistance. The degree of transcription of the lactase gene (*LCT*), OMIM 603202NG_008104 depends on the characteristics of the *MCM6* (*minichromosome maintenance complex 6*) enhancer. Single nucleotide polymorphisms (SNP) of the cis-regulatory element *MCM6*, which are characterized by the replacement of cytosine (C) with thymine (T) in the 13th *MCM6* intron at position 13910 for 14 kb from the *LCT* locus, cause a high penetrance of the lactase gene. The average level of lactase activity in European children with the genotype C/C 13910 is 6.5 U/g (adult-type lactase deficiency, LD), while the activity of lactase with the C/T genotype is 29.9 U/g (heterozygous lactase persistence), and with genotype T/T 50 U/g (homozygous lactase persistence) (1).

Our study focuses on solving one of the global medical and social problems in pediatrics caused by a significant spread of childhood obesity in an urbanized society (2) with *LCT* genotypes, studying the factors that cause chronic inflammation.

The aim of this work was to study the factors that induce insulin resistance within obesity in children caused by a genetically determined impairment of lactose digestion.

MATERIALS AND METHODS

To build a mathematical model for predicting the probability of insulin-resistant obesity in the early diagnostic stage, out of 109 children aged 6–18 years, two groups were identified. The main group ($n = 56$) was presented by children with insulin-resistant obesity according to the recommendations of The European Society of Endocrinology and the Pediatric Endocrine Society (3). The control group ($n = 53$) was formed by children with no signs of insulin-resistant obesity.

Genotyping was carried out at the polymorphic locus of the *LCT* gene by real-time polymerase chain reaction (RT-PCR) in 109 obese children from 6 to 18 years of age in a certified laboratory Synevo. The material for the study was venous blood.

Genotyping for signs of allelic polymorphism of the c.-13910 T>C *LCT* gene was started on the extraction of genomic DNA from peripheral blood leukocytes using the AmlPrime DNA-Sorb B reagent kit according to the manufacturer's instructions (CFX96 (BioRad), USA). In order to recognize alleles in the studied samples, the following classification was used: Allele 1 – allele, the indicated position of the C → T replacement; Allele 2 is the allele indicated after the C → T replacement position. Amplifications were performed on a CFX 96 amplifier (BioRad, USA) with the addition of up to 20 ng of isolated reaction mixture containing: 20 μg of SNP Genotyping Assay Mix (Applied Biosystems, USA) and 2 ng of Universal PCR Master Mix buffer solution (Applied Biosystems, USA). In the process of amplification, a simultaneous hybridization – fluorescence detection was

performed on samples using fluorescent probes on a DNA analyzer according to the Assays-onDemand protocols for SNP genotyping (No 4331183, Applied Biosystems, USA). Thus, three types of amplification products were determined: determination of the fluorescent signal only from the VIC dye – homozygote for gene 1 (genotype C/C 13910), simultaneously fluorescent signals from the dye VIC and FAM – heterozygote for genome 2 (genotype C/T 13910), fluorescent signal only from FAM dye – homozygote for genome 2 (T/T 13910 genotype).

To detect basal insulinemia and glycaemia in venous blood, the Immunochemical Test Method with Electrochemiluminescent Detection (ECLIA) in the Synevo laboratory was used. We calculated the level of sensitivity of peripheral tissues to insulin, the insulin resistance index HOMA (Homeostasis Model Assessment), using the formula (1):

$$\text{HOMA-IR} = (\text{fasting glycaemia (mmol/L)} \times \text{fasting insulin } (\mu\text{AU/L})) / 22.5 \quad (1).$$

HOMA-IR is one of the clinical criteria for insulin resistance. An increase in insulin resistance was observed with HOMA-IR > 95th percentile, respectively, with percentile curves recommended by the IDEFICS Consortium for the European population according to the age and gender of the child (4).

Hydrogen breath test with lactose load, 25 g (HBTL). The concentration of hydrogen in exhaled air was determined by the Gastro + Gastrolyser gas analyzer of the British company Bedfont® Scientific Ltd in parts per million (ppm) with an accuracy of ± 1 ppm. Automatic data analysis was performed using GastroCHART software (Bedfont® Scientific Ltd Station Road, Harrietsham, Maidstone, Kent, ME17 1JA, UK). Duration HBTL was 3 hours at intervals of 30 minutes. A test was considered positive when the level of hydrogen in exhaled air increased after 60 minutes, by more than 20 ppm compared to the basal level and clinical symptoms of lactase intolerance (abdominal pain, flatulence, and diarrhea) appeared on a visual-analogue scale for the next eight hours observations (5).

In determining the gradation of quantitative variables, generally accepted ideas about the risk factors of obesity complicated with insulin resistance were used (3, 6–8).

The algorithm for constructing a prognostic model included the following steps (9):

- Calculation of weighted prognostic (diagnostic) coefficients (PC) for each factor using the sequential analysis of Wald and determining the Kullback's informative coefficient (I) for each factor;
- Selection of factors with sufficient informativeness ($I \geq 0.5$) and the compilation of a prognostic model that included only those signs that had sufficient informativeness;
- Calculation of the total score of prognostic factors (ΣPC) for each observation in the study groups;
- Definition of logistic regression parameters by total points and calculation of the theoretical probability of insulin-resistant obesity for each observation;
- Development of a scale for assessing the probability of insulin-resistant obesity by the total score;
- Evaluation of the developed prognostic model with the use of ROC analysis and other statistical criteria.

To predict the disease, we carried out a sequential procedure for summing up diagnostic (prognostic) factors. Prognostic factors corresponded to the signs identified in the patient before reaching the diagnostic threshold. The choice of diagnostic thresholds (the sum of diagnostic factors), allowing to predict the occurrence of pathology, was carried out using errors of type 1 and type 2, and to achieve an accurate prediction probability of 95% focused on the range: $\Sigma PC \geq 13.0$ – pathology is predicted, or $\Sigma PC \leq -13.0$ – the absence of pathology is predicted. Accordingly, in order to achieve a 99% probability of an error-free forecast, we focused on the range of $\Sigma PC \geq 20.0$ and $\Sigma PC \leq -20.0$, respectively. If the amount was in the range between certain diagnostic thresholds, we concluded that there is not enough information to make a decision about the indicated error levels. The obtained sum of points of prognostic factors (ΣPC) for each observation was calculated taking into account the corresponding weight qualification (Fig. 1) and on its basis, the parameters of logistic regression were determined by formulas (2), (3):

$P = \exp(z) / (1 + \exp(z))$, $z = b_0 + b_1 \times (\Sigma PC)$ (2), where P is the theoretical probability of insulin-resistant obesity in children; ΣPC is the sum of points for a specific patient; b_0 and b_1 are regression coefficients.

The resulting regression equation:

$$P = \exp(1.0976614708555 + (0.11163272142568) \times \Sigma PC) / (1 + \exp(1.0976614708555 + (0.11163272142568) \times \Sigma PC)) \quad (3).$$

Building a regression equation with the determination of threshold values of the total score allowed us to develop a classification by ΣPC to determine children with low, medium and high risk of developing insulin-resistant obesity. The criterion for assigning a child to a particular risk group was the value of the calculated probability P , which ranges from 0 to 1, and a level greater than 0.5 (or 50%) indicates an increased risk and corresponds to ΣPC more than 10 points.

Statistical processing of research results included calculation of Spearman's rank correlation coefficient, Wald sequential analysis with the use of the STATISTICA software package (version 6.1) of the AGAR 909E415822FA serial number, adapted for biomedical research. ROC analysis and construction of ROC curves was carried out in the software package of the trial version of Medical Software Software 17.4 (MedCalc Software, Ostend, Belgium, <https://www.medcalc.org>, 2017).

RESULTS

In 56 children with insulin-resistant obesity (main group) and 53 children without insulin-resistant obesity (control group) aged 6–18, the following lactase gene genotypes were determined. The main group was dominated by the C/C 13910 genotype – in 51.76% of patients (29/56), the C/T 13910 genotype was found in 35.29% of the patients (20/56), the T/T 13910 genotype – only in 12.94% of patients (7/56). In children of the control group, the C/C 13910 genotype was found in 25% (13/53), the C/T 13910 genotype in 54.17% (29/53), the T/T 13910 genotype in 20.83% (11/53). By gender, in the main clinical group, boys

predominated (60.4% versus 39.6%). Then, as among the children of the control group, girls prevailed, the number of which was 58.33% versus 41.66% of boys, but the statistical significance of these differences was not observed ($p > 0.05$).

Risk factors for the formation of insulin resistance correlate most strongly among themselves in the group of examined children with the LCT C/C 13910 genotype. In this group of examined patients, the presence of insulin-resistant obesity correlates with the level of systolic blood pressure (SBP) – $\rho = 0.48$ ($p < 0.05$), the ratio of the waist circumference to the hips (WC/HC) is $\rho = 0.45$ ($p < 0.05$). HOMA-IR index before treatment is $\rho = 0.54$ ($p < 0.05$), which in turn associated with an indicator of a hydrogen respiratory test with lactose load (HBTLL) > 20 ppm ($\rho = 0.41$; $p < 0.05$).

Analysis of the clinical and anamnestic characteristics of children who had various forms of obesity allowed us to separate the risk factors for insulin-resistant obesity at an early diagnostic stage.

To determine the specific weight of each of the 243 analyzed clinical and biological factors, the prognostic factor (PC) was calculated. When comparing the age structure of children with insulin-resistant obesity and metabolically favorable obesity, we found that the minimum risk of insulin resistance is possible at the age from 4 to 8 years (PC –4.5), and the maximum risk – at the age from 12 to 18 years (PC +2.7). Gender features did not have a statistically significant effect on the risk of the formation of metabolically unfavorable obesity.

