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Genetic Variations of Angiotensinogen, Angiotensin Converting Enzyme, and Angiotensin Type 1 Receptor with the Risk of Pulmonary Tuberculosis

Hamidreza Kouhpayeh[1], Mohammad Naderi[1], Zahra Mohammadghasemipour[1], Gholamreza Bahari[2], Nastaran Elahian[3], Mohsen Taheri[3,4], Mohammad Hashemi[3†]

[1] Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran;
[2] Children and Adolescent Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran;
[3] Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran;
[4] Department of Genetic, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
† Deceased

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Abstract: There is little data regarding the impact of renin-angiotensin system (RAS) gene polymorphisms on tuberculosis. The current study designed to survey the possible association between RAS polymorphisms and the risk of pulmonary tuberculosis (PTB) in a sample of the southeast Iranian population. This case-control study was done on 170 PTB patients and 170 healthy subjects. The AGT rs699 C>T, ACE rs4341 C>G and AT1R rs5186 C>A variants were genotyped using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) and ACE rs4646994 (287bp I/D) variant by PCR method. Regarding AT1R rs5186 A>C polymorphism, the findings revealed that AC genotype and C allele significantly

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Mailing Address: Prof. Mohsen Taheri, PhD., Department of Genetic, School of Medicine, Zahedan University of Medical Sciences, Khalije Fars Ave, Hesabi Square, Zahedan 9816743463, Iran; Phone: +98 541 333 721 16; e-mails: mohsen.taheri.gene@gmail.com, taheri@zaums.ac.ir

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decreased the risk of PTB (OR=0.39, 95% CI=0.22–0.67, p=0.001, and OR=0.53, 95% CI=0.25–0.72, p=0.002, C vs. A, respectively). The TC genotype and C allele of AGT rs699 T>C significantly associated with decreased the risk of PTB (OR=0.45, 95% CI=0.28–0.74, p=0.002, TC vs. TT and OR=0.51, 95% CI=0.32–0.80, p=0.005, C vs. T, respectively). The ID genotype of ACE 287bp I/D significantly increased the risk of PTB (OR=1.88, 95% CI=1.12–3.17, p=0.017). Our finding did not support an association between ACE rs4341 C>G variant and the risk of PTB. In summary, the findings revealed an association between AT1R rs5186 A>C, AGT rs699 T>C and ACE 287bp I/D polymorphisms and the risk of PTB in a sample of the southeast Iranian population. Further investigation with higher sample sizes and diverse ethnicities are required to confirm our findings.

Introduction

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (MTB) complex, is still a main public health problem globally and remains the top infectious killer in 2020 (World Health Organization, 2021). The *Mycobacterium tuberculosis* complex constitutes a genetically related group of bacteria that can cause tuberculosis in humans or in animals. The human pathogens are *Mycobacterium tuberculosis*, *Mycobacterium africanum*, and *Mycobacterium bovis*. Among them *Mycobacterium tuberculosis* is the most important human pathogen (Zhang et al., 2022). According to Global Tuberculosis Report 2021, there were expected 10.4 million new TB cases worldwide in 2016 (World Health Organization, 2021). Approximately one third of the world's population is infected with TB. Only about 5–10% infected cases will develop active TB (Taheri et al., 2020; Wu et al., 2020), which proposes a key role of genetic factors in host immunity. Until now, various genes have been recognized as TB candidate genes and variants in these genes have been proposed as risk factors for the disease (Naderi et al., 2014; Taheri et al., 2019; Li et al., 2022).

Renin-angiotensin system (RAS) is usually supposed as an endocrine system that regulates salt, water balance, and blood pressure. Current data have shown that renin and angiotensinogen genes and their products, well-known as local RAS, are also expressed locally at several tissue sites, where they function as major regulators of various physiological and pathophysiological processes (Su et al., 2021; Oosthuizen and Sturrock, 2022). Angiotensin II (AngII), the key effector of the RAS, mediates blood pressure-independent effects leading to target organ damage via diverse inflammatory processes, including activation of nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPK), and Janus-activated kinase-2 (JAK-2)/signal transducers and activators of transcription (STAT) (Kranzhofer et al., 1999; Seyedabadi et al., 2001; Cantero-Navarro et al., 2021). Activation of these signalling pathways may lead to overexpression of pro-inflammatory cytokines, chemokines, cell adhesion molecules, as well as elevation of reactive oxygen species

(ROS) (Hernandez-Presa et al., 1997; Kranzhofer et al., 1999; Seyedabadi et al., 2001; Esteban et al., 2004; Brasier, 2010).

Angiotensin converting enzyme (ACE) is an important component of the renin-angiotensin system (RAS). There are some studies indicating the raised serum levels of ACE in granulomatous diseases (Brice et al., 1995; Kwon et al., 2007; Lopez-Sublet et al., 2018). The cells in the macrophage-phagocytic system within the granulomas secret ACE into circulation (Lopez-Sublet et al., 2018). ACE polymorphism may influence the serum level of ACE. A functional insertion/deletion (I/D) polymorphism in the ACE gene has been recognized (Ristic et al., 2017). The ACE insertion/deletion (I/D) polymorphism is based on a 287bp Alu repeat sequence within intron 16 (Gong et al., 2012; Yigit et al., 2013). The ACE D/D genotype is associated with higher levels of serum ACE (Ay et al., 2007; Lopez-Sublet et al., 2018). Limited study investigated the impact of RAS polymorphisms and the risk of TB (Ogarkov et al., 2008; Zhang et al., 2014) and the findings were controversial. In the present study, we aimed to inspect the possible association between AGT rs699 C>T, ACE rs4341 C>G, ACE 287bp I/D, and AT1R rs5186 C>A variants and the risk of pulmonary tuberculosis (PTB) in as sample of the southeast Iranian population.

Material and Methods

Patients

The present case-control study was achieved on 170 PTB patients and 170 ages and sex matched healthy subjects. The enrolment procedure and the study design are described elsewhere (Kouhpayeh et al., 2012; Naderi et al., 2015, 2016a, b). Briefly, the cases were selected from PTB patients admitted to a university-affiliated hospital (Bou-Ali Hospital, Zahedan, referral center for TB). PTB was diagnosed according to the basis of clinical symptoms, acid-fast bacilli (AFB) smear-positive sputum, chest radiography, and culture positive for *M. tuberculosis*. The controls were unrelated adults chosen through the population without sign, symptom or history of TB. The project was approved by the local ethics committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1396.073) and informed consent was taken from all participants. Extracting of genomic DNA from whole blood samples was done using salting out method.

Genotyping

Genotyping of the variants were performed using PCR-RFLP or PCR method. The primer sequences are shown in Table 1. In each 0.20 ml PCR reaction tube, 1 µl of genomic DNA (~100 ng/µl), 1 µl of each primer (10 µM), and 10 µl of 2X Prime Taq Premix (Genet Bio, Korea) and 7 µl ddH_2O were added.

Amplification was done with an initial denaturation step of 5 min at 95 °C followed by 30 cycles of 30 s at 95 °C, annealing at 68 °C for AGT rs699, 60 °C for AT1R

Table 1 – Primers sequences used for detection of the gene polymorphisms

Polymorphisms	Sequence (5'→3')	Restriction enzyme	Product size (bp)	Annealing temperature (°C)
AGT rs699	F: CCGTTTGTGCAGGGCCTGGCTCTCT R: CAGGGTGCTGTCCACACTGGACCCC	Tth111I	T allele: 165 C allele: 141+24	68
AT1R rs5186	F: AGAAGCCTGCACCATGTTTTGAG R: CCTGTTGCTCCTCTAACGATTTA	DdeI	A allele: 410bp C allele: 292+118	60
ACE rs4341 C>G	F: CGCCAATTTTATTCCAGCTC R: TCGGGTAAAACTGGAGGATG	BsmI	C allele: 200bp G allele: 123+77	58
ACE rs4646994 (287bp I/D)	F: GCCCTGCAGGTGTCTGCAGCATGT R: GGATGGCTCTCCCCGCCTTGTCTC	–	I allele: 599bp D allele: 312bp	66

rs5186, 58 °C for ACE rs4341 and 66 °C for ACE rs4646994 for 30 s and extension at 72 °C for 30 s, final extension was performed at 72 °C for 5 min.

For genotyping, 10 µl of PCR products was digested with appropriate restriction enzyme (Table 1) and then separated by electrophoresis in 2.5% agarose gels.

Statistical analysis

Statistical analysis was achieved using the SPSS 20.0 software. The analysis was done by chi-square test or independent sample *t*-test according to the data. The associations between genotypes and PTB were calculated by calculating the odds ratio (OR) and 95% confidence intervals (95% CI) from unconditional logistic regression analyses. P-value < 0.05 was considered statistically significant.

Results

A total of 340 subjects including 170 confirmed PTB patients (66 males, 104 females; ages 50.1 ± 20.3) and 170 unrelated healthy subjects (79 males, 91 females; ages 49.3 ± 14.8). There was no statistically significant difference between the groups regarding sex and age (P=0.188, and 0.703, respectively).

Genotypes and allele frequencies of AGT rs699 C>T, ACE rs4341 C>G, AT1R rs5186 C>A and ACE rs4646994 (289bp I/D) polymorphisms are shown in Table 2.

Regarding AT1R rs5186 A>C polymorphism, the findings revealed that AC genotype and C allele significantly decreased the risk of PTB (OR = 0.39, 95% CI = 0.22–0.67, p=0.001, AC vs. AA and OR = 0.43, 95% CI = 0.25–0.72, p=0.002, C vs. A, respectively). The TC genotype and C allele of AGT rs699 T>C significantly associated with decreased the risk of PTB (OR = 0.45, 95% CI = 0.28–0.74, p=0.002, TC vs. TT and OR = 0.51, 95% CI = 0.32–0.80, p=0.005, C vs. T, respectively). The ID genotype of ACE 287bp I/D significantly increased the risk of PTB (OR = 1.88, 95% CI = 1.12–3.17, p=0.017). Our finding did not support an association between ACE rs4341 C>G variant and the risk of PTB.

Discussion

A large number of tuberculosis susceptibility genes have been recognized and polymorphisms in these genes have been proposed as risk factors for TB (Bahari et al., 2013; Naderi et al., 2014; Hashemi et al., 2015; Kim et al., 2021; Li et al., 2022). The ACE gene, an important component of the RAS, is one of the candidate genes for TB (Kwon et al., 2007; Zhang et al., 2014). It has been proposed that ACE effect the potency of immunological response, and thus could play potential roles in the pathogenesis of TB (Ogarkov et al., 2008; Nakamura et al., 2018). Individuals with deterioration of the immune system have a much higher risk of falling ill from TB. We examined the possible association between AGT rs699 C>T, ACE rs4341

Table 2 – Frequency distribution of genotypes and allele frequencies of AT1R (rs5186), ACE (rs4341 and 289bp I/D) and AGT (rs699) polymorphisms in PTB and controls

Polymorphisms	Case n (%)	Control n (%)	OR (95% CI)	P
AT1R rs5186 A>C				
Genotype				
AA	148 (87.1)	123 (72.4)	1.00	–
AC	22 (12.9)	47 (27.6)	0.39 (0.22–0.67)	0.001
CC	0 (0.0)	0 (0.0)	–	–
Allele				
A	318 (93.5)	293 (86.2)	1.00	–
C	22 (6.5)	47 (13.8)	0.43 (0.25–0.72)	0.002
ACE 287bp I/D				
Genotype				
II	36 (21.2)	51 (30.0)	1.00	–
ID	101 (59.4)	76 (44.7)	1.88 (1.12–3.17)	0.017
DD	33 (19.4)	43 (25.3)	1.09 (0.58–2.03)	0.792
Allele				
I	173 (50.9)	178 (52.4)	1.00	–
D	167 (49.1)	162 (47.6)	1.06 (0.78–1.43)	0.759
ACE rs4341 C>G				
Genotype				
CC	54 (31.8)	48 (28.2)	1.00	–
CG	70 (41.2)	93 (54.7)	0.67 (0.41–1.10)	0.113
GG	46 (27.1)	29 (17.1)	1.41 (0.77–2.58)	0.266
Allele				
C	178 (52.4)	189 (55.6)	1.00	–
G	162 (47.6)	151 (44.4)	1.14 (0.85–1.53)	0.442
AGT rs699 T>C				
Genotype				
TT	137 (80.6)	111 (65.3)	1.00	–
TC	33 (19.4)	59 (34.7)	0.45 (0.28–0.74)	0.002
CC	0 (0.0)	0 (0.0)	–	–
Allele				
T	307 (90.3)	281 (82.6)	1.00	–
C	33 (9.7)	59 (17.4)	0.51 (0.32–0.80)	0.005

PTB – pulmonary tuberculosis; OR – odds ratio; CI – confidence intervals

C>G, ACE 287bp I/D, and AT1R rs5186 C>A polymorphisms and the risk of PTB in as sample of the southeast Iranian population. Our findings showed that AC genotype and C allele of AT1R rs5186 polymorphism significantly decreased the risk of PTB. We found a significant association between rs699 T>C variant and PTB. So that the TC genotype and C allele significantly decreased the risk of PTB. The ID genotype of ACE 287bp I/D significantly increased the risk of PTB. Our finding did not support an association between ACE rs4341 C>G variant and the risk of PTB.

To the best of our knowledge, there is only one report concerning the impact of RAS on TB. Zhang et al. (2014) performed a case-control study with the aim to assess the association between ACE 287bp I/D and PTB in Chinese population. In contrast to our findings, they reported that the I/D polymorphism was not associated with susceptibility to PTB.

ACE elevated production of ROS and the activation of redox-dependent signalling cascades are critically involved in AGT II activities (Touyz, 2003). AGT II binds to AT1R and triggers intracellular superoxide production (Griendling and Ushio-Fukai, 2000; Mollnau et al., 2002; Kimura et al., 2005). AGT II also increases nitric oxide (NO) generation (Pueyo et al., 1998), and since the reaction of NO with superoxide generates peroxynitrite, it can stimulate the production of ROS and reactive nitrogen species (RNS) and reduce NO availability (Pueyo et al., 1998; Mollnau et al., 2002). The serum levels of ACE were not significantly different between patients with active PTB and healthy control subjects. While, an inverse relationship between the diameter of the cutaneous reaction to tuberculin and serum ACE levels was found (Grange et al., 1984). This inverse association results may be due to competition for receptor sites for the related signal molecules on the macrophage cell surface (Grange et al., 1984).

There are some limitations in the study, one of which is relatively small sample sizes. Second limitation is that we did not determine the plasma levels of ACE. Despite the limitation, our results provide new data regarding the impact of AT1R, AGT, and ACE polymorphisms on PTB in a sample of the southeast Iranian population, which could be useful for futures studies.

In summary, our finding proposed that AT1R rs5186 A>C and AGT rs699 T>C significantly decreased and the ACE 287bp I/D polymorphism significantly increased the risk of PTB. Our findings require replication in a larger independent genetic association study.