The risk of insulin resistance increases the complicated antenatal period (PC +3.6); hereditary burden of metabolic syndrome (PC +2.9); the presence of overweight in early childhood (PC +3); the presence of pneumonia at puberty (PC +7); tallness or physical development at the time of the study, above the 67.34th percentile (PC +1.8); violation of puberty initiation (PC +5.8); acne vulgaris (PC +6). Studying the eating behavior of children with various forms of obesity based on food diaries has shown that the average duration of food increases the risk of insulin-resistant obesity to less than 10 minutes (PC +4.5). Then, as a quiet meal during 20 minutes reduces the risk of insulin resistance (PC –6.7). Significantly increases the probability of insulin resistance and nutritional composition. For example, daily consumption of red meat, sausages, potatoes, rice, margarine, sugary drinks increases the risk of the formation of insulin-resistant obesity (PC +6.4). On the contrary, daily consumption of fresh vegetables and fruits in the form of 2–3 portions (children's palms) reduces the risk of insulin resistance (PC –5.5). Lifestyle modification requires reducing non-academic use of a computer/TV or other gadgets to 3 hours per day (PC +4.9). Clinical manifestations of LD in a patient increased the risk of possible insulin-resistant (PC +2.6), as did the presence of the lactase C/C 13910 gene genotype (PC +5.8). The presence of the genotype C/T 13910 has a protective effect on the risk of obesity (PC –2.9). However, the lowest risk of insulin-resistant obesity was observed in children with the genotype T/T 13910 (PC –12).

The ranking according to the Kullback's (I) informative coefficient showed that the first places among the factors

that predict insulin-resistant obesity in children at an early diagnostic stage are occupied by the genotype of the lactase gene ($I = 3.49$), the level of basal insulinemia ($I = 3.02$) and the lack of daily use up to 2–3 portions of fresh vegetables and fruits ($I = 2.71$), these factors and others have a high informative significance ($3.0 > I \geq 1.0$).

A moderate informative (diagnostic) significance ($1.0 > I \geq 0.50$) was characteristic of the pathological course of pregnancy in the proband mother. Low prognostic information ($0.5 > I \geq 0.20$) is established for the presence of chickenpox in history, the prevalence of fast food in the diet. These factors have been removed from the prognostic model, table 1.

Tab. 1 Prediction table for insulin-resistant obesity in children at an early diagnostic stage before the onset of insulin resistance.

Prognostic sign	Gradation of the sign	PC	I
Lactase gene genotype	T/T 13910	-12.0	3.49
	C/T 13910	-2.9	
	C/C 13910	5.8	
The level of basal insulinemia, mklU/ml	0.88–18.35	-0.8	3.02
	≥ 18.36	9.0	
Daily consumption of up to 2–3 portions of fresh vegetables and fruits	No	7.6	2.71
	Yes	-5.5	
The presence of pneumonia in history	No	-5.2	2.51
	Yes	7.0	
Duration of non-academic time spent at a computer/TV	< 1 hour	-6.1	2.42
	1–2 hours	0.1	
	> 3 hours	4.9	

Average meal duration	up to 10 minutes	4.5	2.33
	10–20 minutes	0.6	
	more than 20 minutes	-6.9	
Presence of acne vulgaris	No	-4.8	2.19
	Yes	6.0	
Daily consumption of red meat, sausages, potatoes, rice, margarine, sugary drinks	No	-1.1	1.74
	Yes	6.4	
Violation of the age norms of puberty initiation	No	-3.2	1.64
	Yes	5.8	
The age of the child at the time of inspection	4–8 years	-4.5	1.51
	8–12 years	-5.2	
	12–17 years	2.7	
Hereditary burden of metabolic syndrome	No	2.4	1.16
	Yes	2.9	
The level of physical development of the child in percentiles	<33.67	-8.0	1.05
	33.67–67.34	-1.2	
	>67.34	1.8	
Being overweight at an early age	No	-3.4	1.01
	Yes	3.0	
Pathological course of pregnancy	No	-2.8	0.96
	Yes	3.6	

Thus, we identified 14 the most prognostically and diagnostically significant clinical and biological predictors of the formation of insulin-resistant obesity in children from 6 to 18 years old, with sufficient informative prognostic significance ($I \geq 0.5$), figure 1.

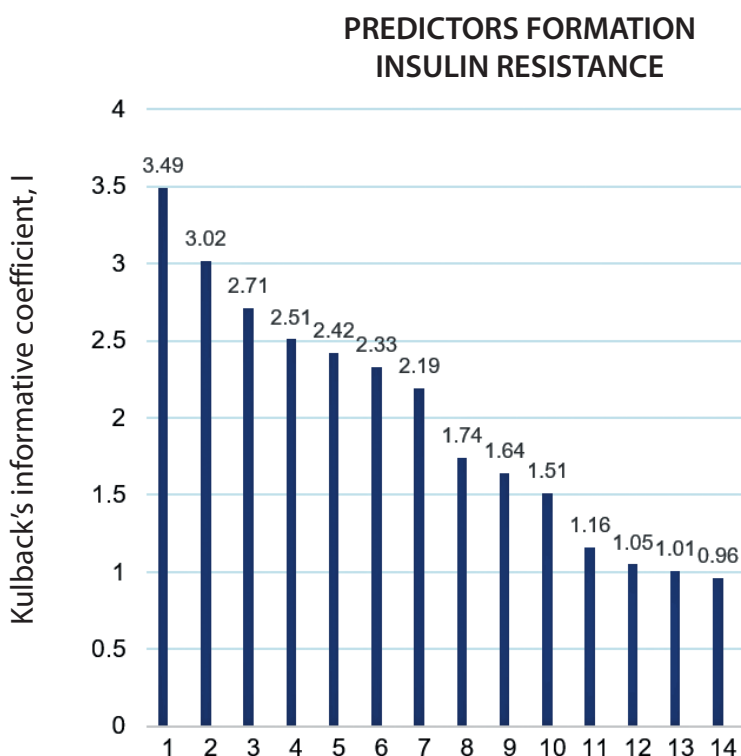


Fig. 1 Predictors of the formation of insulin-resistant obesity in children.

1. The genotype of the gene lactase C/C 13910
2. The level of basal insulinemia more than 18.36 mklU/ml
3. Lack of daily eating of up to 2–3 portions of fresh vegetables and fruits
4. Postponed pneumonia aged 11–14 years
5. Non-academic use of gadgets for more than 3 hours
6. Duration of food up to 10 minutes
7. Presence of acne vulgaris
8. Daily consumption of red meat, sausages, potatoes, rice, margarine, sugary drinks
9. Violation of puberty initiation
10. Age from 12 to 17 years
11. Hereditary burden of metabolic syndrome
12. The level of physical development of more than 67.34 percentiles
13. Overweight at an early age
14. Pathological maternal pregnancy

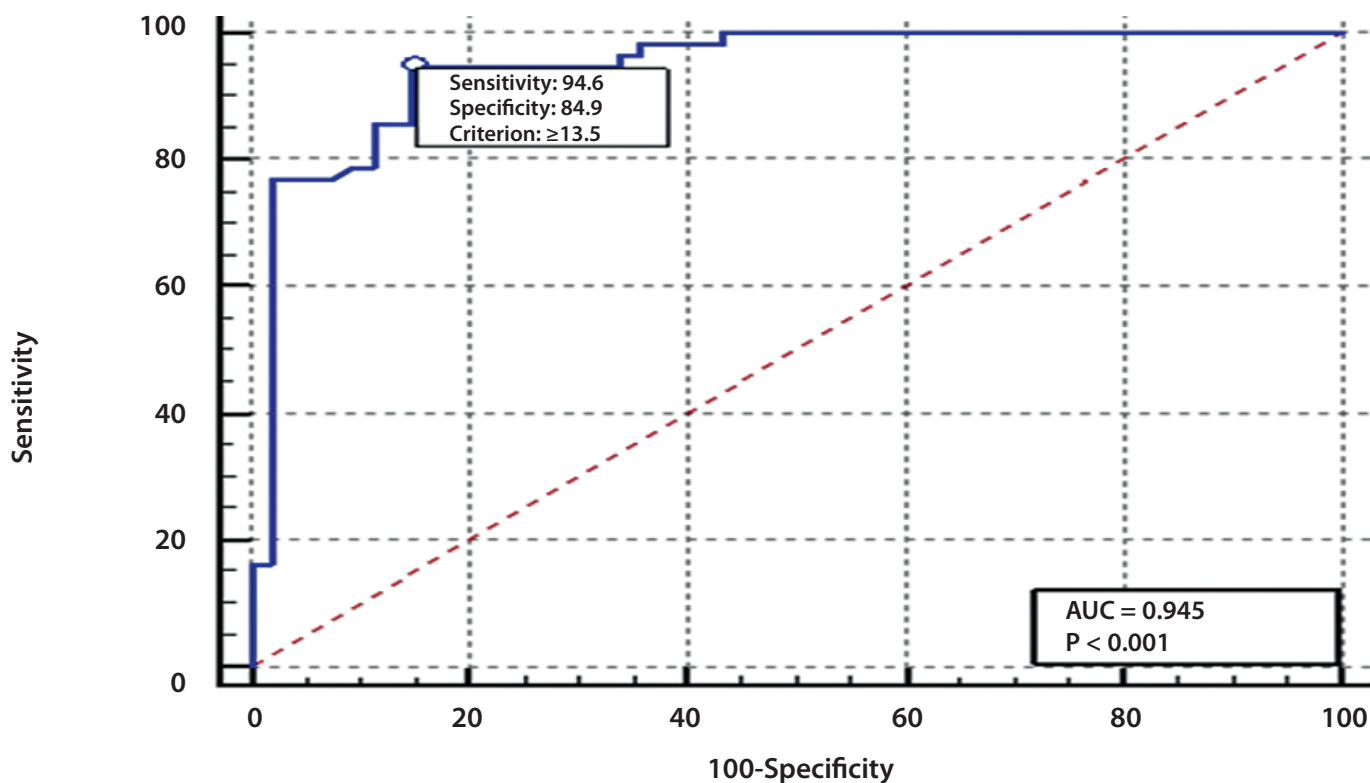


Fig. 2 Operational characteristics of a model for predicting morbid obesity in children at an early diagnostic stage according to ROC analysis.

The developed prognostic model for the development of morbid obesity in children has excellent operational characteristics, figure 2.