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Outcome after Neuro-interventional Treatment of Intracranial Aneurysm (as a First Treatment Modality)

Hossein Ghanaati[1], Aryoobarzan Rahmatian[2,3], Mohammad Reza Amiri-Nikpour[3], Davar Altafi[3], Morteza Taheri[3,4], Seyed Bahaadin Siroos[3], Madjid Shakiba[1], Reza Elahi[1], Mahsa Alborzi Avanaki[1]

[1] Advanced Diagnostic and Interventional Radiology Research Center (ADIR), Emam-Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran;
[2] Department of Neurology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran;
[3] Iranian Center of Neurological Research, Department of Neurovascular Intervention, Tehran University of Medical Sciences, Tehran, Iran;
[4] Iran University of Medical Sciences, Tehran, Iran

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Key words: Intracranial aneurysm – Endovascular treatment – Simple coiling – Stent assisted coiling – Flow diversion stenting – Flow disruption

Abstract: Endovascular treatment is widely applied as the first-line treatment for intracranial aneurysms and includes simple coiling (SC), stent-assisted coiling (SAC), flow diversion stent, and flow disruption stent. The present study is a retrospective cohort study performed in Imam Khomeini Hospital, Department of Neurovascular Intervention, between March 2016 and March 2021. A total number of 229 patients with intracranial aneurysms who underwent therapeutic intravascular interventions were enrolled, of which 89 were treated with SC, 111 with SAC, 25 with flow diversion stent, and 4 with flow disruption stent. The mean age of the subjects was 51.8±12.6 years, and 51.1% were male. Modified Raymond-Roy classification (MRRC) was used to define the occlusion outcome. The success rate, considered as Class I and Class II of MRRC at treatment time was 89% (94.4% in SC, and 84.7% in SAC), which was increased to 90.9% (94% in SC, 93% in SAC, 69.6% in flow diversion stenting, 100% in flow disruption) at 6-month follow-up, and 84.6% (80.8% in SC, 87.8% in SAC, 78.3% in flow diversion stenting, and 100% in flow disruption) at 12-month follow-up. The mean

Mailing Address: Dr. Mohammad Reza Amiri-Nikpour, Iranian Center of Neurological Research, Department of Neurovascular Intervention, Imam Khomeini Hospital Complex, Tohid Square, 1419733141, Tehran, Iran; Phone: +989 124 909 216; e-mail: reza.nikpor@gmail.com

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modified Rankin Scale (mRS) before the procedure was 0.05±0.26 which was increased to 0.22±0.76 after the procedure, 0.22±0.76 at 6 months, and 0.30±0.95 at 12 months (P<0.001). Similar to previous studies, the present study demonstrates that neurovascular intervention can treat ruptured aneurysms as the first therapeutic modality with favourable outcomes. A double-blind, randomized clinical trial is needed to eliminate the confounding factors and better demonstrate the outcome.

Introduction

Intracranial aneurysm is the abnormal bulging of the intracerebral vessels that stands the world's third most common cerebrovascular disease with an incidence of 3.6–6% of the population. Moreover, the intracranial aneurysms are the leading cause of subarachnoid hemorrhage (SAH) and are responsible for 22–25% of mortalities due to cerebrovascular diseases (Wardlaw and White, 2000; Juvela, 2003). The treatment options for a brain aneurysm include surgical treatment, e.g., clipping, wrapping, etc., and endovascular intervention. Endovascular intervention is widely applied as the first-line treatment for brain aneurysms following the International Subarachnoid Aneurysm Trial (ISAT) study (Molyneux et al., 2002, 2005), in which authors compared long-term efficacy of surgical treatment with endovascular intervention. They concluded that the probability of death and dependency is higher in clipping than in endovascular procedures. At the same time, the likelihood of disability-free survival was higher in endovascular procedures than in surgical clipping at ten years. Therefore, in terms of efficacy, these two methods were considered almost equal (Molyneux et al., 2015).

Several methods have been developed for intravascular intervention of intracranial aneurysms. The first method was simple coiling (SC), which is filling aneurysms with the coil. SC has some limitations, such as limitations in treating wide-neck aneurysms and a high risk of aneurysm recurrence (Wiebers et al., 2003; Gallas et al., 2005; Iijima et al., 2005; Li et al., 2006; Pouratian et al., 2006). These limitations resulted in the advent of new endovascular approaches, e.g., balloon-assisted coiling (BAC), stent-assisted coiling (SAC), flow diversion stenting, and flow disruption approach (Pierot and Wakhloo, 2013). These methods have been widely studied in terms of their clinical efficacy and long-term complications (Juvela, 2003; Zhang et al., 2022).

One of the most critical issues in treating intracranial aneurysms is the condition of neurological functions after procedures in treated subjects. Neurologic dysfunctions significantly affect the patient's quality of life and may include a variety of symptoms in different functions, including language, memory, consciousness, and attention (Gao et al., 2022). Therefore, cognitive dysfunction after treating intracranial aneurysms is a matter of concern. The present study investigated the clinical features and neurologic status at discharge, 6, and 12-month follow-ups using the modified Rankin Scale (mRS).

Material and Methods

Subjects

The present study is a retrospective cohort study that evaluates 229 subjects who had undergone endovascular treatment for a brain aneurysm in Imam Khomeini Hospital's Department of Neurovascular Intervention, Tehran, Iran, between March 2016 and March 2021.

Inclusion criteria were as follows: 1) patients with ruptured saccular intracranial aneurysm who were diagnosed with magnetic resonance imaging (MRI) or computed tomography (CT)-angiography; 2) patients aged more than 18 years; 3) patients for whom endovascular treatment included SC, SAC, flow diversion stenting, and flow disruption.

Exclusion criteria were as follows: 1) fusiform or dissecting aneurysm; 2) association with intracranial arteriovenous malformation (AVM), 3) previous coiled or clipped intracranial aneurysm; 4) immunosuppressed patients; 5) active infectious disease (endocarditis, meningitis); 6) follow-up less than a year; 7) absolute contraindication to the contrast agent; 8) platelet lower than 50,000 at admission; 9) known coagulopathy; 10) international normalized ratio (INR) more than three without a history of oral anticoagulant ingestion; 11) advanced atherosclerotic stenosis; 12) advanced vascular tortuosity in intracerebral vasculature; 13) treatment-resistant vasospasm interfered with navigation during the intervention; 14) history of cognitive dysfunction before the onset of the aneurysm.

The hospital file was evaluated, and the required data, including the demographic variables (age, sex, the history of antiplatelet usage, hypertension [HTN], diabetes mellitus [DM], hyperlipidemia [HLP], and smoking history); clinical variables (subarachnoid hemorrhage, rupture of the intracranial aneurysm, modified Rankin Scale at pre-procedure phase, discharge, 6-month, and one-year follow-up); radiologic variables (the location, size, and shape of aneurysm); treatment-related variables (anesthesia type, different endovascular approaches); and the post embolization outcome (Raymond-Roy occlusion classification in pre-procedure phase, 6- and 12-month follow-up), were collected.

Location of the aneurysm

Considering the location, the aneurysms were classified into the following groups: 1) anterior communicating artery (AComA) and anterior cerebral artery (ACA); 2) terminal internal carotid artery (ICA) and posterior communicating artery (PComA); 3) proximal ICA; 4) middle cerebral artery (MCA); 5) basilar artery; 6) vertebral artery and posterior cerebral artery (PCA).

The size of the aneurysm

Considering the size, the aneurysms were classified according to the sac diameter, neck diameter, and dome/neck ratio:

Sac diameter (according to the Unruptured Cerebral Aneurysm Study [UCAS] classification):

- Small: the maximum diameter of an aneurysm less than 5 mm
- Medium: the maximum diameter of an aneurysm between 5 and 10 mm
- Large: the maximum diameter of an aneurysm between 10 and 25 mm
- Giant: The maximum diameter of an aneurysm is more than 25 mm

Neck diameter:

- The maximum neck diameter of less than 4 mm
- The maximum neck diameter of equal to or more than 4 mm

Dome/neck ratio:

- less than 1.5
- equal to or more than 1.5

Evaluation of outcome

The primary angiographic results after treatment were assessed by the Raymond-Roy occlusion classification (RROC) and divided into three categories: Class I: complete obliteration; Class II: residual neck; Class III: residual aneurysm. The treatment was considered successful in the cases with complete occlusion and residual neck (Class I and II). The follow-up (F/U) angiography was performed at six months and 12 months. After reviewing angiographic images, RROC was used to determine the neurologic function of the subjects.

Intraoperative contrast agent extravasation or presence of coil out of aneurysm was in favor of aneurysm rupture. The intra-arterial contrast agent stagnation, intraluminal filling defect, disappearance of the distal vessels, and ischemic signs and symptoms during angiography at primary, 6 months, and 12 months were considered thromboembolic events. In the cases with a functional neurological deficit (FND) or acute headache, the bleeding and rebleeding were evaluated by CT scan and digital subtraction angiography (DSA).

The clinical neurologic status at the pre-procedure phase, discharge, 6-month, and one-year follow-up was evaluated by mRS. mRS is a test evaluating the neuro-cognition disability of the patients and can include a range of 0–5 in which a score of more than two is considered the worst outcome.

Statistical analysis

After collecting the data and required variables, a statistic specialist analysed the data using SPSS 16 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp). The continuous variables were reported as mean ± SD (standard deviation). Categorical variables were reported by number and percentage. Comparison between groups was performed using the chi-square test, Mann-Whitney U test, Kruskal-Wallis test, or ANOVA, based on the type of variables. The Kolmogorov-Smirnov test was used to assess the normality of data. The significant value for this study was considered 0.05.

Ethical considerations

The local institutional ethics committee approved the study, and informed consent was obtained from each participant.

Limitations

The limitations of this study were the short follow-up of the patients, loss of follow-up in some cases, and possible hospital file deficiency.

Results

Two hundred twenty-nine patients were enrolled in the study. The mean age of patients was 51.8 ± 12.6 (12–84 years). One hundred seventeen patients were male (51.1%), and 112 were female (48.9%). Eighty-nine patients were treated by SC, 111 by SAC, 25 by flow diversion stenting, and four by flow disruption. Risk factors of cardiovascular disease, clinical features, radiologic features of aneurysm, follow-up assessments, and technical aspects of procedures were assessed. Table 1 shows the detailed demographic data of patients in four different treatment groups. The distribution of the demographic data was not significantly different between each intervention group.

The most prevalent bleeding type among the subjects was SAH, seen in 184 patients (80.3%). Other bleeding types, intraventricular hemorrhage (IVH) and intraparenchymal hemorrhage (IPH) were seen in 32 (14%) and 13 (5.7%) patients,

Table 1 – Demographic characteristics of the patients in terms of intervention type

		Whole patients	SC (No.=89)	SAC (No.=111)	FD (No.=25)	F Dis. (No.=4)	P-value
Gender	M F	117 (51.1) 112 (48.9)	47 (52.8) 42 (47.2)	57 (51.4) 54 (48.6)	12 (48) 13 (52)	1 (25) 3 (75)	0.53
Age		51.8 ± 12.6 (12–84)	50.5 ± 12.6 (12–84)	52.4 ± 13.1 (18–81)	52.4 ± 10.3 (31–71)	62.3 ± 10.7 (49–75)	0.39
HTN		94 (41.0)	32 (36.0)	50 (45.0)	9 (36)	3 (75)	0.22
HLP		17 (7.4)	4 (4.5)	12 (10.8)	1 (4)	0	0.36
DM		20 (8.7)	7 (7.9)	9 (8.1)	3 (12)	1 (25)	0.48
Smoking		61 (26.6)	23 (25.8)	30 (27.0)	7 (28)	1 (25)	0.85
CVA		8 (3.5)	3 (3.4)	4 (3.6)	1 (4)	0	0.99

SC – simple coiling; SAC – stent-assisted coiling; FD – flow diversion stenting; F Dis. – flow disruption; HTN – hypertension; HLP – hyperlipidemia; DM – diabetes mellitus; CVA – cerebrovascular accident; F – female; M – male; the data have been presented as No. (%) or mean ± standard deviation

Table 2 – Distribution of different hemorrhage types and Hunt-Hess grading scale among patients with subarachnoid hemorrhage (SAH)

		Whole patients	SC (No.=89)	SAC (No.=111)	FD (No.=25)	F Dis. (No.=4)	P-value
Bleeding type	SAH	184 (80.3)	72 (80.9)	89 (80.2)	20 (80)	3 (75)	0.94
	IVH	32 (14.0)	11 (12.4)	16 (14.4)	4 (16)	1 (25)	
	IPH	13 (5.7)	6 (6.7)	6 (5.4)	1 (4)	0	
Hunt-Hess grading in SAH patients	I	17 (9.2)	10 (13.9)	7 (7.9)	0	0	0.33
	II	136 (73.9)	50 (69.4)	66 (74.2)	18 (90)	2 (66.7)	
	III	31 (16.8)	12 (16.7)	16 (18)	2 (10)	1 (33.3)	

SC – simple coiling; SAC – stent-assisted coiling; FD – flow diversion stenting; F Dis. – flow disruption; IVH – intraventricular hemorrhage; IPH – intraparenchymal hemorrhage

Table 3 – Distribution of aneurysms locations in all patients and each group separately

Location	Whole patients	SC (No.=89)	SAC (No.=111)	FD (No.=25)	F Dis. (No.=4)	P-value
ACA, AComA	78 (34.1)	46 (59)	30 (38.5)	2 (2.5)	0	0.003
PComA	6 (2.6)	3 (50)	3 (50.0)	0	0	
Terminal ICA	46 (20.1)	6 (13)	28 (60.9)	11 (23.9)	1 (2.2)	
Proximal ICA	26 (11.4)	5 (19.2)	15 (57.7)	6 (23.1)	0	
MCA	38 (16.6)	17 (44.7)	17 (44.7)	4 (10.6)	0	
Basilar A	26 (11.4)	9 (34.6)	13 (50.0)	1 (3.8)	3 (11.5)	
PCA, Vertebral A	9 (3.9)	3 (33.3)	5 (55.6)	1 (11.1)	0	

SC – simple coiling; SAC – stent-assisted coiling; FD – flow diversion stenting; F Dis. – flow disruption; ACA – anterior cerebral artery; AComA – anterior communicating artery; PComA – posterior communicating artery; ICA – internal carotid artery; MCA – middle cerebral artery; PCA – posterior cerebral artery

respectively. The most common bleeding type in treatment groups was SAH. The distribution of the different bleeding types among different groups did not show statistical differences (P=0.94) (Table 2). Hunt-Hess grading (HHG) scale was assessed in patients with SAH. The most prevalent HHG scale was grade 2 (in all patients and each treatment group). There was no statistical association between the treatment group and HHG (P=0.33) (Table 2).

The distribution of aneurysm locations in each intervention territory is mentioned in Table 3. The most common type of aneurysm location was ACA and AComA

Table 4 – Association between aneurysm features and different treatment groups

		Whole patients	SC (No.=89)	SAC (No.=111)	FD (No.=25)	F Dis. (No.=4)	P-value	Post-hoc test
Height size (mean ± SD)		8.96 ± 4.98	6.93 ± 2.97	10.37 ± 5.65	9.80 ± 5.44	9.75 ± 4.35	<0.001	SC<(SAC= FD=F Dis.)
Width size (mean ± SD)		5.98 ± 3.35	4.49 ± 2.17	6.87 ± 3.70	7.00 ± 3.51	8.00 ± 2.45	<0.001	SC<(SAC= FD=F Dis.)
Neck size (mean ± SD)		4.16 ± 2.21	2.64 ± 0.91	5.05 ± 2.18	5.36 ± 2.71	6.00 ± 0.82	<0.001	SC<(SAC= FD=F Dis.)
Aneurysm size categories	small (<4 mm)	10 (4.4)	7 (70.0)	1 (10.0)	2 (20.0)	0	<0.001	
	intermediate (4–10 mm)	156 (68.1)	70 (44.9)	71 (45.5)	12 (7.7)	3 (1.9)		
	large (>10 mm)	63 (27.5)	12 (19.0)	39 (61.9)	11 (17.5)	1 (1.6)		
Neck size categories	small (<4 mm)	111 (48.5)	79 (71.2)	25 (22.5)	7 (6.3)	0	<0.001	
	wide (≥4 mm)	118 (51.5)	10 (8.5)	86 (72.9)	18 (15.3)	4 (3.4)		
D/N ratio	<1.5	127 (55.5)	20 (15.7)	84 (66.1)	20 (15.7)	3 (2.4)	<0.001	
	≥1.5	102 (44.5)	69 (67.6)	27 (26.5)	5 (4.9)	1 (1.0)		

SC – simple coiling; SAC – stent-assisted coiling; FD – flow diversion stenting; F Dis. – flow disruption; SD – standard deviation; D/N – dome-to-neck

(34.1%), followed by terminal ICA and PComA (22.7%), MCA (16.6%), proximal ICA and basilar artery (11.4%), PCA and vertebral artery (3.9%), and PComA (2.6%), respectively. Different treatment methods were not accomplished similarly in various anatomical aneurysms (P=0.003). Most patients with ACA-AComA aneurysms were treated with SC, while most patients with terminal ICA and PComA were treated with SAC.