Sensitivity 94.64%, specificity 84.91% and their generalized indicator is the area under the ROC curve $AUC = 0.945$ ($p < 0.001$) with 95% CI 0.884–0.980. Test predict a negative result –83.16%. The proportion of correct prediction of the patient's actual belonging to one group or another (whether it is insulin-resistant obesity or not) was 87.16%, which indicates a high consistency in the actual distribution of observations for the presence of pathological obesity and distribution based on the prognostic model.

DISCUSSION

The presented model for predicting insulin-resistant obesity in children confirmed the data of previous studies on the importance of lifestyle modification: nutrition and physical activity (2, 3, 10). Excess lactose in the diet of modern humans causes clinical manifestations of genetically determined lactase deficiency (11). A survey conducted by Lucyna Ostrowska et al. (12) showed that the ability to digest lactose in adult men correlates with low basal hyperinsulinemia. In individuals with the C/C 13910 genotype, reduction of the signaling pathway Gal-9/Tim-3 stimulates the synthesis of pro-inflammatory cytokines and forms a metabolically unfavorable insulin-resistant phenotype.

According to our study, the maximum risk of insulin-resistant was observed at the age from 12 to 17 years. The presence of pneumonia, precisely during puberty (13), which is characterized by the presence of physiological in-

sulin resistance significantly, worsens the risk of metabolic adverse obesity in children.

The data obtained coincide with the results of studies demonstrating the relationship between inhibition of excess galectin-9/Tim-3 signaling pathway by excess lactose and the level of invasiveness of pathological factors (14).

Violation of the initiation of sexual development has gender dissociation and is associated with the development of normogonadotropic hypogonadism in boys and the syndrome of isolated adrenarche in girls. At the same time, activation of contrinsular hormones explains accelerated physical development and high height with insulin resistance. Chronic hyperinsulinemia, which is primarily compensatory, to support euglycemia, leads to the activation of lipolysis in visceral adipose tissue with the release of free fatty acids (FFA), which is an important metabolic risk factor for dyslipoproteinemia and leptin-resistance progression. FFA, among which acetate (60%), butyrate (15–20%), propionate (20–25%), which also accumulate when excessive use of lactose in the intestinal lumen, interact with the receptor GRP41 enteroendocrine cells of the intestine, causing an increase in the synthesis of the intestinal peptide YY, which slows down the intestinal transit time and reduces the feeling of fullness (7).

Despite the progress achieved in endocrinology and molecular genetics, the problem of childhood obesity remains highly relevant and requires further research on the influence on key metabolic risk factors.

CONCLUSION

The main risk factors for the development of pathological obesity in the early diagnostic stage are the genotype of

the lactase C/C 13910 gene, basal hyperinsulinemia and the lack of daily consumption of up to 2–3 portions of fresh vegetables and fruits.

To increase the effectiveness of planning personalized preventive and rehabilitation measures for overweight patients, it is proposed that practical doctors use the algorithm we developed for predicting pathological obesity in children with different genotypes of the lactase gene at an early diagnostic stage. The use of lactase gene genotyping and a HBTLI in all overweight patients is recommended to exclude lactose maldigestion. Children with obesity, with the genotype of the C/C 13910 lactase gene and a HBTLI index less than 19 pmm, must be prescribed low-lactose diet therapy with the calculation of the level of lactose tolerance according to the innovative “Low-lactose diet” computer program that we have presented (15). In children with obesity, with the genotype of the C/C 13910 lactase gene and lactose maldigestion with a HBTLI index of more than 20 pmm, it is necessary to use a combination of low-lactose diet therapy and exogenous lactase drug at a dose of 3000 ALU three times a day for 1 month (16).

Prevention of insulin-resistant obesity in a child must begin from the moment of birth during the preparation of a genetic passport, especially when there is the hereditary burden of the metabolic syndrome, the pathological course of pregnancy in the mother, macrosomia and the presence of the C/C 13910 genotype of the whole family.

AUTHORS' CONTRIBUTIONS

AA was responsible for the idea and study design, looked over the articles and extracted the data. AN analyzed the data and interpreted it. Both authors reviewed the paper and approved the final manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: There is no funding source.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The submissions were reviewed by the Ethics Committee

of the State Institution “Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine” (meeting minutes No. 2 of February 10, 2016 and minutes from meeting No. 5 of February 7, 2018). Informed consent: Informed consent was obtained from all individual participants included in the study.

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Primary Hyperparathyroidism Manifesting with Severe Hypercalcemia in a Nonagenarian Man: Pitfall of Common Imaging Techniques, Localization by ^{18}F -Choline Positron Emission Tomography/Computed Tomography and Successful Management with Calcimimetics

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ABSTRACT

A nonagenarian hypertensive man with chronic kidney disease (CKD) was admitted to the emergency department for gastrointestinal symptoms and worsening symptoms of depression. Severe hypercalcemia (15.3 mg/dL) was found and he was hospitalized. Fluids, loop diuretics and glucocorticoids were administered intravenously, which partially reduced calcium levels over a few days and improved his clinical condition. PTH levels proved increased (306 pg/mL) and 25-OHD levels were reduced; primary hyperparathyroidism (PHPT) was diagnosed. Neck ultrasonography (USG) did not show parathyroid enlargement, nor did $^{99\text{m}}$ Technetium-sestamibi (SESTAMIBI) scintigraphy reveal hyperfunctioning parathyroid glands. By contrast, ^{18}F -choline PET/CT evidenced a nodule located close to the oesophagus, behind the right thyroid lobe, which proved compatible with a hyperfunctioning parathyroid gland. Since the patient declined surgery, and zoledronate was unfit owing to areas of rarefaction of the jaw, the calcimimetic cinacalcet was started; the dosage was progressively titrated up to 120 mg/day with normalisation of calcium levels over time. PTH levels, however, proved erratic and showed an upward trend over the first year of therapy; however its levels partially decreased following increase of vitamin D levels by replacement therapy. Cinacalcet is a useful and safe drug, which can normalise calcium levels and improve the clinical condition, even in very old patients with severe PHPT who decline or are unfit for surgery.

KEYWORDS

primary hyperparathyroidism; hypercalcemia; ^{18}F -Choline PET/CT; cinacalcet

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INTRODUCTION

The incidence of primary hyperparathyroidism (PHPT) increases with age and reaches 95 to 196 per 100,000 in patients aged 70 to 79 years, with a 2-fold higher rate in females (1). Nonetheless, PHPT is overlooked and under-treated in the old population (1, 2).

PHPT generally presents with mild hypercalcemia, which is discovered by routine biochemical screening in over 90% of patients, who are mostly asymptomatic. Occasionally, elderly patients present with pain due to a bone fracture which causes mobility impairment, and thus reduced autonomy. So-called abdominal groans and psychic moans, are rarely the first clue to diagnosing PHPT but should be properly investigated. By contrast, renal involvement (nocturia, polyuria and nephrolithiasis) is not uncommon (3). Finally, a few patients have potentially life-threatening hypercalcemia (3). Neck ultrasonography (USG) and ^{99m}Tc-sestamibi (SESTAMIBI) scintigraphy are the most frequently used morphological and functional imaging techniques to detect pathological parathyroid glands; however, ¹⁸F-choline Positron Emission Tomography (PET)/Computed Tomography (CT) is being increasingly used in difficult/equivocal cases (4). Although parathyroidectomy remains the only definitive cure for PHPT, surgery is rarely advocated in elderly adults, because of concerns about comorbidities (1); in addition, calcimimetics have opened up a new therapeutic era.

Here, we describe the case of a nonagenarian man who complained of multifaceted symptoms and was diagnosed with symptomatic severe hypercalcemia due to PHPT, in whom the usual imaging techniques failed to detect the parathyroid tumour which was successfully located by ¹⁸F-choline PET/CT. The patient, who declined surgery, was successfully managed by means of calcimimetics.

CASE REPORT

A 90-year-old man suffering from nausea, hyporexia, constipation and psychomotor impairment was brought to the emergency department of our hospital. Anamnesis revealed hypertension on therapy with irbesartan plus hydrochlorothiazide, stage 3 chronic kidney disease (CKD), dyslipidemia, and recent-onset depression on polypharmacotherapy (sertraline, trazodone and prazepam). The patient was alert and apyretic but poorly cooperative; his blood pressure was high (160/100 mmHg). Blood tests revealed increased creatinine levels: 1.6 mg/dL (n.v. 0.7–1.2) and severe hypercalcemia: 14.5 mg/dL (n.v. 8.2–10.2), which was equivalent to 15.3 mg/dL when corrected for reduced (3 g/dL, n.v. 4–5.5) albumin levels. Chest X-ray, electrocardiogram (QTc: 410 ms) were normal and arterial blood gas analysis did not show acidosis; abdominal X-ray showed faecal retention without air-fluid levels. Saline infusion was started and 125 mg methylprednisolone was administered intravenously (i.v.). The patient was hospitalized in our Internal Medicine ward; he was alert but slow, and complained of nausea. His albumin-corrected calcium level was 15.5 mg/dL. Saline (2 L/day), methylprednisolone (80 mg/day i.v.), furosemide (40 mg/day i.v.)

and metoclopramide (20 mg/day i.v.) were started, and hydrochlorothiazide (known to reduce calcium excretion) was withdrawn. The following day, calcium was slightly reduced: 14.2 mg/dL; phosphorus levels were reduced: 2.3 mg/dL (n.v. 2.5–4.5), whereas PTH levels proved increased: 306 pg/mL (n.v. 15–65, intact PTH immunoassay); 25-OHD levels were reduced: 11 ng/mL (n.v. ≥ 30), creatinine levels: 1.6 mg/dL (glomerular filtration rate: 45 mL/min) were unchanged, and thyroid function (TSH: 1.8 μ U/mL) was normal.

A diagnosis of PHPT was made. On the second day of therapy, calcium levels were reduced to 13.5 mg/dL, remaining unchanged on the third day; at this time-point, however, a 15% overall reduction in calcium levels from the baseline had led to clinical improvement in the patient's condition. Neck USG showed subcentimetric isoechoic thyroid nodules with peripheral vascularization, and no enlarged parathyroid glands. Abdominal USG did not show kidney stones. Dual ^{99m}Tc-pertechnetate/SESTAMIBI subtraction scintigraphy did not reveal hyperfunctioning parathyroid glands also in the delayed 2h-scan. CT of the thorax and mediastinum, without contrast, evidenced a 12 mm ovoid solid formation, of possible parathyroid origin, localized in the right para-oesophageal space (Figure 1 a, b, c). ¹⁸F-choline PET/TC evidenced an oval-shaped 12 mm nodule (SUV: 8.1) located close to the oesophagus, behind the middle third of the right thyroid lobe, which proved compatible with a hyperfunctioning parathyroid gland (Figure 1 d, e). Dual-energy X-ray absorptiometry revealed wrist (T score: -1.9) and lumbar spine (T score: -1.3) osteopenia. The combined intravenous therapies decreased only partially calcium levels: 12.4 mg/dL (day 10, %: -25% vs baseline values). With a view to administering zoledronate i.v. (at a reduced dosage owing to CKD), in order to further reduce calcemia, jaw X-ray was performed; this revealed several areas of bone rarefaction which would have put the patient to high risk of developing osteonecrosis and for which orthognathic surgery was advised. This was declined by the patient and the drug was not therefore administered. Since the patient refused surgery, the calcimimetic cinacalcet 60 mg/day in two split doses was started; after 3 days, calcium levels were unchanged and the patient, who felt well, was discharged; frequent calcium checks were scheduled. Ten days later, since calcium levels were unchanged (12.6 mg/dL) cinacalcet was increased to 90 mg/day; calcium levels proved reduced (11 mg/dL) four weeks later. However, on the following evaluation calcium levels was 11.9 mg/dL and cinacalcet was increased to 120 mg/day in two split doses with steady normalisation of calcium levels (range: 9–10 mg/dL) thereafter, whereas PTH levels were erratic and showed an upward trend during the first 1-year follow-up examination (Figure 2). After the normalisation of calcium levels, cholecalciferol (25,000 IU/month) was started, and the dose was increased (50,000 IU/month) over time with increase in 25-OHD levels: 20 ng/mL. PTH levels partially decreased following the increase in 25-OHD levels (Figure 2). Patient's gastrointestinal symptoms did not relapse, and depression symptoms improved significantly and only sertraline was left in therapy. Long-term clinical and biochemical follow-up was scheduled.

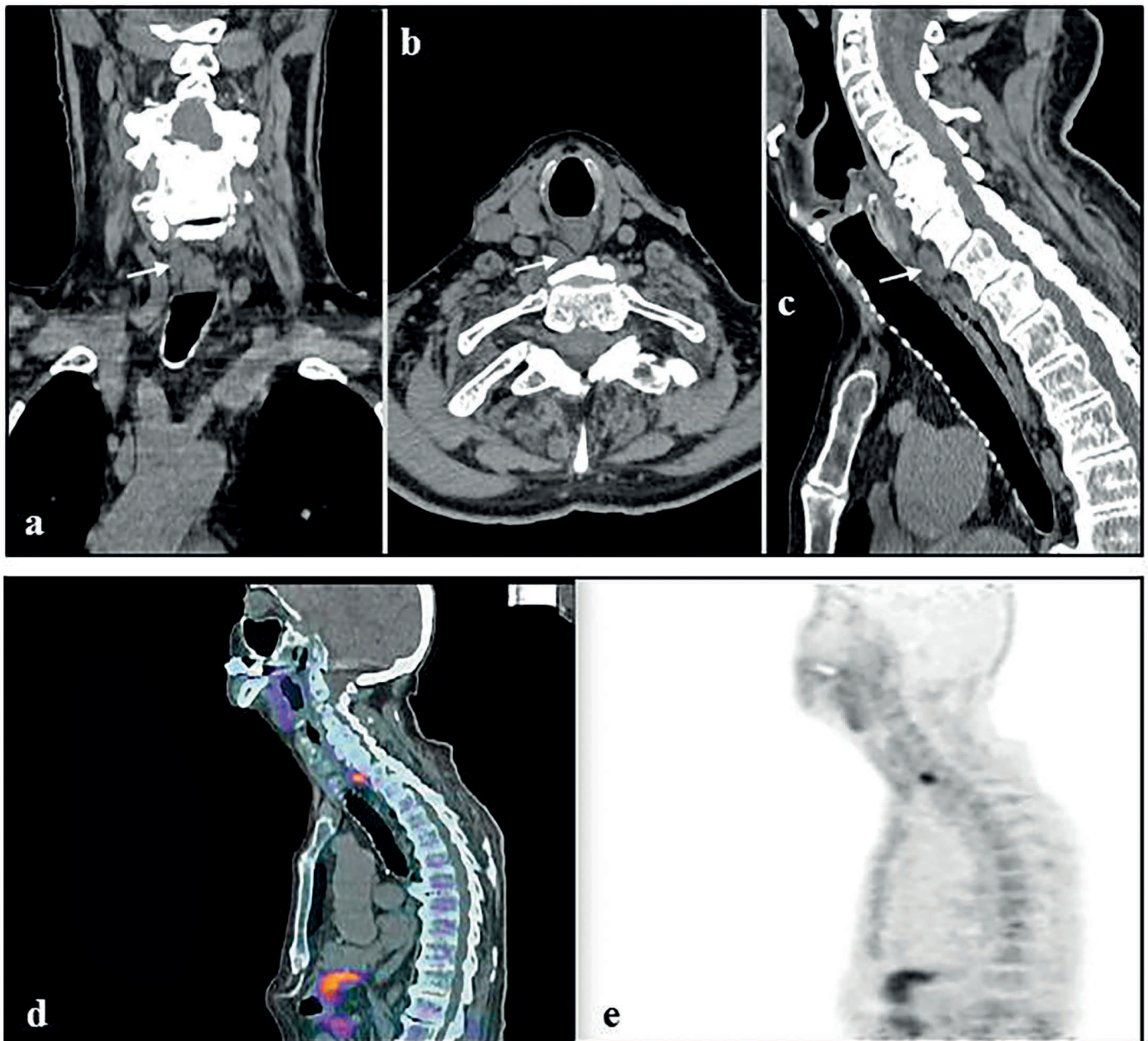


Fig. 1 Computed tomography, without contrast, showed a 12 mm ovoid solid formation (arrow) (a: coronal section, b: transaxial section, c: sagittal section) of possible parathyroid origin, localized in the right para-oesophageal space. Fused ^{18}F -Choline PET/TC (d) and PET (e) showed a high focal uptake (SUV max 8.1) of the tracer by a 12 mm hypodense right para-oesophageal nodule (arrow), which was compatible with a hyperfunctioning parathyroid gland.

DISCUSSION

In the elderly, PHPT is often asymptomatic or displays non-specific symptoms; rarely life-threatening hypercalcemia occurs. In this scenario, polypharmacy can further increase calcium levels (thiazides) or impair bone health (glucocorticoids); vitamin D deficiency may weaken skeleton status. Two of these harmful issues (thiazides and vitamin D deficiency) were present in our patient's clinical history.

In a recent Italian prospective multi-centre study on 604 patients (83% females, mean age: 61 ± 14 years) with mild PHPT (mean PTH levels: 163.0 pg/mL, mean calcium levels 10.9 mg/dL), the authors ascertained that 40.7% patients were "symptomatic", having at least one of the following features: (i) nephrolithiasis, either symptomatic or

asymptomatic (i.e., discovered on USG evaluation) (29.1%); (ii) clinical fragility fractures (11.6%); (iii) symptoms such as nausea, vomiting and constipation (5.6%) (5). Although not specifically designed for elderly patients, this study highlights that symptoms of PHPT, unless overt, must be investigated.

PHPT must be investigated in patients admitted to the emergency department in whom hypercalcemia is ascertained. Unfortunately, the calcium ion is not routinely included in the blood test array in this setting. In a retrospective cross-sectional study performed in an emergency department, Lindner et al. reported that hypercalcemia (mean calcium levels: 10.9 mg/dl) was found in 0.7% of 15,000 patients in whom calcium was assayed. Only 26% of hypercalcemic patients had symptoms that were probably related to hypercalcemia, such as nausea, weakness