The association between aneurysm features and different treatment groups is demonstrated in Table 4. The mean height, width, and neck size of aneurysms were 8.96 ± 4.98 mm (2–29 mm), 5.98 ± 3.35 mm (2–20 mm), and 4.16 ± 2.21 mm (2–17 mm), respectively. Aneurysms treated with the SC method were smaller than the other three treatment methods (P<0.001). A similar pattern was seen for aneurysm width and aneurysm neck size (i.e., the aneurysms treated with the SC method had smaller width and neck size considering the other three methods) (both P-values < 0.001).

After the categorization of aneurysm size (<4 mm, 4–10 mm, and >10 mm) and aneurysm neck size (<4 mm, and ≥4 mm), we assessed the distribution of different treatment methods in each size category. As we can see in Table 4, this association was statistically significant; in fact, small aneurysms were treated more with SC, while large aneurysms were treated more with SAC. In addition, aneurysms with small necks were treated more with SC, while wide necks were treated more with the SAC method (both P-values < 0.001). On the other hand, aneurysms with a dome-to-neck (D/N) ratio lower than 1.5 were treated more with SAC, while other aneurysms were treated more with the SC method (P-value < 0.001).

The type of all aneurysms was saccular. All neuro-intervention procedures were conducted via femoral puncture, and a bi-plane angiography machine was performed all except one. In 67 patients, the procedure was performed under general anesthesia (29.3%). Table 5 describes the intervention method and treatment sessions conducted for patients. In most patients, the embolization was completed in the first session (144 patients, 62.9%; 57 in SC, 61 in SAC coiling, 22 in flow diversion stenting, and 4 in flow disruption), while for 79 patients, the procedure was completed in the second session (34.5%; 30 in SC, 46 in SAC, and 3 in flow

Table 5 – Association between the number of treatment sessions and different treatment groups

		Whole patients	SC (No.=89)	SAC (No.=111)	FD (No.=25)	F Dis. (No.=4)	P-value
Session time	first	144 (62.9)	57 (64.0)	61 (55.0)	22 (88)	4 (100)	0.04
	second	79 (34.5)	30 (33.7)	46 (41.4)	3 (12)	0	
	third	6 (2.6)	2 (2.2)	4 (3.6)	0	0	

SC – simple coiling; SAC – stent-assisted coiling; FD – flow diversion stenting; F Dis. – flow disruption

diversion stenting). For six patients, a third session was required to complete the procedure (2.6%; 2 in SC and 4 in SAC).

As mentioned, the modified Raymond-Roy classification assessed clinical outcomes of aneurysm occlusion (MRRC). This assessment was performed immediately after the procedure and in 6- and 12-month follow-ups. We compared the data of different treatment methods. In addition, we compared aneurysm filling in each treatment method at successive times (immediately after the procedure and in 6- and 12-month follow-ups).

In the present study, the initial RROC Class I, RROC Class II, and RROC Class III were achieved in 73.5%, 15.5%, and 11% of the patients, respectively. These rates were 77.5%, 16.9%, and 5.6% in the SC group and 70.3%, 14.4%, and 15.3% in the SAC group, respectively. At the 6-month follow-up, the RROC Class I, RROC Class II, and RROC Class III were achieved in 69.4%, 21.5%, and 9.1% of the patients, respectively. These rates were 69.9%, 24.1%, and 6% in the SC group, 68%, 25%, and 7% in the SAC group, 69.6%, 0, and 30.4% in flow diversion stenting, and 100%, 0, and 0 in flow disruption, respectively. At the 12-month follow-up, the RROC Class I, RROC Class II, and RROC Class III were achieved in 69.1%, 15.5%, and 15.5% of the patients, respectively. These rates were 65.1%, 15.7%, and 18.1% in the SC group, 69.4%, 18.4%, and 21.2% in the SAC group, 78.3%, 0, and 21.7% in flow diversion stenting, and 100%, 0, and 0 in flow disruption, respectively. The aneurysm filling was not statistically different between treatment methods in each time session. In addition, the rate of aneurysm recurrence at successive times for each treatment method is not high enough to make statistical significance.

Most aneurysms stayed unchanged with time; however, some recurred in 6 and 12 months. The rate of worsening in aneurysm filling with time was more than the rate of improvement in aneurysm filling (Table 6). Comparison of 12-month follow-up with the after-procedure situation was statistically significant for the SC group but was not significant in the SAC group. This means a higher rate of aneurysm recurrence was observed in the SC group compared to the SAC group. More precisely, the difference in successive time sessions in SC groups showed that the main worsening of aneurysm filling occurred when we compared the data from 6 and 12 months and the comparison of data from after the procedure and 12 months. Data demonstrated that the recurrence of aneurysms accelerated after six months. The comparison of successive time sessions for SAC did not show a significant difference (all P-values greater than 0.31). In addition, a comparison of 6 months and 12 months in the FD (flow diversion stenting) group did not show a significant difference (P-value = 0.16).

Table 7 describes the distribution of adverse events following the intervention. Aneurysm rupture during the procedure occurred in 8 patients (3.5%; 3 in SC and 5 in SAC). Intraprocedural thromboembolism events were seen in 21 patients (9.2%; 7 in SC, 10 in SAC, 3 in flow diversion stenting, and 1 in the flow disruption group). Intraprocedural thromboembolism was more common than the six and

Table 6 – Aneurysm filling after procedure and successive follow-ups based on modified Raymond-Roy classification in all patients and terms of treatment groups

MRRC		Whole patients	SC (No.=89)	SAC (No.=111)	FD (No.=25)	F Dis. (No.=4)	P-value
MRRC class after procedure	I	147 (73.5)	69 (77.5)	78 (70.3)	–	–	0.16
	II	31 (15.5)	15 (16.9)	16 (14.4)	–	–	
	III	22 (11.0)	5 (5.6)	17 (15.3)	–	–	
MRRC class after 6 months	I	145 (69.4)	58 (69.9)	68 (68.0)	16 (69.6)	3 (100)	0.62
	II	45 (21.5)	20 (24.1)	25 (25.0)	0	0	
	III	19 (9.1)	5 (6.0)	7 (7.0)	7 (30.4)	0	
MRRC class after 12 months	I	143 (69.1)	54 (65.1)	68 (69.4)	18 (78.3)	3 (100)	0.50
	II	32 (15.5)	14 (15.7)	18 (18.4)	0	0	
	III	22 (15.5)	15 (18.1)	12 (21.2)	5 (21.7)	0	
P-value (3 F/U sessions)		0.07	0.015	0.92	–	–	
Comparison of 0 and 6-months							
Worse		37 (1 to 2: 27; 2 to 3: 3; 1 to 3: 7)	18 (1 to 2: 13; 2 to 3: 1; 1 to 3: 4)	19 (1 to 2: 14; 2 to 3: 2; 1 to 3: 3)	–	–	
No change		117 (1: 108; 2: 7; 3: 2)	53 (1: 49; 2: 4; 3: 0)	64 (1: 59; 2: 3; 3: 2)	–	–	
Improve		29 (3 to 2: 11; 2 to 1: 15; 3 to 1: 3)	12 (3 to 2: 3; 2 to 1: 9; 3 to 1: 0)	17 (3 to 2: 8; 2 to 1: 6; 3 to 1: 3)	–	–	
P-value		0.21	0.12	0.79	–	–	

MRRC	Whole patients			SC (No.=89)			SAC (No.=111)			FD (No.=25)			F Dis. (No.=4)			P-value
Comparison of 0- and 12-months																
Worse	48	1 to 2 2 to 3 1 to 3	24 5 19	26	1 to 2 2 to 3 1 to 3	13 4 9	22	1 to 2 2 to 3 1 to 3	11 1 10	–			–			
No change	102	1 2 3	98 1 3	46	1 2 3	44 0 2	56	1 2 3	54 1 1	–			–			
Improve	31	3 to 2 2 to 1 3 to 1	7 19 5	11	3 to 2 2 to 1 3 to 1	1 10 0	20	3 to 2 2 to 1 3 to 1	6 9 5	–			–			
P-value	0.008			0.002			0.39			–			–			
Comparison of 6- and 12-months																
Worse	31	1 to 2 2 to 3 1 to 3	12 10 9	18	1 to 2 2 to 3 1 to 3	6 8 4	13	1 to 2 2 to 3 1 to 3	6 2 5	0	1 to 2 2 to 3 1 to 3	0 0 0	0	1 to 2 2 to 3 1 to 3	0 0 0	
No change	156	1 2 3	125 19 12	58	1 2 3	48 7 3	74	1 2 3	58 12 4	21	1 2 3	16 0 5	3	1 2 3	3 0 0	
Improve	20	3 to 2 2 to 1 3 to 1	2 14 4	7	3 to 2 2 to 1 3 to 1	1 5 1	11	3 to 2 2 to 1 3 to 1	1 9 1	2	3 to 2 2 to 1 3 to 1	0 0 2	0	3 to 2 2 to 1 3 to 1	0 0 0	
P-value	0.09			0.028			0.31			0.16			1			

SC – simple coiling; SAC – stent-assisted coiling; FD – flow diversion stenting; F Dis. – flow disruption; MRRC – modified Raymond-Roy classification; F/U – follow-up

Table 7 – Association between the adverse events and different treatment groups

		Whole patients	SC (No.=89)	SAC (No.=111)	FD (No.=25)	F Dis. (No.=4)	P-value
Rupture during procedure		8 (3.5)	3 (3.4)	5 (4.5)	0	0	0.78
Thromboembolic events	intra-procedure	21 (9.2)	7 (7.9)	10 (9.0)	3 (12)	1 (25)	0.80
	in 6-month	6 (2.6)	2 (2.2)	3 (2.7)	1 (4)	0	0.75
	in 12-month	3 (1.3)	1 (1.1)	2 (1.8)	0	0	0.95
Rebleeding in 1 year		15 (6.6)	5 (5.6)	8 (7.2)	2 (8)	0	0.80
Morbidity	at discharge	18 (7.9)	8 (9.0)	7 (6.3)	3 (12)	0	0.49
	after 6-month	17 (7.4)	6 (6.7)	9 (8.1)	2 (8)	0	0.94
	after 12-month	19 (8.3)	8 (9.0)	9 (8.1)	2 (8)	0	0.96
Mortality	in hospital	16 (7.0)	5 (5.6)	9 (8.1)	1 (4)	1 (25)	0.74
	after 6-month	4 (1.7)	1 (1.1)	2 (1.8)	1 (4)	0	0.58
	after 12-month	2 (0.9)	0	2 (1.8)	0	0	0.61

SC – simple coiling; SAC – stent-assisted coiling; FD – flow diversion stenting; F Dis. – flow disruption

Table 8 – Association of mRS between different treatment groups

mRS		Whole patients	SC (No.=89)	SAC (No.=111)	FD (No.=25)	F Dis. (No.=4)
before procedure	0	209 (91.3)	81 (91)	102 (91.9)	22 (88)	4 (100)
	1	7 (3.1)	5 (5.6)	1 (0.9)	1 (4)	0
	2	3 (1.3)	0	2 (21.8)	1 (4)	0
	3	8 (3.5)	2 (2.2)	5 (4.5)	1 (4)	0
	4	2 (0.9)	1 (1.1)	1 (0.9)	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
at discharge	0	191 (83.4)	76 (85.4)	92 (82.9)	20 (80)	3 (75)
	1	7 (3.1)	3 (3.4)	2 (1.8)	2 (8)	0
	2	7 (3.1)	1 (1.1)	5 (4.5)	1 (4)	0
	3	4 (1.7)	4 (4.5)	0	0	0
	4	3 (1.3)	0	2 (1.8)	1 (4)	0
	5	1 (0.4)	0	1 (0.9)	0	0
	6	16 (7.0)	5 (5.6)	9 (8.1)	1 (4)	1 (25)
in 6 months	0	189 (82.5)	77 (86.5)	89 (80.2)	20 (80)	3 (75)
	1	5 (2.2)	2 (2.2)	2 (1.8)	1 (4)	0
	2	6 (2.6)	1 (1.1)	4 (3.8)	1 (4)	0
	3	6 (2.6)	3 (3.4)	2 (1.8)	1 (4)	0
	4	3 (1.3)	0	3 (2.7)	0	0
	5	0	0	0	0	0
	6	4 (1.7)	1 (1.1)	2 (1.8)	1 (4)	0
in 12 months	0	184 (80.3)	75 (84.3)	86 (77.5)	20 (80)	3 (75)
	1	6 (2.6)	2 (2.2)	3 (2.7)	1 (4)	0
	2	9 (3.9)	3 (3.4)	5 (4.5)	1 (4)	0
	3	5 (2.2)	3 (3.4)	1 (0.9)	1 (4)	0
	4	3 (1.3)	0	3 (2.7)	0	0
	5	0	0	0	0	0
	6	2 (0.9)	0	2 (1.8)	0	0

SC – simple coiling; SAC – stent-assisted coiling; FD – flow diversion stenting; F Dis. – flow disruption; mRS – modified Rankin Scale

12 months thromboembolic events. Moreover, the SC group had the highest rate of thromboembolic events.

Similarly, 6-month and 12-month morbidities more commonly occurred at discharge in the SC group. Rebleeding in one year was also commonly observed in the SC group (15 cases). In-hospital, at 6-month, and 12-month mortality was more common in the SC group.

Regarding the clinical neurological situation, we assessed the mRS among the patients (Table 8). Among all patients, the mean mRS before the procedure was 0.05 ± 0.26, which increased to 0.22 ± 0.76 after the procedure, to 0.22 ± 0.76 in

6 months, and to 0.30 ± 0.95 in 12-month follow-up (P<0.001). Pairwise comparisons of mRS between different sessions were statistically significant for all comparisons before the procedure and at discharge, before the procedure and six months, and before the procedure and 12-month follow-up (all P-values < 0.001). This means deterioration of neurological status after the procedure. Comparisons of mRS between discharge and 6-months and 12-months were not statistically significant (P-values = 0.16 and 0.12), and the comparison between 6- and 12-months was borderline (P=0.049).