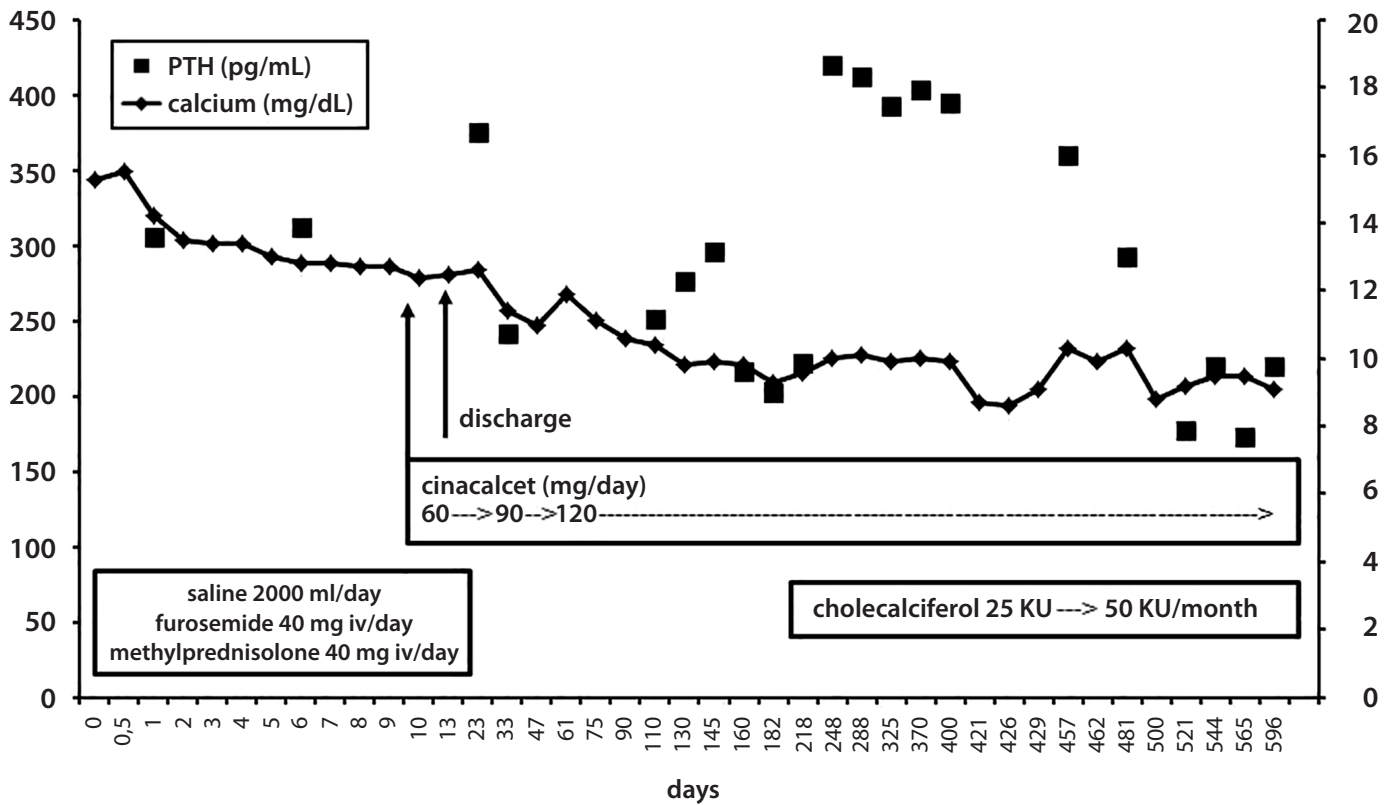


Fig. 2 PTH and albumin-adjusted calcium levels throughout hospitalization and during follow-up in the patient studied.

and disorientation; PHPT was found in only 8% of these patients (6).

Taken together, these studies showed that gastrointestinal symptoms are a common feature of PHPT. In our elderly patient, some of these symptoms (nausea, hyporexia and constipation) were the main reason for his admission to the emergency department, where severe hypercalcemia (subsequently related to PHPT) was ascertained; his symptoms improved following a moderate reduction in calcium levels, and vanished as calcemia normalised over time. Therefore, the so-called abdominal groans, although *per se* non-specific, should prompt an evaluation of the calcium level and, if this is increased, of PTH levels.

Although often deemed unspecific and poorly correlated to calcium levels, neuropsychiatric symptoms, such as depression, may be indicators of hypercalcemia, which is often overlooked.

In our patient, apathy and reduced mental and physical performance, had been ascribed to depression by a neurologist, and specific therapy had been started, with poor benefit. Severe hypercalcemia secondary to PHPT was only subsequently diagnosed and treated. As the patient's calcium levels first decreased and then normalised over time with combined therapies, his depression symptoms greatly improved and specific therapy was reduced.

In our very old hypertensive patient, severe hypercalcemia was managed by combined therapies. First of all saline was infused which expanded the intravascular space, and promoted diuresis and calcium excretion. Loop diuretic (furosemide) was used in order to prevent a potential volume overload with subsequent congestive heart failure induced by saline. Although loop diuretics reduce calcium reabsorption in the loop of Henle, they can para-

doxically increase calcemia by causing volume depletion when used improperly without fluid replacement (7).

In addition, before an etiological diagnosis of hypercalcemia was made, glucocorticoids were used. Once the diagnosis of PHPT was made, the drug was tapered and withdrawn. Glucocorticoids reduce the intestinal absorption of calcium and increase urinary calcium excretion but they have potential complications also in the short-term therapy and are ineffective in PHPT; glucocorticoids still have a significant role in the management of hypercalcemia secondary to granulomatous diseases or haematological malignancies (7).

In our patient, neck USG failed to detect the pathological parathyroid gland, which was subsequently localized behind the trachea by ¹⁸F-choline PET/TC. Ectopic gland can be difficult to detect by USG, particularly those in the retro-tracheal region, because of the poor acoustic window due to the tracheal air column (8). MIBI SPECT, which displays tridimensional images, is the functional imaging technique of choice for the localization of parathyroid adenomas. However, it displays misleading imaging results in nearly one third of patients, particularly in presence of small parathyroid adenomas (4). In our case, planar MIBI scintigraphy, also in the delayed 2h-scan, failed to detect the hyperfunctioning parathyroid gland, which was localized by ¹⁸F-choline PET/TC close to the oesophagus, behind the middle third of the right thyroid lobe. This imaging technique is based upon the avid uptake of choline, a precursor of the cellular membrane component phosphatidylcholine, by hyperfunctioning parathyroid cells. ¹⁸F-choline PET/TC, although it cannot yet be recommended as the first-line approach, is superior to MIBI SPECT/CT, particularly for the localization of small parathyroid adenomas (4).

Parathyroidectomy, the only curative treatment for PHPT, has a high success rate when performed by experienced surgeons. However, elderly patients with PHPT are seldom referred for parathyroidectomy, because they are unfit owing to their associated comorbidities, which may increase the surgical risk (1, 2). Our nonagenarian patient declined surgery and was successfully treated with the calcimimetic cinacalcet. This drug is indicated for the treatment of patients with PHPT who are unfit or decline parathyroidectomy, or in whom hypercalcemia relapses after surgery.

Cinacalcet increases the sensitivity of the calcium-sensing receptor on the parathyroid cells to the inhibitory action of extracellular calcium; hence it reduces PTH secretion and, consequently, serum calcium. However, it has no effect on bone mineral density (9).

In a double-blind, randomized, placebo-controlled study involving 67 subjects (78% females, mean age: 72 years) with moderate PHPT (mean PTH levels: 164.0 pg/mL, calcium levels: 11.7 mg/dL) unfit for parathyroidectomy, and normal 25-OHD levels, Khan et al. found that cinacalcet (median dosage 60 mg/day) normalized calcium levels in 76% of patients, and reduced PTH levels by 24% vs the baseline. Cinacalcet was generally well tolerated, causing only minor side-effects, such as nausea and myalgias (9).

In our patient, cinacalcet was titrated up to 120 mg/day and normalised calcium levels over time with no side-effects, whereas PTH levels proved erratic and showed an upward trend at least during the first year of therapy. This finding is in contrast with the results of the study of Khan et al.; however in our report the PHPT were more severe as featured by higher PTH and calcium levels, and 25-OHD levels were reduced.

The initial increase of PTH levels might be due to several factors which interplayed: vitamin D deficiency secondary to both patient's old age and to stage 3 CKD, decreased PTH metabolism secondary to CKD, and, not least, methodological bias since most but not all PTH samples were assayed in the laboratory of our hospital. In particular, hypovitaminosis D may have worsened the primary hyperparathyroidism by further stimulating PTH synthesis and secretion, resulting in secondary hyperparathyroidism that superimposed on the pre-existing PHPT. However, once plasma vitamin D levels increased by replacement therapy, PTH levels partially decreased.

In summary, PHPT is an often overlooked disease in the elderly, and can present with a wide range of symptoms,

often unspecific, which must be investigated. In this frail population, calcium levels can rarely be life-threatening as ascertained in our patient, and require a timely and proper management. Although parathyroidectomy is the treatment of choice for PHPT whenever possible, calcimimetics are a safe and effective alternative therapeutic tool in this subset of patients, too.

INFORMED CONSENT

Oral Informed consent has been obtained by the patient

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Mucopolysaccharidosis Type I in Children, a Forgotten Diagnosis Responsible for Undiagnosed Musculoskeletal Complaints: Report of Two Cases

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ABSTRACT

Mucopolysaccharidoses (MPS) are a subgroup of lysosomal storage disorders. The underlying mechanism of MPS disorders are deficiency in specific enzymes which leads to accumulation of partially degraded glycosaminoglycans (GAGs) in various tissues. A wide variety of manifestations are reported but musculoskeletal complaints are common among them. In milder forms of MPS, musculoskeletal complaints are presenting symptoms. Delays in diagnosis due to unspecific and mild symptoms is common. Misdiagnosis of MPS as juvenile idiopathic arthritis and other inflammatory arthritis disorders is frequent. Early diagnosis and treatment prevents irreversible cellular damages and is a key factor in efficacy of enzyme replacement therapy. In this study we described two MPS patients with musculoskeletal complaints who were not diagnosed for a period of time. Although musculoskeletal manifestation are common in a variety of clinical conditions, their presence at low ages or co-occurrence of other manifestations (such as cardiac, respiratory, neurologic, etc.) in multiple systems should prompt evaluation of patients for MPS and other metabolic disorders. The rheumatologists' awareness on MPS should be promoted to achieve timely diagnosis and subsequent early treatment.

KEYWORDS

mucopolysaccharidoses; glycosaminoglycan; hand drop; juvenile idiopathic arthritis; pediatric rheumatology

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INTRODUCTION

Mucopolysaccharidoses (MPS) are a subgroup of lysosomal storage diseases which are caused by progressive deposition of glycosaminoglycans (GAGs) in tissues. This deposition is due to enzymatic defects which impair degradation of GAGs (1). Seven types of MPS are described with specific enzymatic deficiencies. In addition to the type of deficient enzyme, the amount of deficiency is correlated with severity of the disease. GAG accumulation in cells occurs in various organ systems which leads to markedly reduced lifespan, multiple organ failures and cognitive function deterioration (2). Musculoskeletal complaints are present in nearly all types of MPS and represent the most common presenting symptom in these patients (3). Skeletal dysplasia, decreased joint mobility, short stature and Carpal tunnel syndrome are among the most prevalent musculoskeletal symptoms in MPS (1). Due to extensive involvement of different body organs, variety of clinical manifestations and rarity of MPS, misdiagnosis and delay to diagnosis are frequent. Usually patients with musculoskeletal complaints are referred to rheumatologists and they are frequently diagnosed with more prevalent conditions such as Juvenile idiopathic arthritis (4). Some efficacious treatments are developed for specific types of MPS (including MPS type I which is the most common form of MPS). Enzyme replacement therapy has shown promising outcomes in patients suffering from MPS type I. The most important factor regarding the efficacy of this treatment is timely diagnosis and early initiation of therapy (5–6). Of note is that the damages caused by MPS is irreversible and treating after the establishment of these changes within the body tissue yields not benefit for sufferers. Almost all patients with MPS remain undiagnosed for a relatively long period of time without receiving appropriate treatment (7). To improve outcomes in patients with MPS, it is necessary to raise awareness among pediatricians, rheumatologists and pediatric rheumatologists about MPS disorders. Once it was reported that just a small proportion (under 20 percent) of these medical care providers and specialists could diagnose MPS accurately (8). So to raise suspicions about MPS and consequent investigations and interventions to avert irreversible damages, publishing various case reports on different clinical scenarios of MPS might be helpful. In the article, we describe two undiagnosed MPS patients with different clinical presentations.