In the SC group, comparison of mRS before the procedure with mRS at discharge and mRS in 6 months and mRS in 12 months were all significant or borderline (P-values = 0.003, 0.065, and 0.036, respectively) in favour of slight deterioration of clinical situation and function. Comparison of all time pairs after the procedure (for example, comparison of discharge mRS with six months mRS) did not show a statistical difference. A similar scenario occurred in the SAC group. In the SAC group, all comparisons of mRS in pre-procedure time with after-procedure time points (including discharge mRS, six months mRS, and 12 months mRS) showed statistically significant differences; P-values were as follows: <0.001, 0.002, and 0.001, respectively. This favours slight deterioration of the patient's clinical situation and neurological function. Comparisons of mRS in all time points after the procedure did not show any statistically significant difference, meaning no significant clinical change (improvement or deterioration) in the clinical condition and function of the patients after the procedure and with time spent. FD group and F. Dis (flow disruption) did not show statistically significant changes in mRS before and after procedures (all P-values greater than 0.05).

Discussion

Following the publication of the ISAT study in 2002, endovascular treatment became the mainstay of intracranial aneurysm treatment in many centers (Molyneux et al., 2002). Moreover, it is one of the standard treatment modalities for both ruptured and unruptured intracranial aneurysms (Ferns et al., 2009). Another fundamental study with a similar background is the Barrow Ruptured Aneurysm Trial (BRAT) study, which demonstrated that the one-year outcome after treatment of ruptured aneurysms was better with coil embolization than with surgical clipping. The authors followed the patients for ten years and concluded that there was no significant difference in clinical outcome between the two treatment groups. On the other hand, while coiling had a significant advantage for posterior circulation aneurysms at one year, this study did not show the significant difference at longer follow-up. Moreover, the complete occlusion rate was significantly lower (22% vs. 93%), and the rate of cross over to the opposite treatment method was higher (36% vs. 1%) in coil-assigned patients (Spetzler et al., 2019).

Coiling has some limitations; first, as an initial treatment, all the aneurysms are not completely occluded; second, the patients with the ruptured aneurysm are at risk of early rebleeding; and third, there is a risk of reopening the aneurysm even in the case with adequate initial coil packing (Ferns et al., 2009). The factors that may play a role in initial incomplete coil occlusion include the large size of the aneurysm, intraluminal thrombosis, low packing density, initial incomplete occlusion, the duration of follow-up, ruptured aneurysm, aneurysm in the posterior circulation, and the large neck to dome ratio (Ferns et al., 2009).

A study evaluated 35 symptomatic or asymptomatic saccular aneurysms in Egypt, in which, similar to our study, females and males had the same distribution. However, the mean age of the patients was lower than our study (45.7 ± 13 vs. 51.8 ± 12.6). Similar to our findings, hypertension was the most common risk factor for intracerebral aneurysm. 96.8% of the patients were symptomatic. 32.2% of the patients were in grades 1 and 2 of H and H (Hunt and Hess scale) grade, and 67.8 in grades 3 and 4. The complete occlusion rate was 82.9%, the residual neck rate was 11.4%, and the residual sac rate with contrast within the coil mass was 2.9%. Follow-up demonstrated that 23 patients had mRS 0, 1, 2 (good outcome), and eight patients 3, 4, 5, 6 (poor outcome). Two patients died after surgery. The mean duration of follow-up was 33.03 ± 15.96 months. Moreover, at follow-up, the permanent complete occlusion, the residual neck, and the residual aneurysm rates were 79.3%, 10.3%, and 10.3%, respectively. There was no case of delayed ischemia or rebleeding. Two patients had a significant recurrence and needed retreatment. This study's risk factors for poor outcome were a history of myocardial infarction (MI), H and H grade, mRS, fisher grade, vasospasm, and vasospasm-related infarct. The immediate angiographic results showed no risk factor. Multivariate analysis demonstrated that H and H grade, mRS at admission, fisher grade, vasospasm-related infarct, and shunt-needed hydrocephalus were related to poor outcomes (Elewa, 2018).

In a study, Ferns and his co-workers (2009) systematically reviewed the studies with more than 50 patients who underwent coiling and had sufficient follow-up. 65.4% of patients had ruptured aneurysms. The mean duration of follow-up was 14.1 months, and 66.6% of aneurysms were in the anterior circulation. The initial complete occlusion, near-complete, and incomplete occlusion rates were 62.3, 29.5, and 8.2%, respectively. The sufficient initial occlusion rate was 91.2%, and the incomplete occlusion rate was 8.8%. In the follow-up, the complete, near-complete, and incomplete occlusion rates were 61.5, 22.5, and 15.8%, respectively. The sufficient complete rate was 83.4%, and the incomplete occlusion rate was 16.6%. Reopening and retreatment rates were 20.8 and 10.3%, respectively (Ferns et al., 2009).

In a multicenter study, Sophie Gallas and her colleagues (2005) evaluated 705 ruptured intracranial aneurysms treated with Guglielmi Detachable Coils. The initial complete occlusion rate was 72.6%, subtotal occlusion was 25%, and incomplete

occlusion was 2.4%. On extended follow-up (mean 36 months), the complete, subtotal, and incomplete occlusion rates were 73.9, 25.9, and 0.17%, respectively. The overall mortality was 11.4%, and the procedure-related mortality was 1.4%. The morbidity was 8.6% (Gallas et al., 2005).

In follow-up, the sufficient occlusion, reopening, and retreatment rates were 92.6, 15.5, and 6.5% in the anterior circulation, and 70.4, 22.5, and 14.5% in the posterior circulation. The reopening and retreatment rates increased with the increased aneurysm size. Although the rupture of an aneurysm was demonstrated as a risk factor for the reopening of the aneurysm in other studies, the ruptured aneurysm had a higher sufficient occlusion rate and lower reopening and retreatment rates (Ferns et al., 2009). These findings are consistent with the results of our study, which showed that coiling of the ruptured aneurysms resulted in adequate occlusion rate.

Raymond et al. (2003) evaluated the decisive factors in angiographic recurrence after endovascular treatment in a study. 501 aneurysms in 466 patients were surveyed. The mean age was 54.20 ± 12 years. 74% of the patients were female. 35.6% of the patients have multiple aneurysms. The recurrence rate was 33.6% in 12.31 months. Minor or stable recurrence was 22.1% in 33.6 month follow-up, and major recurrence was 20.7% in 16 month follow-up. The bleeding rate was 0.8% (3 patients) in 13 months of follow-up, related to angiographic recurrence. The factors related to the recurrence in this study included the treatment in the acute phase of aneurysm rupture, the size of the aneurysm, the width of the neck, the primary suboptimal angiographic results, and the duration of follow-up. The age, sex, and location of the aneurysm had no effect. The major recurrence rate was 25.1% in patients treated after aneurysm rupture and 16.3% in patients without rupture. The larger size of the aneurysm and increased neck width had a solid relation to recurrence. The significant prognostic factors for recurrence or regression were an aneurysm of more than 10 mm, a ruptured aneurysm, residual neck, residual aneurysm, and longer follow-up (Raymond et al., 2003).

Laurent Pierot et al. (2020) evaluated the one-year bleeding and rebleeding rate following intracranial aneurysm coiling. They followed 1,140 patients for 12 months. The bleeding rate was 0.0% and 1.0% in patients with unruptured and ruptured aneurysms. Multivariate analysis demonstrated that incomplete aneurysm occlusion after initial treatment (2.0% in incomplete aneurysm occlusion vs. 0.2% in complete aneurysm occlusion) and dome-to-neck ratio (1.5 ± 0.5 with rebleeding vs. 2.2 ± 0.9 without rebleeding) were associated with the rebleeding occurrence (Pierot et al., 2020). In our study, the 1-year rebleeding rate of the ruptured aneurysms in the SC and SAC groups were 5.6 and 7.2%, respectively. The underlying cause of the higher rebleeding rate in our study might be the smaller sample size and differences in aneurysm size and type between the two communities.

Petra Cimflova et al. (2021) reported the result of their study in 2020. They evaluated clinical and radiologic outcomes in 23 patients with M2 and more distal aneurysms who underwent flow diversion. The complete or near-complete occlusion

occurred in 70% of the patients in 30-month follow-up. Moreover, 70% of patients had an mRS score of excellent (0 and 1) within six months of follow-up (Cimflova et al., 2021). However, in our study, the mRS score of zero in the flow-diversion group was observed in 88% of the population before discharge, which was preserved at 80% in the subsequent follow-ups.

Imamura and his co-workers (2020) retrospectively surveyed 5,358 patients. The intraprocedural rupture rate, ischemic complications, and rebleeding rate were 4.1, 4.2, and 1.2%, respectively. The factors related to intraprocedural rupture included female, bifurcation type, a size less than 5 mm, emergency treatment, local anesthesia, and balloon-assisted coiling. The factors which were effective in ischemic complications included poor grade in World Federation of Neurosurgical Societies (WFNS) grading, width neck, and stent-assisted coiling. The affective factors in rebleeding included poor WFNS grading, bifurcation type, width neck, and body filling as the initial result (Imamura et al., 2020).

After endovascular treatment, Laurent Pierot and co-workers (2008) evaluated the immediate clinical outcome for unruptured intracranial aneurysms. Six hundred forty-nine patients were analysed. Coiling, SAC, and BAC were performed in 54.9, 37.3, and 7.8% of the patients. Endovascular treatment was unsuccessful in 4.3% of the patients. The complications were observed in 15.4% of the patients (7.1% thromboembolic events, 2.6% intraprocedural rupture, and 2.9 device-related events). The complications which resulted in a temporary or permanent neurologic deficit occurred in 5.4% of the patients. The one-month morbidity and mortality were 1.7 and 1.4%, respectively (Pierot et al., 2008).

In a study by Chalouhi and colleagues in 2013, compared to coiling, treatment with pipeline embolization device (PED), which is a type of flow diversion, showed a lower need for retreatment of the unruptured aneurysms. The results of our study also showed similar findings. As shown in Table 5, there was a lower need for second and third-session treatments in the F Dis. Group (Chalouhi et al., 2013).

The results of our study also showed that compared to the 6-month (69.9%), RROC in the flow diversion group was improved at 12 months (78.3%). Interestingly, the RROC was reduced at 6 and 12 months in the SC and SAC groups. Therefore, it could be concluded that flow diversion is more effective in RROC at six and 12-month follow-ups. However, according to the nature of this study which is a retrospective study and the small number of patients in the flow diversion group, these data could not be generalized. Therefore, a larger prospective randomized clinical trial is required to compare the efficacy of coiling with flow diversion.

Conclusion

Similar to the other studies, the results of the present study are promising and demonstrate the effectiveness of the neurovascular intervention as a first therapeutic

modality for ruptured and unruptured aneurysms. A double-blind, randomized clinical trial that compares the patients who underwent coiling and flow diversion is needed to eliminate the efficacy and confounding factors affecting the outcome of ruptured intracranial aneurysm subjects.

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Raised First Trimester Thyroid Peroxidase Antibodies May Predict First Trimester Miscarriage: A Case Control Study

Boniface Ago[1,2], Enya Okpani[2], Sylvester Abeshi[1,2], Lawson Ekpe[3]

[1] Department of Obstetrics and Gynaecology, University of Calabar, Calabar, Nigeria;
[2] Department of Obstetrics and Gynaecology, University of Calabar Teaching Hospital, Calabar, Nigeria;
[3] Department of Chemical Pathology, University of Calabar, Calabar, Nigeria

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Abstract: Miscarriages constitute a significant aspect of failed pregnancies and a source of worry for the patient and caregiver. Some of the causes of miscarriages remain unknown. Immunological conditions such as thyroid autoimmunity could play significant roles. Our objective was to determine the relationship between raised thyroid peroxidase antibodies and first trimester miscarriages in a low resource setting. This was a case control study at the Gynaecological Clinic of the University of Calabar Teaching Hospital, Nigeria; from 14th February 2020 to 13th January 2021, involving 145 cases who had first trimester miscarriages, and their matched controls who had apparently normal pregnancies, at same gestational ages. Sera of venous blood from both participants and controls were analysed for thyroid peroxidase antibodies using enzyme-linked immunosorbent assay, and analysed using SPSS version 20, and GraphPad Prism 8.4.3 statistical software. Being a civil servant and low social status had significant odds for first trimester miscarriage. Raised thyroid peroxidase antibodies in the first trimester had 10-fold odds for miscarriage. Odds ratio 10.34, 95% CI: 3.22 to 32.98, P-value = 0.0001. The test had a sensitivity of 89.66% and specificity of 54.41%. The positive predictive value was 17.93%, while the negative predictive value was 97.93% and a likelihood ratio of 1.966. Rising thyroid peroxidase antibodies in early pregnancy could be a predictor for miscarriage. This is so because patients with raised thyroid peroxidase antibodies in the first trimester had a 10-fold risk of having a first trimester miscarriage.

Mailing Address: Assoc. Prof. Boniface Ago, MD., Department of Obstetrics and Gynaecology, University of Calabar, Unical Hotel Road, Etta Agbor, Calabar, Nigeria; Phone: +234 802 321 53 19; e-mail: bonifaceago@yahoo.com

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Introduction

First trimester miscarriage is a challenging experience for both the patient and the physician, it is a source of maternal anxiety and yet commonly encountered in clinical practice. Up to 75% of fertilized ova and at least 15% of clinically recognized pregnancies never survive to birth (Jurkovic et al., 2013; Stagnaro-Green, 2015). First trimester miscarriage is defined as the loss of pregnancy before 13 weeks of gestation (American College of Obstetricians and Gynecologists' Committee on Practice Bulletins – Gynecology, 2018). Both ectopic and molar pregnancies are not usually included in this definition. Most miscarriages occur early as seen in a study where over 80% of cohort participants had first trimester pregnancy losses (Lawani et al., 2022) with approximately half occurring before or just after a missed menses. It is thought that an overwhelming majority of miscarriages are the result of chromosomal abnormalities (Jurkovic et al., 2013). In a study in Jos, Nigeria, thyroid peroxidase antibodies were positive in 11.4% of women with first trimester miscarriage as against 4.5% of pregnant women who have had a previous normal delivery without miscarriage (Samson et al., 2018). In India, Bhattacharyya et al. (2015) reported a prevalence of 10.87% of miscarriages among women with positive thyroid peroxidase antibodies as against 4.8% in women who were negative for thyroid peroxidase antibodies.

Considering the fact that, the exact cause(s) of first trimester miscarriage is not known in over 50% of cases (Twig et al., 2012), this has become a topic of consideration by researchers. Several aetiological reasons have been put forward. Immunological disorders are known causes of first trimester miscarriage, these include: rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus, antiphospholipid antibody syndrome (APS), thyroid disorders among others. There is evidence that thyroid autoimmunity is an important risk factor for miscarriage and preterm birth, as it has been reported that the presence of thyroid autoantibodies is relatively common in women of reproductive age (Stagnato-Green, 2015). Studies have shown that the prevalence of thyroid autoantibodies in women of reproductive age range from 5 to 20%, and in about 22.1% of women seeking fertility in Damascus (Aljarad et al., 2019). Even in women with biochemically normal thyroid function, studies have reported presence of thyroid autoantibodies, particularly thyroid peroxidase (TPO) antibodies in 10–15% of normal individuals (Frohlich and Wahl, 2017). Pregnant women who are euthyroid but who are positive for thyroid peroxidase antibodies are at increased risk for miscarriages, preterm birth, pregnancy-induced hypertension, intrauterine growth restriction, and intrauterine fetal demise (Rajput et al., 2017).

Miscarriages in women with positive thyroid autoantibodies occur within the first trimester of gestation (Lata et al., 2013) when the fetus is critically dependent on maternal thyroid hormones. Therapeutic interventions aiming at modulating the immune system of women with autoantibodies have included intravenous

immunoglobulin administration. This treatment resulted in an increase in the percentage of women who had successful pregnancies (Lata et al., 2013). An earlier study (Negro et al., 2006) divided thyroid autoantibody positive women into two groups. One group was treated with levothyroxine (LT4) in a dose based on the thyroid stimulating hormone (TSH) starting values. The other group was not treated. A third group served as a normal general population control. The TSH values of the untreated group remained significantly higher than those of the other two groups during the entire gestational period. TSH and free thyroxine (FT4) values in the treated group were not significantly different from the control group. The treated group and the controls showed a similar miscarriage rate (3.5 and 2.4%, respectively), which was lower than that of the second group (13.8%) who did not receive LT4. The conclusion of this study was that thyroid autoantibody positive women have an increased risk of miscarriage but when given the benefit of treatment with thyroid hormone, they behave as the normal ones.

It is necessary to study the association of thyroid autoantibodies in early pregnancy and first trimester miscarriages in Calabar.

Aims and Objectives

Specific aim

To determine the relationship between presence of thyroid autoantibodies and first trimester miscarriages at University of Calabar Teaching Hospital.

Specific objectives

1) To study the sociodemographic characteristics of women with first trimester miscarriage and their matched controls.
2) To determine the odds of raised thyroid peroxidase antibody on first trimester pregnancy.
   Null hypothesis: There is no relationship between raised thyroid peroxidase antibody and first trimester miscarriage.
   Alternate hypothesis: A relationship does exist between raised thyroid peroxidase antibody and first trimester miscarriage.

Methods

Study design

This was a case control study.

Study setting

This study was conducted in the Department of Obstetrics and Gynaecology of the University of Calabar Teaching Hospital, Calabar. The hospital is located within

the Calabar metropolis providing tertiary health services to more than 3 million people in Cross River State and also serves as a referral center for adjourning States of Akwa Ibom, Abia, Benue as well as the Republic of Cameroun and Equatorial Guinea. The cases and controls were studied from 14th February 2020 to 13th January 2021.

Participants

The study population comprised 145 consenting women presenting with miscarriage within 12 weeks of gestation in the gynaecological emergency unit of the University of Calabar Teaching Hospital (UCTH), Calabar, Nigeria. These participants are referred to as "cases". The "control" group comprised 145 consenting pregnant women (within 12 weeks of gestation) who have had a previous successful pregnancy, without history of previous miscarriage, matched for maternal age and gestational age with the "cases", and presented for routine antenatal care.

The research was conducted in line with requirement for the conduct of research on human subjects. It was registered with University of Calabar Teaching Hospital Research and Ethics Committee with number NHREC/07/10/2012 and assigned approved protocol number UCTH/HREC/33/692.

Inclusion criteria:

1) Women presenting with miscarriages at or before 12 weeks of gestation.
2) Maternal age and gestational age-matched women with normal pregnancies at or before 12 weeks of gestation.

Exclusion criteria:

1) Women who refuse to give consent.
2) Obese pregnant women.
3) Maternal age more than or equal to 40 years.
4) Women known to have rheumatoid arthritis, chronic renal disease, diabetes mellitus or antiphospholipid syndrome.

Variables

The outcome measure was first trimester miscarriage. The exposure was raised thyroid peroxidase antibody in first trimester pregnancy.

Data sources/sampling method and data collection

Women who presented in the gynaecological emergency unit with miscarriage (≤ 12 weeks) were the case group, while the control group were women matched for age, and gestational age with apparently normal pregnancy at that time. Convenient sampling technique was used in selecting controls to match the cases. Consent was taken from the respondents before data collection.

Test procedure

Blood samples were collected by venepuncture of the antecubital vein aseptically. About 5 ml of blood was collected into a plain vacutainer tube using a 5 ml syringe. It was left undisturbed to clot at room temperature over 15–30 minutes. The sample was centrifuged at 2,500–3,000 rpm for 5–10 minutes, after which it separates into upper liquid layer, and the serum, below the upper layer, was stored at –20 °C before analysis. The product kit used was Accubind-ELISA microwells, Monobind, CA 92630 USA. Assays were done according to the kit's manufacturer's specification. One hundred µl of calibrators, controls and prediluted samples were drawn into the wells, and incubated for 30 minutes at room temperature (20–28 °C). The content of each microwell was discarded and washed 3 times with 300 µl of wash solution. One hundred µl of enzyme conjugate was added into each well and then incubated for 15 minutes at room temperature. The contents of the microwells were discarded and washed 3 times with 300 µl of wash solution; one hundred µl of tetramethylbenzidine (TMB) substrate solution will be added into each well and incubated at room temperature for 15 minutes. The reaction was stopped by adding 100 µl of stop solution to each well of the modules and incubate for 5 minutes at room temperature. Optical density at 450 nm was read and results calculated. The optical density (OD) is proportional to the antibody concentration, which is interpreted using a standard chart. Values above 35 IU/ml are considered positive for the presence of anti-TPO autoantibodies (Loh et al., 2016). However, our laboratory used values above 40 IU/ml as positive.

Quality control for the assays were ensured using pooled sera as control specimen run in duplicate with each assay batch and the inter and intra-batch coefficient of variations computed to examine the analytical precision.

All samples were subjected to the same processing to eliminate bias.

A researcher-administered questionnaire was used to collect information on the sociodemographic characteristics of the participants (cases and controls).

Estimation of sample size

The sample size was estimated using the formula (Charan and Biswas, 2013):

$$n = \frac{\{P_1(1-P_1) + P_2(1-P_2)\} \times (Z_\alpha + Z_\beta)^2}{(P_1-P_2)^2}$$

Where:

n = number of sample size in each of the group

P_1 = proportion of positive TPO autoantibodies among women with miscarriage (0.11 in a similar study) (Samson et al., 2018)

P_2 = proportion of positive TPO autoantibodies in the control group (0.05 in the same study)

$Z\text{-}_{\alpha/2}$ = value of standard normal distribution corresponding to a significance level of alpha (1.96 for two-sided test at the 0.05)
$Z\text{-}_{\beta/2}$ = value of standard normal distribution corresponding to the desired level of power (0.84 for a power of 80%)

$$n = \frac{\{(0.11\times0.89 + 0.05\times0.95)\} \times (1.96 + 0.84)^2}{(0.06)^2} \qquad n \approx 134$$

The sample size was scaled up to 145 for the cases and 145 for the controls.

Analyses of the sociodemographic characteristics of the cases and controls were performed using SPSS software (version 20.0) and presented as frequencies and percentages. Categorical and continuous variables were compared using chi-squares and Fischer's exact tests as appropriate, and P-values are shown in Table 1.

The result of thyroid peroxidase antibodies of the participants (cases and controls) is presented in Table 2. The sensitivity, specificity, positive and negative predictive values as well as the likelihood ratio were also determined using GraphPad Prism software, and the result presented in Table 3.

Apart from age and ethnicity other sociodemographic characteristics were compared by a contingency analysis using GraphPad Prism software. The results are presented in Figure 1.

The result of thyroid peroxidase antibodies was used to dichotomize those with raised antibodies (> 40 IU/ml) from those (≤ 40 IU/ml) between cases and controls. The values as shown in Table 2 were analysed for contingency using GraphPad Prism software version 8.4.3, San Diego, California. Fishers' exact test determined a two-sided P-value between the groups. The odd ratios (OR) and 95% confidence intervals (CI) are shown in Figure 2. A P-value of < 0.05 was considered as statistically significant.

Results

Table 1 shows sociodemographic characteristics of the participants. The mean age of the women with miscarriages and control groups was 29.87 ± 3.84 years. Education of the women was not significantly different at P-value < 0.05, however, the distribution of their occupation, husband's education and occupation and social class were significantly different.

Table 2 shows the distribution of participants according to result of antibody test for the cases and controls. There were 26 patients with miscarriage and 3 in the control group who had thyroid peroxidase antibodies above 40 IU/ml. In Table 3, the values in Table 2 were subjected to contingency analysis for sensitivity, specificity, positive and negative predictive values. The sensitivity of the test was 89.7%, specificity 54.4%, positive predictive value 17.9%, negative predictive value 97.9% and a likelihood ratio of 1.97.

Table 1 – Sociodemographic characteristics of women with first trimester miscarriages (cases) and their matched controls

Variables	Cases (n=145)		Control (n=145)		Chi-square	P-value
	frequency	%	frequency	%		
Age group						
≤ 35	135	93.1	135	93.1		
> 35	10	6.9	10	6.9		
Education						
Primary	32	22.2	19	13.3		
Secondary	84	57.8	103	71.1	5.90	0.0514
Tertiary	29	20.0	23	15.6		
Ethnicity						
Efik	23	15.9	23	15.9		
Ibibio	29	20.0	23	15.9		
Obudu	42	29.0	16	11.0	30.52	<0.00001*
Igbo	26	17.9	19	13.1		
Others	25	17.2	64	44.1		
Occupation						
Housewife	32	22.1	52	35.9		
Student	10	6.9	22	15.2		
Trader	45	31.0	42	28.9	23.05	0.00012*
Civil servant	32	22.1	9	6.2		
Self employed	26	17.9	20	13.8		
Husband education						
Primary	26	17.9	10	6.9		
Secondary	71	49.0	106	73.1	18.70	0.000086*
Tertiary	48	33.1	29	20.0		
Husband occupation						
Self employed	29	20.0	42	29.0		
Civil servant	48	33.1	19	13.1		
Trader	52	35.9	48	33.1	32.09	<0.00001*
Unemployed	10	6.9	6	4.1		
Artisan	6	4.1	30	20.7		
Social class						
Low	61	42.1	12	8.3		
Middle	61	42.0	120	82.8	54.90	<0.00001*
High	23	15.9	13	8.9		

*significant P-value < 0.05

Figure 1 shows a comparison of five sociodemographic characteristics listed in Table 1.

Figure 1A compared participants' occupation. Being a civil servant was at increased risk for miscarriage (OR 4.28, 95% CI 3.06 to 9.44).

Table 2 – Results of thyroid peroxidase antibody among women with first trimester miscarriage and matched controls

Data analysed	Thyroid peroxidase > 40 IU/ml	Thyroid peroxidase ≤ 40 IU/ml	Chi-square	P-value
Cases	26	119		
Controls	3	142	20.2682	<0.00001
Column total	29	261		

Table 3 – Results of sensitivity, specificity, positive and negative predictive values as well as likelihood ratio of the test

Effect size	Value (%)	95% confidence interval (%)
Sensitivity	89.66	73.61 to 96.42
Specificity	54.41	48.34 to 60.34
Positive predictive value	17.93	12.54 to 24.98
Negative predictive value	97.93	94.09 to 99.44
Likelihood ratio	1.966	

Figure 1B compared participants' educational status. There was no increase in odds when primary level of education was compared with other levels, P-value was 0.0635.

Figure 1C compared participants' husbands' level of education. Women whose husbands had only primary level education were at increased odd for miscarriage (OR 2.95, 95% CI 1.38 to 6.04).

Figure 1D compared participants' husbands' occupation. Women whose husbands were civil servants were at increased odd for miscarriage (OR 3.28, 95% CI 1.80 to 6.04).

Figure 1E compared social status of participants. Women of low social status were at increased odd for miscarriage (OR 8.05, 95% CI 4.11 to 15.26).

In Figure 2, there were 26 positive cases within the group of women who had first trimester miscarriage, and 3 among those of matched controls. We identified two groups; one with raised thyroid peroxidase > 40 IU/ml and another with values ≤ 40 IU/ml, which were analysed. The OR was 10.34 with a 95% CI of 3.22 to 32.98, and P-value 0.0001.

Discussion

Social class, husband education and occupation as well as wife occupation are significant risk factors for miscarriage as shown in Table 1. The odd ratios in Figure 1A, D and E, infer that being a civil servant, especially of low socioeconomic

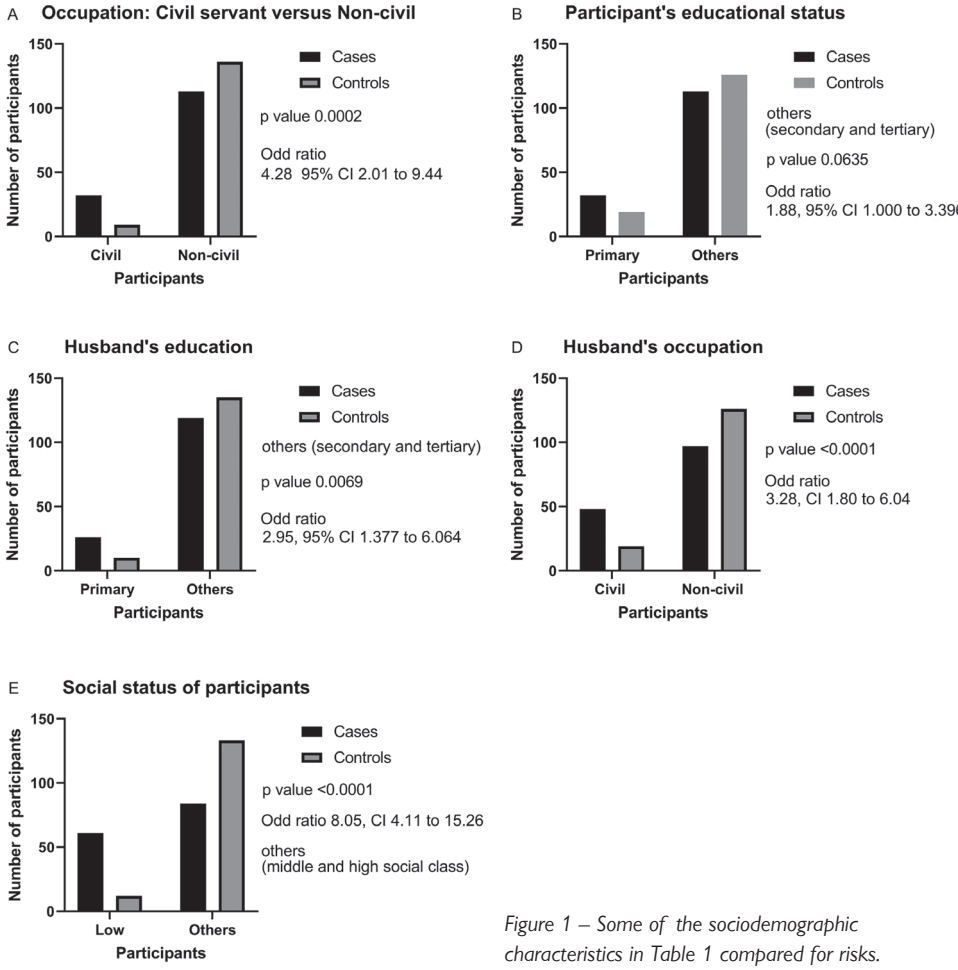

Figure 1 – Some of the sociodemographic characteristics in Table 1 compared for risks.

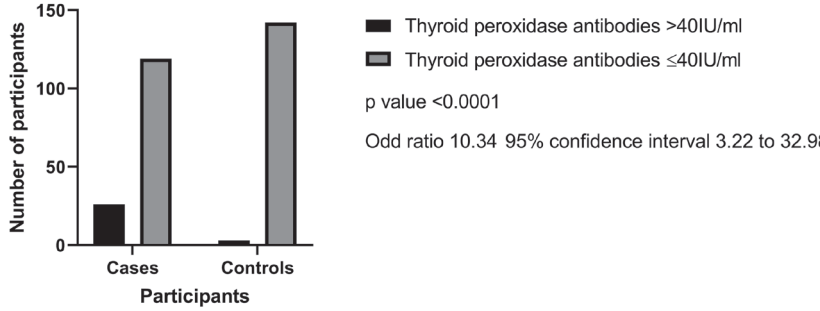

Figure 2 – Bar chart showing the number of participants with raised thyroid peroxidase antibody (levels > 40 IU/ml) among women with first trimester miscarriage (cases) and their matched controls in the University of Calabar Teaching Hospital, Calabar.

status was significantly associated with increased odd for miscarriage. This is a significant finding and a wakeup call for policy implementers in the Nigerian civil service. Low socioeconomic status, on its own, is associated with adverse pregnancy outcomes (Thomson et al., 2021).