CASE REPORTS

CASE 1

The first case is a 10-year old boy who developed wrist drop at the age of 9 years. He is the first child of relative parents and did not have any particular problem since birth and afterwards. Results of all the other clinical examinations were normal. He does not show cognitive impairment, goes to school and is intellectually normal. To find out the extent of nerves' involvement, electromyography (EMG) and nerve conduction velocity (NCV) tests were conducted. Right flexor carpi radialis, left abductor pollicis brevis

and left opponens pollicis muscles had reduced or absent activity. Both median nerves had no sensory and motor activity. It was concluded that there was bilateral chronic severe median nerve injury above wrist. Brain MRI was normal. Biochemical routine laboratory tests were normal. There were not clinical symptoms for metal poisoning including lead and copper and ceruloplasmin, serum copper and lead level were within normal ranges. Then MPS was suspected. In urine testing, GAGs level was elevated. Electrocardiography and echocardiography was normal and ophthalmologic examination didn't show corneal clouding and any other abnormality.

To confirm the diagnosis, tandem mass spectrometry from dried blood spot was conducted. Five enzymes were checked. No Glycosaminoglycan alpha-L-iduronohydrolase activity was detected compatible with MPS I diagnosis. Genetic analysis revealed two homozygous missense mutations on p.Asp203Asn. After diagnosis, more evaluation for other abnormality were normal, including skeletal abnormality (by X-ray), abdominal ultrasonography and pulmonary function test.

CASE 2

The second case is an 8-year old boy with joint deformity and decreased range of motion predominantly in small joints of upper extremities. Decrease range of motion was detected in proximal interphalangeal (PIP) and distal interphalangeal (DIP) joints. Mild decrease in range of motion of elbow, knee and hip were also found. Pain was intermittent without particular pattern. There was no history of fever, rash and morning stiffness. This patient was primarily diagnosed as Juvenile idiopathic arthritis. He is the second child of relative parents delivered through normal vaginal delivery and his birthweight was 4 kg. His first sibling is a healthy female. His symptoms were developed at 6 years of age. Patient's growth and development was normal. There was history of umbilical and inguinal hernias in 1 and 4 years old age, respectively. Routine biochemical tests were normal. No significant erosion was seen on X-ray. Abdominal ultrasonography revealed no abnormal findings. Echocardiography showed aortic insufficiency and mitral regurgitation. Ophthalmologic assessment yielded no defects. Absence of inflammatory signs and morning stiffness, involvement of large and small joints, involvement of small joints of upper extremities and relative parents raised the suspicion of metabolic disorders. Further urine evaluations showed raised GAGs level which was suggestive of MPS. Tandem mass spectrometry from dried blood spot was conducted. Decrease glycosaminoglycan Alpha-L-iduronohydrolase activity was detected. And genetic study confirmed the diagnosis of MPS I.

DISCUSSION

In this study we introduced two cases with musculoskeletal problems who were finally diagnosed with MPS I. This condition is a multi-systemic and progressive disorder which can lead to death, finally (9). This rare condition oc-



Fig. 1 Small joint contracture and normal facies in our patient with MPS type I.

curs in one out of 100,000 newborns. The inheritance pattern is via autosomal recessive pathway (10, 11); thus MPS is more prevalent in children of relative parents, as in our reported cases. The inherited genetic defect is responsible for deficiency of glycosaminoglycan alpha-L-iduronohydrolase. This defect impairs the degradation of GAGs and results in accumulation of GAGs in tissues which causes cellular damages. MPS type I is divided into three subgroups which include Scheie, Hurler-Scheie and Hurler syndromes. Scheie is the least severe and Hurler is the most severe form of the disease. Severe forms are usually diagnosed very early in the course of the disease due to obvious cognitive and mental impairment. They are not expected to live long and almost all of them die before 10 years of age without treatment. Hurler-Scheie patients are regarded as intermediate severity MPS. They may be free of mental impairment but still short life expectancy is seen due to cardio-pulmonary complications without treatment (2). The diagnostic problem emerges in the cases of Scheie syndrome who are the patients with the mildest form of MPS. These patients do not have characteristic facial features, cognitive impairment or any other specific symptom. Diagnosis in these patients is so diffi-

cult due to the fact that clinical suspicion of MPS is not usually prompted (12). Although biochemical evaluations (urinary analysis) is so helpful but it is not sensitive in 100% of cases. To confirm the diagnosis, analysis of enzymatic activity is necessary and genetic assessment to figure out the responsible mutations is usually conducted (12).

The common symptoms of MPS can be summarized as joint contractures and skeletal deformities, cardiovascular involvement, corneal clouding, decreased visual acuity, hepatosplenomegaly, hernia and obstructive airway disease. As in our patients who presented with musculoskeletal manifestation, these complaints are the most common symptoms of MPS at the onset and course of the disease (13).

Considering neuropathy, wrist drop and foot drop are reported in Wilson's disease we assessed Wilson's disease and ruled out it (14, 15). All types of MPS except MPS types III and IX are associated with joint involvement (13). These complications are consequent to infiltration of soft tissues (ligaments, tendons, joint capsules, etc.) by GAGs. These changes lead to altered and malfunctioning skeletal alignment (1, 16). Musculoskeletal manifes-

tations can mimic various rheumatologic disorders and lead to misdiagnosis such as juvenile idiopathic arthritis (13). The articular symptoms of MPS are not inflammatory-type. Stiffness is not maximum at morning and does not alter in severity with rest or activity. Tenderness, swelling and other signs of inflammation are not found. Inflammation markers are also not on the rise. Another point that proves non-inflammatory nature of MPS is that these symptoms do not improve with anti-inflammatory treatments (13, 17). Although any joint can be involved in severe types, articular symptoms mainly occur in phalangeal joints in MPS. Involvement of interphalangeal joints results in claw hand deformity and impairment of hand function (7, 12). While proximal interphalangeal (PIP) or metacarpophalangeal (MCP) joints involvement is more common in inflammatory conditions, DIP involvement is more probable in MPS (13, 18).

Carpal tunnel syndrome in children is very uncommon and should be carefully evaluated on presentation (19). Carpal tunnel syndrome is a common phenomenon in MPS patients which is responsible for more than 50% of carpal tunnel syndrome in pediatric patients. Infiltration of GAGs in flexor retinaculum and surrounding tissues of median nerves leads to thickening of these tissues and puts the nerve under pressure. Nerve compression is the underlying cause of carpal tunnel syndrome and related symptoms (19). Diagnosis of carpal tunnel syndrome faces two difficulties in MPS patients: The first one is that carpal tunnel syndrome might present with atypical manifestations in these patients. Pain and numbness may be absent as in our patients which was manifested with wrist drop. The second one is that probable cognitive impairment and mental retardation in MPS patients can delay the diagnosis (20, 21).

Another finding in one of our patients was histories of umbilical and inguinal hernia. Hernia raises clinical suspicion on several conditions such as hypothyroidism, trisomy 21 and MPS in pediatric patients. Hernias are among the most common extra-skeletal findings in MPS patients. For instance, A recent study has reported that 66% of MPS patients had umbilical and inguinal hernias (22). Most hernias need no surgical treatment except hernias with larger than 2 centimeters size. One of our patients had aortic insufficiency and mitral regurgitation on echocardiography. It is reported that cardiac symptoms, particularly mitral and aortic valve disease, murmur, cardiomyopathy, and cardiomegaly are common in MPS (23). Presence of extra-skeletal manifestation, especially those which are not common in pediatric age group, should be taken seriously as a clue for the diagnosis of metabolic disorders.

In this study, we used urine GAGs for screening MPS. Urine GAG tested by combination of semiquantitative Berry spot and 1,9-Dimethyl-Methylene Blue that have been used for the quantification of sulfated glycosaminoglycans. Berry spot test is not enough sensitive for MPS type III and IV (24), so we added 1,9-Dimethyl-Methylene Blue test in our center for screening and false negative has been reduced, seriously (25). It has low specific for diagnosis and after positive GAGs, enzymatic assay should be considered for confirmation.

CONCLUSION

Although musculoskeletal manifestation are common in a variety of clinical conditions, their presence at low ages or co-occurrence of other manifestations (such as cardiac, respiratory, CNS, etc) in multiple systems should prompt evaluation of patients for MPS and other metabolic disorders. Early diagnosis and treatment is necessary for prevention of irreversible damages in MPS patients. The rheumatologists' awareness on MPS should be promoted to achieve timely diagnosis and subsequent early treatment.

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CONFLICT OF INTEREST

None

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Drug-induced Hemolysis in G6PD Deficiency: an Unusual Presentation of a Common Clinical Condition

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) deficiency can present a diagnostic dilemma owing to the varying degrees of disease severity and the wide range of precipitating factors. Here, we report a case of a 56-year-old man who presented with signs and symptoms of heart failure and, during the course of treatment, developed intravascular hemolysis. On investigation, he was found to be G6PD deficient. Following discontinuation of the fixed-dose combination of isosorbide dinitrate and hydralazine, the clinical condition of the patient improved, and there were no further episodes of hemolysis. The case highlights the need for a high degree of suspicion of G6PD deficiency in patients with unexplained signs and symptoms of intravascular hemolysis.