In Tables 2 and 3, there were 26 patients with first trimester miscarriage and 3 in the control group who had thyroid peroxidase antibodies above 40 IU/ml. This method of testing has a sensitivity of 89.7% and a negative predictive value of 97.9%. In Figure 2, patients with raised thyroid peroxidase antibodies in the first trimester had a 10-fold odd of having a first trimester miscarriage. It could be inferred that testing for thyroid peroxidase antibodies in early pregnancy could detect those who might be at risk for miscarriage because of thyroid autoimmunity.

One of our study limitation was the challenge of getting pregnant women coming to book at or before 12 weeks of pregnancy. Most pregnant women book after the first trimester, with about 24.8% booking in the first trimester (Oliobi et al., 2019).

In Table 2, thyroid autoimmunity as evidenced by raised thyroid peroxidase antibodies in this study was present in 26 of 145 (17.9%) of women with first trimester miscarriages, and in three of 145 (2.1%) of matched controls. In the study by Lata et al. (2013), the miscarriage rate was 13.8% in the thyroid autoantibody positive group and 2.4% in the antibody negative group. In a similar study, Bhattacharyya et al. (2015) reported a miscarriage of 10.87% among thyroid peroxidase positive pregnant women against 4.8% in the thyroid peroxidase negative pregnant women in the first trimester. Samson et al. (2018) reported autoimmunity of 11.4% in Jos, North Central Nigeria, and 9% prevalence documented by Jibril et al. (2015) in Zaria. Our study design was case control, which is not a prevalence study. However, the prevalence of 17.9% in our study was deduced from a subset of women with miscarriage who had raised thyroid peroxidase antibodies above 40 IU/ml. In addition, our control group were pregnant women who had no previous history of miscarriage rather than those negative for thyroid peroxidase antibodies. Samson et al. (2018) did a case control study using 44 patients with miscarriage at mean gestational age of 11.57 ± 4.3 weeks (cases) and 44 pregnant women with previous history of delivery without miscarriage at mean gestational age of 17.9 ± 4.9 weeks as control. This study compared the thyroid peroxidase antibodies on both groups with finding of 11.4% in cases and 4.5% in the control. The difference was not statistically significant. Our study had a larger population and the participants' ages and gestational ages were matched. Our finding of 17.9% positivity for thyroid peroxidase antibodies in cases and 2.1% in the control group was statistically significant.

In Table 1 the sociodemographic characteristics of the women recruited in this study revealed that level of education of the women was the only factor that was not statistically significant. Poor husband education and occupation may mean

poor feeding (poor iodine intake) and high risk to environmental toxicants, which may affect thyroid function (Brent, 2010). All the twenty-nine patients (including controls) who had raised thyroid peroxidase antibodies were aged 35 or less. This does not refute the fact that thyroid disease can occur at any age as variously reported in Nigeria (Okafor et al., 2019). Our study focused on patients with miscarriage, but tested thyroid peroxidase antibodies in comparison with matched controls.

The patients in the control group who had raised thyroid peroxidase antibodies in first trimester were counselled on the need to take levothyroxine to prevent adverse pregnancy outcome in the second or third trimester. Although raised thyroid peroxidase antibodies in pregnant euthyroid women increased the risk of miscarriages and preterm birth, and treatment with levothyroxine decreased miscarriage rate (Thangaratinam et al., 2011; Dal Lago et al., 2021). However, Dhillon-Smith et al. (2019) in a randomized controlled trial involving 19,585 women in 49 hospitals in the United Kingdom failed to demonstrate significant difference in the pregnancy outcomes. Further research in different communities is necessary to elucidate the role of levothyroxine in preventing adverse pregnancy outcomes in this group of patients.

Conclusion

There was a 10-fold odd of having first trimester miscarriage in pregnant women with raised thyroid peroxidase antibodies. This study buttressed the fact that endocrine disorder is a significant contributor to first trimester pregnancy loss.

This study establishes a local data on thyroid autoimmunity and first trimester miscarriage, which could serve as template for a broad-based and multi-center study on this topic in order to improve on its validity.

Early assay of rising thyroid peroxidase antibody in first trimester may predict a pregnancy that may miscarry if not protected.

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Laryngotracheoesophageal Cleft Type IV in a Preterm Neonate. A Case Report and Literature Review

Rozeta Sokou[1,2], Anastasia Batsiou[1], Aikaterini Konstantinidi[1], Paschalia Kopanou-Taliaka[1], Marina Tsaousi[2], Maria Lampridou[1], Evangelia-Filothei Tavoulari[1], Konstantinos Mitropoulos[1], Paraskevi Liakou[1], Stamatina Willadara-Gamage[1], Petros V. Vlastarakos[3], Zoi Iliodromiti[2], Theodora Boutsikou[2], Nicoletta Iacovidou[2]

[1] Neonatal Intensive Care Unit, "Agios Panteleimon" General Hospital of Nikea, Piraeus, Greece;
[2] Neonatal Department, Medical School, National and Kapodistrian University of Athens, Aretaieio Hospital, Athens, Greece;
[3] 2nd ENT Department, National and Kapodistrian University of Athens, Attikon University Hospital, Athens, Greece

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Abstract: We present a case of a preterm neonate with a type IV laryngo-tracheo-oesophageal cleft, an uncommon congenital malformation, resulting from the failure of separation of the trachea and the oesophagus during fetal development, often associated with other deformities as well. Data in the literature shows that the long-term morbidity from the entity has declined over the last decades, even though prognosis remains unfavourable for types III and IV. This report emphasizes the complex issues neonatologists are faced with, when treating neonates with this rare disorder in the first days of life, what will raise suspicion of this rare medical entity, and that direct laryngoscopy/bronchoscopy finally depicts the exact extension of the medical condition. At the same time extensive evaluation for coexisting congenital anomalies should be performed. For all the above reasons, these neonates should be treated in specialized tertiary pediatric centers for multidisciplinary prompt management, which may improve, the outcome.

Mailing Address: Rozeta Sokou, MD., MSc., PhD., Neonatal Intensive Care Unit, "Agios Panteleimon" General Hospital of Nikea, 3 Dimitrios Mantouvalos Str., 18454 Piraeus, Greece; Neonatal Department, Medical School, National and Kapodistrian University of Athens, Aretaieio Hospital, 76 Vasilissis Sofias Str., 11528, Athens, Greece; e-mail: sokourozeta@yahoo.gr

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Introduction

Laryngo-tracheo-oesophageal cleft (LTC) is a rare congenital anomaly, characterized by deficient anatomical separation of the oesophagus and the upper respiratory tract, at the level of the larynx and trachea. The incidence varies from 1 in 10,000 to 20,000 live births, with a male:female ratio of 5:3 (Griffith and Liversedge, 2014). The first reported case was made by Richter in 1792 in a newborn with feeding difficulties and recurrent aspiration (Richter, 1792; Benjamin and Inglis, 1989; Griffith and Liversedge, 2014). Laryngeal clefts comprise 0.3–0.5% of all the congenital anomalies of the larynx (Griffith and Liversedge, 2014). The classification into 4 types of clefts, as proposed by Benjamin and Inglis in 1989, is the most commonly used, as depicted in Figure 1.

Type I is a supraglottic cleft, above the vocal cords. Type II is a partial cricoid cleft, below the level of the vocal cords. Type III is a total cricoid cleft, extending through the cricoid cartilage to the cervical trachea/oesophagus. Type IV is

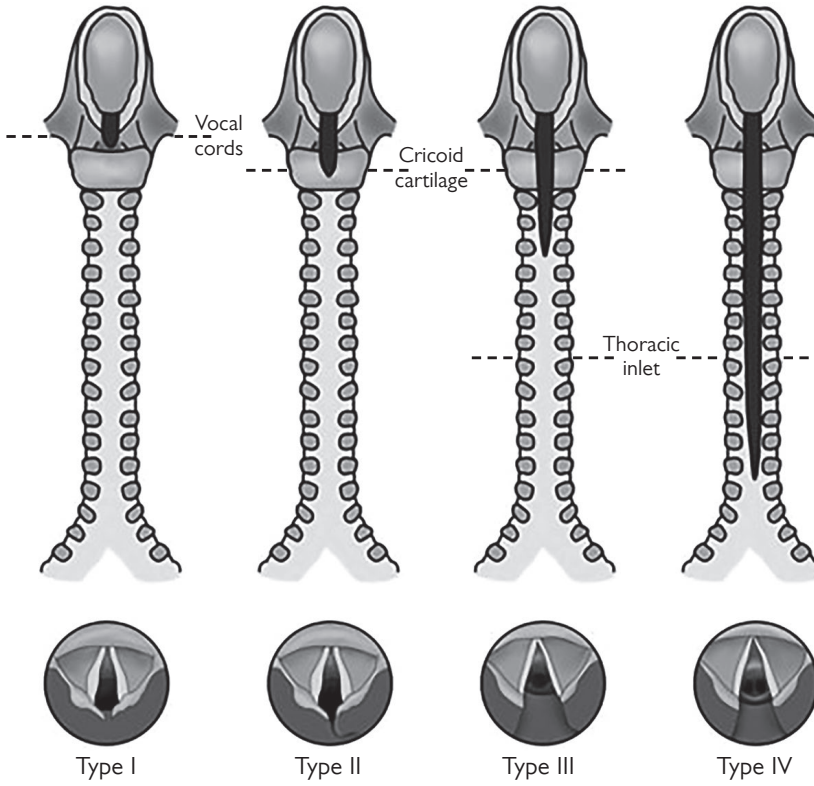

Figure 1 – Types I–IV of laryngo-tracheo-oesophageal cleft based on the classification of Benjamin and Inglis (1989).

a laryngo-esophageal cleft extending to the thoracic trachea (Benjamin and Inglis, 1989). Significant controversy surrounds the embryological origin of trachea-oesophageal anomalies. A traditional theory suggests that the separation of the foregut is the result of merging of the lateral ridges arising from the lateral foregut wall. This process begins cranially and extends caudally and thus a septum is created, dividing the foregut into a ventral component, the laryngotracheal tube and a dorsal component, the oesophagus (His, 1880; Merei and Hutson, 2002). In 1984, O'Rahilly and Müller used a "respiratory tap" analogy to describe the formation of the respiratory tract. According to their theory, the pulmonary anlage, arising ventrally from the foregut mesenchyma, extends caudally forming in the process the tracheoesophageal septum. Later Kluth et al. (1987) indicated that the respiratory diverticulum arises from the ventral aspect of the foregut and continues to elongate, forming a stalk from which the trachea will develop. In 2009 Brown and James in a systematic review of the literature proposed that the trachea and oesophagus develop from 2 completely different parts of the trilaminar embryonic disc, but due to the cephalic folding, they approximate and form a common tracheoesophageal chamber that is later divided. In other words, the respiratory primordium is formed *in situ* as a ventral component of the foregut and does not consist of a protrusion of the gut. Disruptions during the embryonic development of the tracheobronchial system and the oesophagus can lead to various anatomical deformities of the area (Jöhr et al., 2003).

Aspiration episodes and airway obstruction are the usual clinical signs of a tracheal cleft. The clinical presentation depends on the type and extend of the cleft, and on other coexisting congenital anomalies (Jorgensen et al., 2018). Infants with type I usually have non-specific symptoms, such as stridor, feeding difficulties or swallowing disorders, and may remain undiagnosed for a long period. Infants with types II and III present with pulmonary tract infections, due to significant aspiration (Leboulanger and Garabédian, 2011). Infants with type IV cleft display the most serious clinical presentation, with early respiratory distress and difficulty in maintaining mechanical ventilation.

Congenital malformation of the gastrointestinal tract (16–67%) (Leboulanger and Garabédian, 2011), such as trachea-oesophageal fistulae, oesophageal atresia or anal atresia are the most common coexisting anomalies (Roth et al., 1983). Congenital malformations from the respiratory tract (2–9%), the cardiovascular system (16–33%), the genitourinary tract (14–44%), as well as the craniofacial malformations (5–15%) are less frequent (Leboulanger and Garabédian, 2011). Laryngeal cleft may represent a congenital malformation within the context of various syndromes, such as the Opitz G/BBB syndrome (50% of children with this syndrome have a LTC [Shehab and Bailey, 2001]), 22q11 monosomy, the Pallister Hall syndrome, the VATER/VACTERL association, the CHARGE syndrome (Leboulanger and Garabédian, 2011), DiGeorge, Cayler cardiofacial and velocardiofacial syndrome (Griffith and Liversedge, 2014).

Endoscopy sets the diagnosis of LTC, makes possible the differential diagnosis for various other medical conditions, and may also reveal other coexisting malformations (Leboulanger and Garabédian, 2011).

Management of LTC depends on the type of cleft. First of all, satisfactory ventilation must be maintained, for which endotracheal intubation may be needed (Leboulanger and Garabédian, 2011). In types III and IV, adequate nutrition of the baby via a nasogastric tube or with parenteral nutrition (due to high risk of aspiration), may be required (Seidl et al., 2021). As for the closing of the cleft, various techniques have been published, both endoscopically and by open surgical approach (Seidl et al., 2021). For types III and IV an open surgical approach is the mainstay (Seidl et al., 2021).

We aimed at presenting a premature neonate, admitted to a tertiary neonatal intensive care unit, with the diagnosis of LTC type IV, based on the classification of Benjamin and Inglis (1989). This case provides educational points on the clinical signs and conditions, that could aid the neonatologist to make a prompt diagnosis of this rare developmental anomaly. We focus on the difficulties while treating the patient and the team-oriented management that is crucial for optimizing the outcome of this uncommon, yet ominous deformity.

Case report

A 32-week of gestation female neonate with birth weight of 1,470 g was born to a healthy 38-year-old Gravida 2 Para 1 mother, via an emergency caesarean section due to non-reassuring fetal status. Pregnancy was uneventful until 28 weeks when oral glucose tolerance test (OGTT) confirmed gestational diabetes mellitus, and ultrasonography showed polyhydramnios (confirmed at the caesarean section) with absent fetal stomach. At birth the neonate was floppy, apneic with heart rate < 100 beats/min. Resuscitation with sustained inflation was provided with inadequate response, and the neonate was intubated with great difficulty by the attending neonatologist with a size 3 endotracheal tube (ET). On admission to neonatal intensive care unit (NICU), the neonate was connected on a ventilator on volume guarantee mode. The achievement of delivering appropriate tidal volume to the neonate was impossible, and large air leak of 80–90% was recorded. Due to vigorous respiratory effort of the neonate, ventilation mode was changed to pressure control. In order to confirm or to rule out oesophegeal atresia, a size 6-8FR nasogastric tube (NGT) was sited with great difficulty as well. Chest X-ray showed air in the stomach, and the tip of NGT *in situ* (Figure 2).

Early after birth, the neonate developed respiratory distress and received one dose of surfactant, maintaining adequate respiratory drive thereafter. Vital signs and physical examination were otherwise normal. The difficult intubation procedure

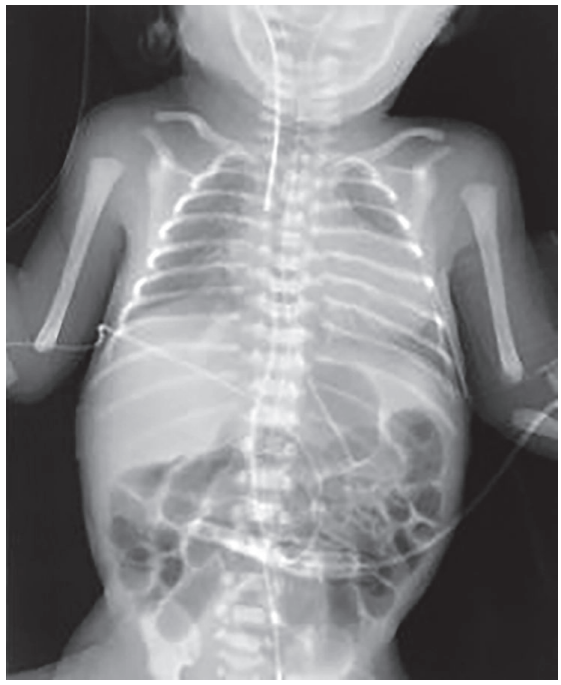

Figure 2 – Chest X-ray with nasogastric tube into the stomach.

with a large air leak, and the aspiration of gastric content during endotracheal suctioning raised the suspicion of H-type tracheoesophageal fistula. Pediatric surgical consultation was carried out, and a barium swallow study was scheduled for the next day, provided that the patient's clinical condition would be permissive. However, during the ensuing hours the neonate's pulmonary status gradually deteriorated, and the barium swallow study was deferred. Echocardiography and abdominal ultrasonography were normal, and cranial ultrasonography depicted grade II intraventricular hemorrhage. Reintubation, following accidental extubation, on the 3rd day of life proved to be a challenge. Abnormal upper airway anatomy and inability to visualize the entry of the trachea and oesophagus raised suspicion of a laryngeal cleft. The infant was transferred to a tertiary pediatric hospital for diagnostic laryngotracheobronchoscopy by pediatric specialists. The next day, after accidental extubation, the neonate developed pneumothorax, while multiple attempts for reintubation were performed. At laryngoscopy, the diagnosis of laryngeal cleft type IV was confirmed by otolaryngologist (ear, nose, throat – ENT). Unfortunately, due to the severity of the neonate's condition no documentation of the endoscopic examination was obtained. Soon after, the patient died, prior to genetic counselling that had been scheduled. Parents did not consent to postmortem examination.

Discussion

We present a case of a premature neonate, with type IV laryngo-tracheo-oesophageal cleft, a rare congenital malformation which to the best of our knowledge, is the first to be reported in Greece.

Laryngo-tracheo-oesophageal cleft is often associated with other congenital anomalies, such as tracheobronchial, gastrointestinal and cardiac malformations (Seidl et al., 2021). Seidl et al. (2021) reported that half (4 patients out of 9) of their cohort, were diagnosed with a genetic syndrome. Unfortunately, in our case, no genetic counselling was performed. Type IV LTC is the rarest form of laryngeal clefts and carries the worst prognosis. Martha et al. (2021) in a literature review of laryngeal cleft studies from 2010 to 2021, reported only 3 (0.32%) out of 1,033 patients with clefts, to have type IV.

No specific pathognomonic prenatal findings associated with laryngeal clefts exist. The polyhydramnios, observed in the index patient, is a very common finding described in many cases of airway clefts (Carr et al., 1999; Alnemri et al., 2010; Seidl et al., 2021), and is attributed to impaired fetal swallowing. Absent stomach bubble, as reported in our case, is also a prenatal fetal finding in LTCs. Kawaguchi et al. (2005) reported that polyhydramnios was noted in 5 of 6 fetuses, and absent stomach bubble in 2 others on prenatal sonography. The association between LTCs and premature birth, supported by the literature, is also exemplified in our case, as the neonate was born at 32 weeks of gestation (Moungthong and Holinger, 1997; Seidl et al., 2021).

The diagnosis of laryngeal cleft is difficult to establish, especially early postnatally, and several other entities must be included in the differential diagnosis. A LTC may imitate clinically oesophageal atresia or a tracheoesophageal fistula. Passing a NGT into the stomach rules out esophageal atresia. Barium swallow study, although it can provide adjunct information, may not be able to differentiate between tracheoesophageal fistula (TEF) and severe forms of LTC (III and IV), as contrast medium will be present in the trachea in both cases. Additionally, the 2 entities are often encountered in the same patient, with 20–37% of LTCs being complicated by TEF (Mahour et al., 1973; Fraga et al., 2015; Londahl et al., 2018). Definite diagnosis is set with endoscopy. Additional imaging, such as magnetic resonance imaging (MRI), is not needed to assess the severity of LTC, but it is only used for adjunct information regarding associated malformations (Leboulanger and Garabédian, 2011).

Difficult intubation, repeated displacement of the ET, inadvertent extubation (Moungthong and Holinger, 1997) and inability to achieve appropriate tidal volumes during mechanical ventilation, should raise suspicion for this rare malformation. In our case, neonatologists set suspicion for the malformation clinically, and thus reinforcing the fact that the laryngeal cleft type IV can be identified early postnatally, during endotracheal intubation, by an experienced neonatologist (Jorgensen et al., 2018).

Data in the literature suggests that, in the apnoeic patient, even if upper airway appears normal, a LTC may be present, because the arytenoids are not spontaneously separated. In these cases, widening of the airway can be achieved with rigid bronchoscopy or with positive airway pressure and a flexible bronchoscope (Jöhr et al., 2003). We presume that this was the case in our neonate, who was non-vigorous, floppy and apneic on the 1st day of life, and it would explain why the malformation was not visible during the first intubation attempt. All neonates with severe forms of LTC (types III and IV) face difficulties maintaining mechanical ventilation, and accidental extubation is a common event. An uncuffed ET can easily slip into the oesophagus through the cleft. This situation along with multiple attempts for reintubation, can lead to pneumothorax, as is reported in our case.

Minor clefts, such as type I and II require minimal endoscopical management with usually good prognosis. Type III and IV clefts are much more serious and require early surgical management. Long-term mortality is high in the latter types, due to cleft relapse, pulmonary infections and repeated hospitalizations, but it has dropped from 93% in 1983 (Roth et al., 1983) to 50% (Martha et al., 2021; Seidl et al., 2021). Shehab and Bailey (2001) reported only 10 (33%) cases of type IV successfully treated. Although the survival rate has increased, it is not possible to draw conclusions for the quality of life of these patients. 71% of types III and 89% of types IV required tracheostoma, and about 60% of them needed prolonged ventilation (Seidl et al., 2021). Seidl et al. (2021) reviewed studies until 2020, and reported that 97% of these children had recurrent aspirations, 80% tracheostoma, 80% percutaneous endoscopic gastrostomy, 33% percutaneous endoscopic jejunostomy and 61% survived on mechanical ventilation, with failure to thrive. All 9 patients that were reported in the same study, had sequelae as mentioned above. Shehab and Bailey (2001) reported that severe tracheomalacia was present in 2 out of 6 patients they treated, requiring prolonged positive pressure ventilation. Walker et al. (2017) reported that many patients with a cleft had a coexistent neuromuscular dysfunction, explaining the observed dysphagia and the repeated aspiration episodes. Unfortunately, in our case, the patient died in the 1st week of life immediately after diagnosis was confirmed and before any medical or surgical treatment was attempted. Any neonate with respiratory and feeding problems in the early days of life, should be evaluated for possible laryngeal cleft by an experienced paediatric ENT. A high index of suspicion for the presence of laryngeal cleft in a newborn with a difficult intubation during resuscitation, and inability to achieve appropriate tidal volume delivered with a large air leak, is needed for early detection of such abnormalities. Laryngoscopy or bronchoscopy should be performed to reveal the extent of the malformation. For optimal long-term outcome, a multidisciplinary team, of neonatologists, pediatric ENTs, pediatric surgeons and anesthesiologists, should manage these patients.

Overall, the management of LTC is difficult. Early diagnosis, satisfactory ventilation, protection of the respiratory tract from aspiration and endoscopic or surgical repair are needed. The time from diagnosis to treatment is vital and minimizes the degree

of esophageal tracheitis caused by reflux and the recurrent respiratory infections due to aspiration. Prompt diagnosis of coexisting cardiac malformations, which are very common, is equally important. LTC is often associated with genetic syndromes, and therefore, each case should be evaluated by a geneticist. When all evaluations are completed the best approach for each case should be planned. Nutritional status should be a priority in the preoperative and postoperative management. Increased caloric and protein intake has been linked to optimal growth in these neonates, and also speeds up the wound healing process. Optimizing early nutrition in critically ill neonates and especially those born very preterm and with very low birth weight, reduces mortally, growth failure, adverse neurodevelopmental outcomes and long-term health consequences (Moltu et al., 2021; Gounaris et al., 2022).

Regardless though, to early identification of LTC, the prognosis is still ominous, because of the recurrent pulmonary aspiration that causes repeated respiratory infections, such as pneumonitis and tracheitis, leading to high mortality rate.

Conclusion

This case raises several points. Laryngo-tracheo-oesophageal cleft is a rare congenital malformation of the upper respiratory and gastrointestinal tract. In order to maximize favourable outcome of these infants, a multi-disciplinary approach of specialized physicians is needed. Effective ventilation, early management of gastric reflux, adequate nutrition and endoscopic or surgical management are mandatory. Neonatologists when treating neonates with symptoms of respiratory distress in need of mechanical ventilation support with difficulties to manage, and with additional congenital abnormalities, should keep in mind this rare anomaly. The aim of this case report is to underline that increased awareness is needed for an early diagnosis, especially in long segment LTC types III and IV, which have the highest morbidity.

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A Giant Scrotal Neurofibroma in a Child Masquerading as Filariasis: Uncommon Presentation of a Common Disease

Anurag Singh[1], Gyanendra Singh[2], Anuragani Verma[3], Yogendra Narayan Verma[4]

[1] Department of Pathology, King George Medical University, Lucknow, India;
[2] Department of Pathology, All India Institute of Medical Sciences Rajkot, Gujarat, India;
[3] Department of Microbiology, King George Medical University, Lucknow, India;
[4] Department of Pathology, Ganesh Shankar Vidyarthi, Medical College, Kanpur, India

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Key words: Neurofibroma – Neurofibromatosis type I – Scrotum – Scrotal mass

Abstract: Neurofibroma of the scrotum is a very uncommon benign neoplasm, specifically when it affects teenagers and is not associated with neurofibromatosis type I. To the best of our knowledge, only a couple of cases of neurofibroma in children have been documented. Here, we report a case study of a 17-year-old boy who had a giant scrotal lump for ten years masquerading clinically as filariasis. A provisional diagnosis of benign nerve sheath neoplasm was made based on cytology findings. The lump was surgically removed from the patient, and a histopathological and immunohistochemistry examination established the diagnosis of neurofibroma. The combined clinical, preoperative cytological, histological, and immunohistochemistry findings were not presented in the literature in any of the formerly documented cases of scrotal neurofibroma. The current case expands the spectrum of differential diagnoses for scrotal tumours that clinicians have previously observed.

Mailing Address: Gyanendra Singh, MD., Department of Pathology, AIIMS Rajkot, village Khanderi, Parapipaliya, 360001, Gujarat, India; e-mail: gyanendra002@gmail.com

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Introduction

The spermatic cord and epididymis are the major sites of extra-testicular scrotal tumours. Leiomyoma, fibroma, lipoma, and haemangioma are the most frequent benign mesenchymal scrotal tumours (Gupta et al., 2011). Neurofibroma must be considered in the differential diagnosis of scrotal tumours despite its exceptional rarity. To the best of our knowledge, only a few cases of solitary scrotal neurofibroma in children without a relationship with neurofibromatosis type 1 (NF1) have been reported in the English literature (Türkyılmaz et al., 2004; Jaber et al., 2020). Here, we report an extremely uncommon case of a giant scrotal mass in a child that was clinically masquerading as scrotal filariasis with the demonstration of clinical, cytological, histomorphology, and immunohistochemical findings.

Case report

A 17-year-old boy was evaluated for an isolated, painless, progressively increasing hanging giant scrotal mass that had been present for ten years. The results of a physical examination revealed a massive lump that appeared to originate from the left scrotum and extend up to the left knee, measuring 32.5×8.5 cm (Figure 1A). On palpation, it was difficult to precisely localise its relationship to the spermatic cord and the epididymis. The lump was non-transilluminating, and no hernia was found. An enlarged left inguinal lymph node was also noted, which was firm, mobile, and non-tender on examination. The overlying skin over the lymph node was unremarkable. No abnormality was detected on systemic examination. Ultrasonography revealed a giant hetero-echoic extratesticular mass lesion arising from the left scrotum. Both testes were seen separately from the mass without a hydrocele. Laboratory parameters, including the complete blood count, renal function test, liver function test, and serological examination, were within normal limits. A provisional diagnosis of scrotal filariasis was made based on clinical and radiological evaluation, and the patient was scheduled for fine needle aspiration cytology from the left scrotal mass and inguinal lymph node. The cytology smears from the scrotal mass revealed cellular smears, displaying bland monomorphic spindle-shaped cells with wavy nuclei on a background with few red blood cells. There was no evidence of necrosis or mitotic activity (Figure 1B). The cytology of the scrotal mass was suggestive of benign spindle cell neoplasm, and the possibility of benign nerve sheath neoplasm was considered. The aspiration smears from the lymph node were cellular and showed a reactive population of lymphoid cells on a hemorrhagic background, which was reported as reactive lymphoid hyperplasia (Figure 1C).