KEYWORDS

G6PD deficiency; isosorbide dinitrate; hydralazine; intravascular hemolysis

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common inherited and sex-linked enzymopathies (1). The identification of G6PD deficiency was the result of several converging events, one being the observation that some, but not all, individuals developed hemolysis on administration of 8-aminoquinoline antimalarials (2). The characteristic history and the availability of rapid diagnostic kits for detection of the enzyme deficiency have made the disease detection and management easy in the clinical setting, especially in malaria endemic areas, where the patients typically present with acute symptoms of intravascular hemolysis following culprit drug administration. Owing to the varying degrees of disease severity and the wide range of precipitating factors, the disease sometimes poses a diagnostic dilemma (3–5). Here, we report an unusual presentation of a case of drug-induced hemolysis due to G6PD deficiency in a 56-year-old patient presenting with symptoms of heart failure.

CASE REPORT

A 56-year-old male patient of Asian race presented to the General Medicine outpatient department of Kasturba Medical College Hospital, Mangalore, India, with complaints of swelling of both feet and breathlessness on exertion since four days. He was a known case of type 2 diabetes mellitus on oral medications, glimepiride and metformin. There was no significant past history or family history of any medical illness; no history of hospitalization or prolonged medication intake. On examination, his pulse rate was 120 beats per min and blood pressure was 128/80 mmHg. Jugular venous pulse was elevated, and there was bilateral pitting edema up to the ankle. On systemic examination, there were bilateral basal crepitations, hepatomegaly, and third heart sound was heard in the mitral area. Considering the presenting complaints and the physical examination findings, cardiologist opinion was sought, and two-dimensional echocardiography was done. The echocardiography was suggestive of con-

gestive cardiac failure with ischemic heart disease and severe left ventricular dysfunction with an ejection fraction of 30%. A thin rim of pleural and pericardial effusion was also seen. Ultrasound abdomen showed a normal-sized spleen with minimal ascites and mild pleural effusion. Liver was 14 cm in size with normal echotexture. He was started on tablet digoxin 0.25 mg half-tablet on alternate days, a fixed-dose combination of isosorbide dinitrate 20 mg plus hydralazine 37.5 mg thrice daily, injection furosemide 20 mg intravenously thrice daily, low dose aspirin 75 mg once daily, atorvastatin 10 mg once daily, and ramipril 5 mg once daily. On day 1, the haemoglobin level was 118 g/L; the rest of the blood parameters were normal. On day 2 of the in-hospital stay, he complained of 4–5 episodes of vomiting, which was treated symptomatically. On day 3, the vomiting persisted, and on day 4, physical examination revealed icterus; however, the urine appeared clear. The patient was scheduled to undergo coronary angiography, but the procedure was withheld, and blood investigations ordered, which showed a haemoglobin level of 94 g/L with a total bilirubin of 64.12 micromol/L. The patient was evaluated to ascertain the cause for the drop in hemoglobin level. Stool occult blood was found to be negative. Peripheral smear showed normocytic normochromic anemia with features suggestive of hemolytic anemia. Also, elevated total bilirubin was seen, predominantly unconjugated. The possibility of drug-induced intravascular hemolysis was considered, and a workup for G6PD deficiency was done. Using the dye decolorization test, the patient was found to be positive for G6PD deficiency. Among the prescribed medications, the fixed-dose combination of isosorbide dinitrate plus hydralazine was suspected to be the culprit drug based on the limited evidence available. The drug was withheld, and the rest of the medications were continued. On days 7 and 8, there was a further drop in hemoglobin level to 62 g/L. The opinion of a hematologist was sought, and two units of packed cell transfusion were given. Then, the patient was started on antioxidants and vitamin supplements. No further drop in the hemoglobin level was observed on days 7 and 8, and he was discharged from the hospital with the following medications: ramipril, trimetazidine, furosem-

Tab. 1 Laboratory investigations performed during the hospital stay.

Day of in-hospital stay	Total bilirubin (micromol/L)	Direct bilirubin (micromol/L)	Indirect bilirubin (micromol/L)	Haemoglobin (g/L)	Other laboratory investigations
Day 1	–	–		118	–
Day 4	64.12	17.78	46.34	94	Coombs test – negative Mean corpuscular volume 75.1 fl (reference range, 83.0–101.0) LDH level 354 U/L (reference range, 0–250)
Day 5	86.18	23.42	3.67	84	Peripheral smear – microcytic hypochromic with neutrophilic leucocytosis and features of hemolysis. No Heinz bodies seen. G6PD – decolorization > 6 hours
Day 6	87.21	30.95	3.29	75	–
Day 7	108.58	56.60	3.04	74	Stool occult blood – negative
Day 8	51.64	26.16	1.49	62	Blood transfusion given
Day 9	44.28	24.96	1.1	82	–

G6PD, glucose-6-phosphate dehydrogenase

ide, glimepiride + metformin, ivabradine, pantoprazole, ferrous sulphate, folic acid, and vitamin C tablets. During the follow-up visit two weeks later, his hemoglobin level was 112 g/L, and he was hemodynamically stable. The haemoglobin increased to 130 g/L after a month, suggesting that the initial anemia was due to iron deficiency. Table 1 shows the important lab investigation values during the course of the hospital stay.

DISCUSSION

G6PD is not an uncommon condition in India. The frequency of occurrence in India is 8.5% (6). This is in agreement with the global estimated prevalence of 8% in malaria endemic countries (7). G6PD deficiency can be classified into five classes with class I representing severe deficiency, characterized by chronic nonspherocytic hemolytic anemia and normal erythrocyte function (8). Class IV corresponds to normal enzyme activity and, class V, increased activity. Class II G6PD deficiency, with less than 10% of normal enzyme activity, is more common in Asians and the Mediterranean population. The case reported here is unique in that this middle-aged patient had no previous history suggestive of hemolysis and, during the treatment of the presenting complaints, developed intravascular hemolysis to drug(s) not commonly implicated in causing hemolysis. Except for a report of hemolysis in two patients, there is no substantial evidence to avoid the use of isosorbide dinitrate (9). While some suggest avoiding the drug due to the possible risk of hemolysis, not all have included it in the list of drugs to be avoided (10, 11). There is, however, a recent report of late-life presentation of hemolysis due to G6PD deficiency following administration of intravenous nitroglycerin (12). There is limited evidence implicating hydralazine in G6PD hemolysis (13–15). In fact, we were unable to find any reports of hydralazine-induced hemolysis in G6PD deficiency. The drug has been implicated in hemolysis due to the formation of immune complexes. However, this mechanism is unlikely to be responsible for the acute intravascular hemolysis seen in this patient (13). Also, the Coombs test was negative. The drug monograph of hydralazine states the following hematological adverse reactions: Blood dyscrasias, consisting of a reduction in hemoglobin and red cell count, leukopenia, agranulocytosis, and purpura (16). There is no recommendation regarding its use in patients with G6PD deficiency. The initial episode of vomiting was suspected to be due to the irritant effect of the drugs prescribed. However, the persistence of vomiting followed by the occurrence of jaundice raised the suspicion of intravascular hemolysis. We decided to evaluate the G6PD status of the patient as no other cause for intravascular hemolysis was apparent. Dechallenge confirmed the diagnosis as there was no further intravascular hemolysis, barring the immediate period following drug withdrawal, as determined via the hemoglobin levels, and there was an improvement in the hemoglobin status subsequently despite the continuation of all the other drugs. While furosemide is also known to cause hemolysis in G6PD deficient patients, the improvement in the patient's condition despite the con-

tinuation of furosemide rules it out as the precipitating drug (17). A rechallenge was not performed as there was no pharmacological compulsion for continuing with isosorbide dinitrate plus hydralazine and, hence, would be unethical. Based on the WHO-UMC causality assessment scale and the Naranjo scale, the causality was judged to be probable. We were unable to determine the class of G6PD enzyme variant and confirm which of the two drugs in the fixed-dose combination caused the adverse event.

This case highlights the importance of suspecting the presence of G6PD deficiency in an unrelated clinical setting precipitated by a drug not well known to cause oxidative hemolysis. In population with a G6PD deficiency of 3–5% or more in males, screening of new born infants is recommended (8). Fluorescent spot test, a qualitative assay, is a suitable test for the detection of G6PD deficiency, as compared to the more definitive quantitative tests, in heterozygous males and homozygous females in high-burden, resource-poor areas (18). Early recognition using simple laboratory tests will avoid unnecessary investigations and prolonged hospital stay.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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Ultrashort ssDNA in Retinoblastoma Patients Blood Plasma Detected by a Novel High Resolution HPLC Technique: a Preliminary Report

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ABSTRACT

A significant population of ultrashort (50–150 n) single-stranded DNA fragments were found in exosome-free blood plasma of retinoblastoma patients (6.84 ng mL⁻¹), but not in plasma of healthy donors. An original high resolution HPLC technique has been proposed to reveal and characterize this peculiarity. To solve this task, a novel molecular size exclusion – anion exchange analytical technique was developed. Its applicability to diagnostics and oncogenesis research is quizzed here.

KEYWORDS

Retinoblastoma; blood plasma; cfDNA; ssDNA; HPLC

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INTRODUCTION

The DNA repair machinery damage involves a marked DNA polymerase β hyperexpression and its processivity limitations in retinoblastoma cells (1, 2). This may promote some tumor DNA release as long as the DNA repair related short polydeoxyribonucleotides are going to get lost in a chaotic modulation of chromatin structure (3–5).

So the DNA repair caused release of short single-stranded DNA sequences is likely to contribute to the blood circulated DNA pool in retinoblastoma patients.

To reveal such a contribution, the extra-high resolution ssDNA-detecting tool is needed. It is hardly possible to overestimate this requirement since the conventional agarose gel electrophoresis and PCR procedures are not selective and sensitive enough as long as the pg-ng concentration range for short (smaller than 100n) ssDNA detection in clinical samples is the case (1–5).

Taking this into account, the aim of a present work was to develop a new chromatographic technique suitable to solve the above specified task, a high resolution ssDNA analysis in liquid biopsies.

MATERIALS AND METHODS

Four 5.0 year old male retinoblastoma (2A) patients and four adult male healthy donors were taken for blood plasma cfDNA extraction according to (4, 5). A consequent treatment of DNA extract with exonucleases lambda, III and S1 (3) was followed then by a cascade ultrafiltration on K75/K25 SPM TechSep (Mirabel, France) membranes (6). An enzymatic treatment of samples was conventionally carried out to purify ssDNA (3) aiming for a double-stranded DNA hydrolysis followed by ultrafiltration removal of the resulted free nucleotides and nucleases I, III traces (3, 6). As a negative control, the ssDNA destroying nuclease S1 treatment has been employed (3). The enzymatic treatment steps were performed to engage a known peculiarities of phage I 5'-exonuclease and E. coli 3'-exonuclease III, both digesting double-stranded DNA (7) and Aspergillus oryzae nuclease S1 digesting ssDNA (7). All enzymes were purchased from Worthington Corp., USA. Enzymatic treatment regime: 20 mM Tris-HCl (pH 8.40), 10 mM MgCl₂, 20 mM NaCl, 10 mM DTT, 15 mM EDTA, 60 min, +37 °C (3, 6, 7). A resulted fraction of lambda/III-nuclease resistant 25–75kDa compounds was analysed by size exclusion/anion exchange (SEAE) HPLC.

For this purpose, our original procedure has been employed. Its key parameters are the followings: stationary phase – polymethylamidopropylmethacrylamide; column PRP-X600 AE, 4.6 × 150.0mm, 5.0 micrometer particles, 1.6 meq/mL (Hamilton Corp., USA); 1,800 p.s.i., 22–25 °C, 0.8 mL/min elution rate. Both synchronous linear elution LiCl₂ (0–2.5M) and pH (8.0–4.0) gradients were formed on 100mM Tris/acetonitrile (85:15, v/v). Waters/Hamilton compatible Breeze 200SLE Analytical System, W2998 UV-Detector (254nm), W600E gradient former (Waters, Inc., USA). Sample loading: 80–100 g DNA in 50 microliters of 100mM Tris-HCl (pH 8.0)/acetonitrile (85:15, v/v). All ssDNA measurements and 2.0% agarose gel electro-

phoresis DNA size control were performed as described in (3).

For a positive control, the mixtures of equal amounts (5.0–6.0 ng/mL) of different size Poly(dT) single-stranded sequences were applied onto a column. Poly(dT)50, Poly(dT)100 and Poly(dT)150 species (ThermoFisher Corp., USA) were employed.

RESULTS

The content of ssDNA in the blood plasma of patients is 6.84 ± 0.56 ng mL⁻¹, this DNA population consists of ultra-short fragments (50–150n). In the control, a smaller population of ssDNA (2.40–2.82 ng mL⁻¹) was found consisting of the larger 350–400n sequences (Figure 1). Besides, the blood circulating ssDNA population is found to be heterogeneous in retinoblastoma patients but not in healthy donors (Figure 1).

Noteworthy, the retention time (Rt) values detected in a positive control fractionation runs (see Methods) were found equal to 19–20 min (Poly(dT)50), 16–17 min (Poly(dT)100) and 12–13 min (Poly(dT)150).

DISCUSSION

A separation efficiency shown by our original SEAE-HPLC technique allows to reveal the size/charge – different populations within the ssDNA pool in a cancer patient blood plasma (Figure 1). Noteworthy, this fractionation procedure provides with a far better resolution level as com-

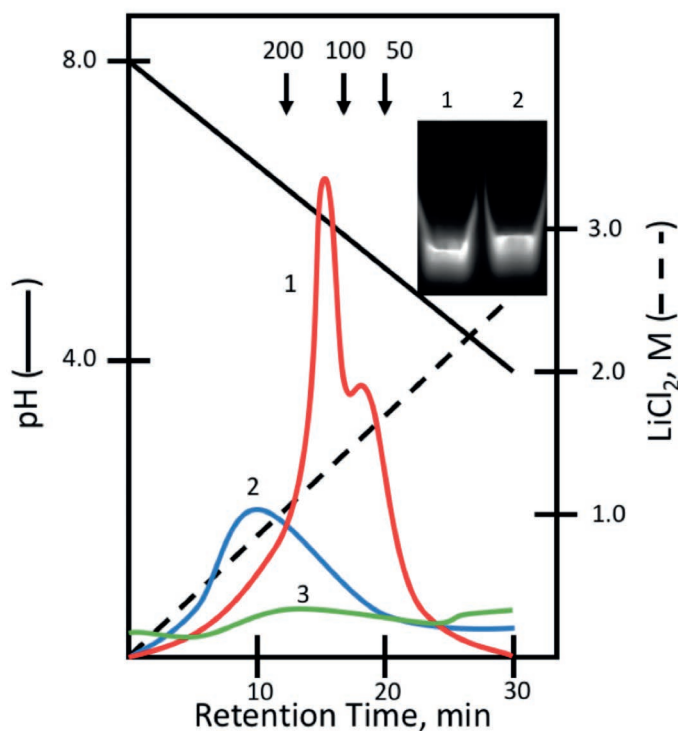


Fig. 1 Fractionation of plasma ssDNA fragments by HPLC on Polymethylamidopropylmethacrylamide. 1 – retinoblastoma patient, 2 – healthy donors, 3 – S1 nuclease control. Arrows show the retention times for 50n, 100n and 200n single-stranded poly(dT) markers (Takara Biomedical Corp., Rep. Korea).

pared to the dPCR engaging DNA size measurements and/or to a routine agarose gel electrophoresis (3–5). Moreover, the PCR engaging DNA size measurement might be an inadequate choice once these DNA fragments have the DNA repair related origin. In this case, the release of ssDNA species would occur in the DNA repair process usually accelerated in malignancies (1–4).

So an original procedure of SEAE-HPLC we proposed can be used then in biochemistry as well as in molecular biology, biotechnology, experimental oncology, analytical and clinical chemistry. Paying attention to a clear heterogeneity of ssDNA pool within the 50–150n molecular size range (Figure 1), a certain advantage of our HPLC analytical tool should be emphasized.

As per the positive control findings (see Results), it seems nothing but a clear sign of high resolution capabilities of our technique.

The molecular lengths of smallest ssDNA ever found in the human blood plasma cfDNA pool, either healthy or any disease diagnosed patients (cancer, infections, autoimmune pathologies) were not smaller than 770n, contents level 6.5–18.0 ng mL⁻¹ (4, 5, 9). This is the reason why we prefer the term “ultrashort” to pay attention to uniqueness of two facts: (1) high resolution of HPLC technique we proposed and (2) very small sizes of ssDNA detected (50–150n). The latter fact, as well as a clear heterogeneity of the retinoblastoma patients ssDNA pool (Figure 1), is an attention catching point with no any reliable explanation beyond. Obviously, this should be a part of agenda of our forthcoming studies.

A probable diagnostic potential of ultrashort ssDNA as the early markers capable of appearing in the blood plasma prior to protein oncomarkers should be further investigated.

The question we might expect: Would it be a possible to claim that our novel method might specifically detect ultra-short cell-free DNA (cfDNA) from retinoblastoma patients? The answer is “no”. Not just cfDNA of a certain size but, exclusively, their initially originated single-stranded species (ssDNA) are in a focus here with an emphasize on their possible DNA-repair related origin.

A remarkably high detection sensitivity of our SEAE-HPLC technique is no doubt helpful for further studies aiming to find out a link between the DNA repair damages in carcinogenesis and the appearance of the broken DNA repair products, short ssDNA (1, 2), in the patients blood plasma. So our method is to be treated as a reliable tool for both oncogenesis research and laboratory diagnostics purposes.

To the best of our knowledge, this is a first report ever on 50–150n ssDNA fragments presented in reliably detected ultra-low concentrations in cancer patient blood plasma to the contrast with healthy controls. On another hand, it should be safe to say that the present study is just a preliminary technical report with a certain potential for further clinical and experimental research.

It should be also outlined that this work deals with a new, previously not known (3–5) combination of molecular size exclusion and anion exchange separation principles in detection of a certain ssDNA populations within a clinical liquid biopsy samples. This itself is a key technological message of a present study.

Our assumption on the DNA repair related origin of the tumor-released ultrashort ssDNA fragments deserves further extensive studies being a new contribution to understanding of the carcinogenesis molecular mechanisms.

Comparing our method with a massive sequencing (MPS) technology (8), we have to state that a random, indiscriminate, nature of the DNA repair resulted short ssDNA fragments makes MPS limited to the DNA size estimation only. A primary structure of DNA chains is not in a focus once the DNA repair related origin of ssDNA has been assumed. So our HPLC approach might be treated as a reasonable alternative to MPS. Being about the same sensitive as MPS, our SEAE-HPLC technique provides nearly the same level of resolution available at ~30–40% lower expenses compared to MPS, once an appropriate HPLC system is in a lab equipment list.

Being a preliminary result, this work is an attention catching signal for those ones involved into a long lasting cfDNA studies of all sorts (3–5) including clinical and analytical chemistry, molecular medicine, etc. After all, this is the first report ever on a SEAE-HPLC version “nicely applicable” for analytical purposes once the blood circulated short ssDNA fragments are in a focus.

CONCLUSIONS

A new chromatographic procedure described is to be taken as an efficient tool for short ssDNA sequences detection in biomaterials which itself means a promising approach to ongoing studies on diagnostic relevance of cfDNA species in oncology.

Noteworthy, a resolution capabilities of this HPLC procedure look better as compared to most known chromatographic DNA separation versions (3), PCR and agarose gel electrophoresis (4, 5).

This work presents data of unconventional cell-free ssDNA isolation that should be of interest to the liquid biopsy technology community.

ABBREVIATIONS

SPM – sulfonated polysulfone membranes
 PRP – polymethylamidopropylmethacrylamide
 cfDNA – cell-free DNA
 ssDNA – single-stranded DNA
 SEAE – size exclusion – anion exchange

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