The complete excision of the left scrotal mass lesion was performed under general anaesthesia. The mass was completely dissected from the surrounding tissue. The testes and epididymis were not involved. The scrotal mass has firmly adhered to

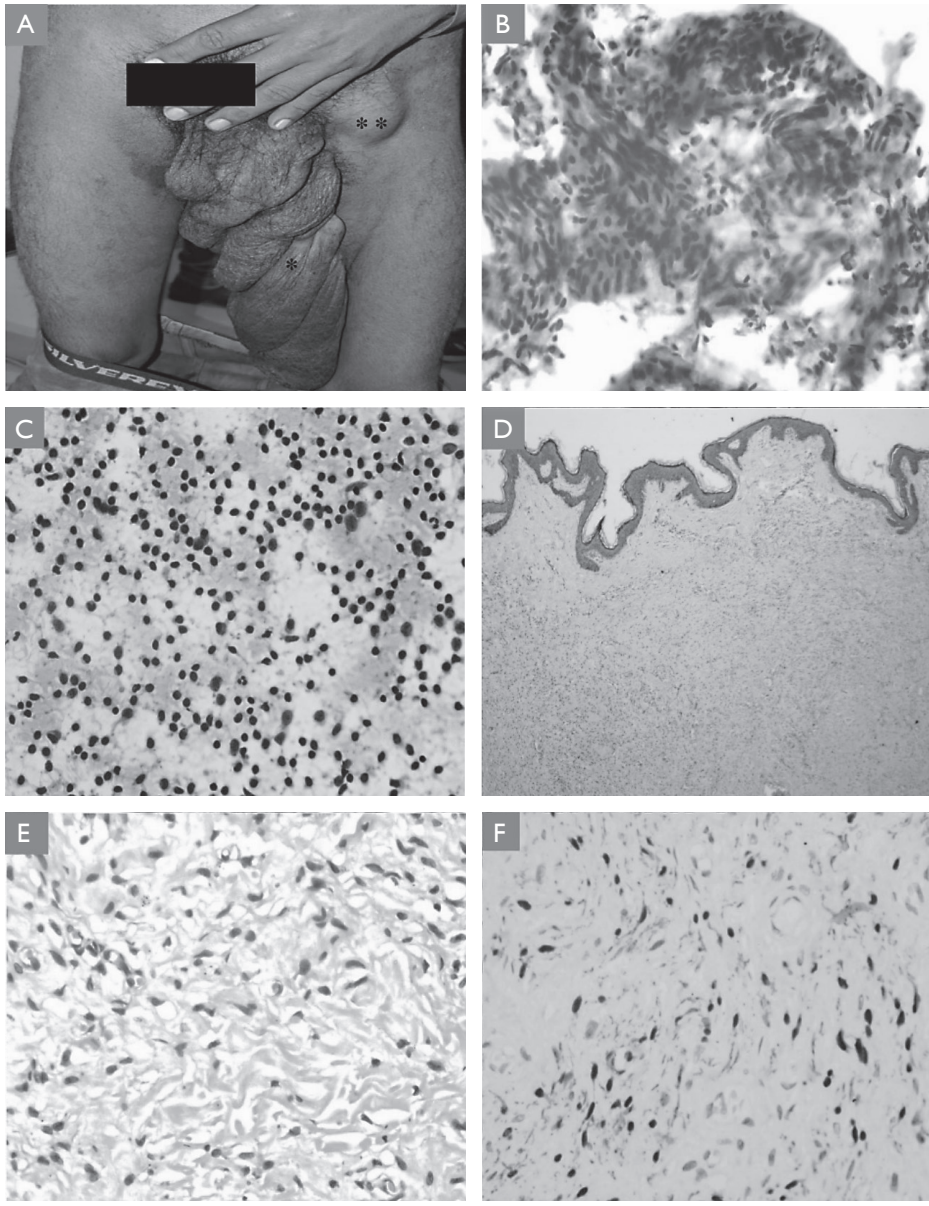

Figure 1 – A) Clinical image of the left scrotal mass and enlarged left inguinal lymph node**; B) cytology smear from left scrotal mass displaying bland monomorphic spindle-shaped cells having wavy nuclei and ill-defined cytoplasm (hematoxylin and eosin [H and E] stain, 400×); C) cytology smears from inguinal lymph node displaying reactive population of lymphoid cells (H and E stain, 400×); D) section showing tumour composed of evenly distributed spindle-shaped cells covered by stratified squamous epithelium (H and E stain, 40×); E) section displaying proliferating spindle-shaped cells with wavy nuclei lying in connective tissue stroma composed of delicate collagen bundles (H and E stain, 400×); F) immunohistochemistry displaying positive expression for S-100 protein in tumour cells.*

the scrotal wall, which signifies that it most likely originated from subcutaneous connective tissue. The external spermatic fascia, ilioinguinal, and genitofemoral nerves were not related to the tumour. The excised mass measured 33×10×6 cm and was covered by skin; the cut surface was gelatinous grey-white with focal areas of haemorrhage. There was no gross necrosis.

Histopathological examination of the resected mass showed a non-capsulated tumour covered by unremarkable stratified squamous epithelium. The tumour was comprised of uniformly dispersed monomorphic spindle-shaped cells with wavy nuclei, bland chromatin, and poorly defined cytoplasm that were embedded in a stroma that showed sclerosis at places. No mitotic figures or necrosis were discernible (Figure 1D and E). Immunohistochemical examination of the tumour showed positive expression of S100 and vimentin (Figure 1F). Smooth muscle actin, cytokeratin, and desmin were negative in tumour cells. The Ki-67 proliferation index was 1%. A definitive diagnosis of neurofibroma was established on the basis of cytology, histomorphology, and immunophenotypic findings. Retrospectively, the patient has been investigated for a possible link with NF-1, but the radiological and genetic study results were negative. During a six-month follow-up, there was no tumour recurrence.

Discussion

Neurofibroma is a benign tumour of the nerve sheath that arises from Schwann cells. They can occur in any part of the central or peripheral nervous system, including the neck, thorax, skull, retroperitoneum, and flexure regions of the extremities (Milathianakis et al., 2004). In children, only a couple of scrotal neurofibroma cases without the association of NF1 have been recorded in the literature (Türkyılmaz et al., 2004; Jaber et al., 2020). The scrotal neurofibroma presents as a painful or painless mass and is reported in patients of all ages (Yoshimura et al., 1990; Issa et al., 1993; Gupta et al., 2011; Jaber et al., 2020). In the present case, a 17-year-old boy presented with a painless giant mass of the scrotum. Although the precise origin of the tumour is frequently unknown in cases of scrotal neurofibroma, the majority of these tumours are extra-testicular. The genitofemoral nerve, epididymis, subcutaneous neural tissue, spermatic cords, and tunics have been the origins of scrotal neurofibroma (Yamamoto et al., 1982; Deliveliotis et al., 2002; Singal et al., 2012). The tumour in the present case has been connected to the subepithelial zone of the scrotal wall, suggesting that the subcutaneous neural tissue was the most likely place of origin. Investigations should focus on the clinical and radiological signs of this disease association with NF1, such as café-au-lait macules, long bone cortical thinning, Lisch iris nodules, optic glioma, and a family history of the disease (Mishra et al., 2002; Hosseini et al., 2012). NF1 was not found in the genetic study, and the present case also had no disease-related clinical, radiological, or family history.

In order to provide a provisional diagnosis for superficial masses, aspiration cytology is the preferred, less intrusive diagnostic technique. However, for definitive diagnosis, histology along with the assimilation of immunocytochemistry, is the recommended method that can provide a conclusive diagnosis. On aspiration smears, it is typically impossible to distinguish between neurofibroma and schwannoma, with the exception of cases where schwannoma exhibits Verocay's bodies on cytology (Gupta et al., 2011). Increased cellularity, numerous mitoses, and the presence of necrosis favour the diagnosis of a malignant peripheral nerve sheath tumour (Pekmezci et al., 2015; Verma et al., 2020; Sharma et al., 2021). In the present case, there was no necrosis or mitosis, and the Ki-67 proliferation index was 1%, supporting the diagnosis of neurofibroma.

The preferred course of treatment for these tumours is complete excision, avoiding orchidectomy. An orchidectomy has been done if the tumour has an intratesticular location or if the testicles and the tumour share a common blood supply. In order to rule out malignancy and avoid orchiectomy, the fine needle aspiration cytology or frozen section is also very beneficial (Gupta et al., 2011; Hosseini et al., 2012). The tumour in the current case was extratesticular and provisionally diagnosed as a benign nerve sheath neoplasm by aspiration cytology; hence, both gonads were left intact. Complete excision of the mass has produced positive outcomes in all previously reported cases, with no residual disease or recurrences.

Conclusion

In the differential diagnosis of scrotal tumours in children, scrotal neurofibroma, a rare benign tumour, should be taken into account. A preoperative diagnosis can be made using radiology and fine-needle aspiration cytology, allowing the right surgical strategy to be established. Excellent outcomes were obtained, with no recurrences following the complete removal of the tumour.

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Recurrent Stroke as a Presenting Feature of Takayasu Arteritis in an Adolescent: A Case Report and Literature Review

Vasiliki Sgouropoulou[1], Efthymia Vargiami[1], Maria Kyriazi[1], Sofia Kafterani[1], Stella Stabouli[1], Georgios Tsigaras[1], Athanasia Anastasiou[2], Maria Trachana[1], Dimitrios Zafeiriou[1]

[1] 1st Department of Paediatrics, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece;
[2] Department of Radiology, Hippokration General Hospital, Thessaloniki, Greece

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Key words: Takayasu arteritis – Adolescent rheumatology – Brain – Stroke

Abstract: Takayasu arteritis is a large vessel vasculitis, characterized by granulomatous inflammation of arterial vessels, that typically affects the aorta, its main branches and pulmonary arteries. Disease diagnosis is a challenge and requires awareness of the condition, as clinical signs can be not specific. We report a case of an adolescent with recurrent stroke diagnosed with Takayasu arteritis. A diagnosis of Takayasu arteritis was established due to angiographic findings in the magnetic resonance angiography in conjunction with systolic blood pressure discrepancy, arterial hypertension and increased acute phase reactants. Takayasu arteritis is a rare cause of ischemic stroke in children. However, stroke may be the first manifestation of the disease. Clinical experience and multidisciplinary approach, including aggressive treatment, is essential for the favourable outcome of the disease and the reduction of the associated morbidity and mortality.

Mailing Address: Vasiliki Sgouropoulou, MD., MSc., PhD., Konstantinoupoleos 49, 54642, Thessaloniki, Greece; Phones: +302 310 892 498, +306 972 232 877; e-mail: vsgouro@hotmail.com

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Introduction

Takayasu arteritis (TAK) is a rare chronic inflammatory disease that typically affects the aorta, its main branches and pulmonary arteries. TAK is categorized as a large vessel vasculitis according to the 2012 International Chapel Hill Consensus Conferences (CHCC) definition (Jennette et al., 2013). Vessel inflammation shows adventitial thickening, leucocytic infiltration of the tunica media and intimal hyperplasia leading to extensive vessel stenosis, which is a major risk factor for stroke. Disease diagnosis is a challenge and requires awareness of the condition and a high index of suspicion, as in the acute early phase symptoms are usually non-specific and specific biomarkers are absent. Clinical features vary depending on the affected vessels. The EULAR/PRINTO/PReS classification criteria for TAK in children are being applied as diagnostic criteria with high sensitivity and specificity, 100% and 99.9% respectively (Ozen et al., 2010). Early detection and aggressive management of the disease are essential for optimizing outcome and reducing the associated morbidity and mortality. We present a case report of an adolescent with recurrent stroke diagnosed with Takayasu arteritis and a review of the literature.

Case report

A 16-year-old male presented to the emergency department with left arm weakness and facial nerve palsy on the left side 15 hours after the onset of the symptoms. His medical history revealed post-Salmonella reactive arthritis at the age of 12 and a smoking habit over the last two years. Additionally, a previous hospitalization 10 days ago was mentioned due to left hemiparesis, left facial palsy and an episode of transient loss of consciousness, followed by confusion, slurred speech and loss of urine. Magnetic resonance imaging (MRI) of the brain had been performed that revealed a recent right ischaemic infarct in the basal ganglia and magnetic resonance angiography (MRA) disclosed narrowing of the A1 segment of anterior cerebral artery, while the findings of the magnetic resonance venography (MRV) of the brain were normal. The patient was evaluated by the team of pediatric neurologists and a thorough diagnostic investigation was performed. Serology tests, including hepatitis B and C virus, human immunodeficiency virus, Toxoplasma, rubella, *Borrelia burgdorferi*, Epstein-Barr virus, measles, Parvovirus B19, Herpes simplex virus and *Mycoplasma pneumoniae* were negative. The patient had been tested for thrombotic risk factors, including anticardiolipin antibodies (IgG, IgM), anti-beta2-glykoprotein I antibodies (IgG, IgM), factor V Leiden, factor II, protein C and S, antithrombin III, hyperhomocysteinemia and lupus anticoagulant and the findings had been normal. Echocardiography had been performed and normal findings had been disclosed. The patient was started on aspirin (100 mg/daily) and as the clinical condition was improved, he was discharged with a follow-up appointment.

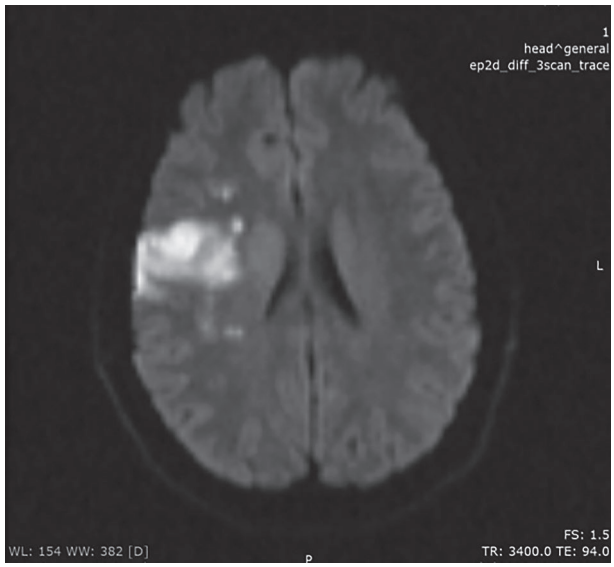

Figure 1 – Brain magnetic resonance imaging showing right middle cerebral artery infarct.

Upon hospital admission, neurological examination revealed left facial nerve palsy, decreased strength and sensation in the left upper extremity and weak reflex response on the left side. Brain MRI revealed right middle cerebral artery infarct (Figure 1) and MRA disclosed narrowing of the right middle cerebral artery, right anterior cerebral artery and right posterior communicating artery. MRA of the neck also unveiled narrowing and thrombosis of the right internal carotid artery (Figure 2). The patient was additionally started on anticoagulant therapy with low

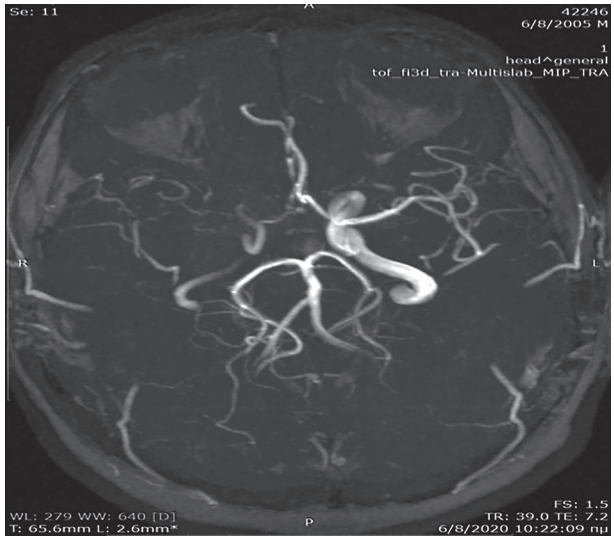

Figure 2 – Magnetic resonance angiography of cerebral arteries showing absence of flow in the right internal carotid.

molecular weight heparin (LMWH). The laboratory results were normal. On day 3 the patient developed high grade fever, without further deterioration of his neurologic condition. The laboratory results revealed neutrophilic leukocytosis (white blood cell count 19.5 K/UI [Neu 91.6%]) and elevated acute phase reactants, C-reactive protein 108.6 mg/l (N: <6.0) and erythrocyte sedimentation rate (ESR) 13 mm/h (N: 1–10). Blood and urine culture and nasal swab for SARS-CoV-2 by RT-PCR were negative. Chest X-ray, ultrasound scan of the abdomen and computed tomography angiography (CTA) of the thoracic and the abdominal aorta were unremarkable. Upon clinical examination, the patient showed systolic blood pressure discrepancy. Blood pressure of the right lower extremity was 144/66 mm Hg and of the left lower extremity 95/56 mm Hg. There were no decreased or absence of peripheral artery pulses. Based on clinical examination, laboratory tests and imaging findings the patient was diagnosed with Takayasu arteritis. Assessment of disease activity was achieved using the Paediatric Vasculitis Activity Score (PVAS), which was calculated at 20/63. The patient was treated with intravenous methylprednisolone pulse (1 g/day for 3 consecutive days), followed by oral prednisolone (60 mg/day). Cyclophosphamide was also administered at a dose of 500 mg/m^2. There was an amelioration of the patient's clinical symptoms, including improved mobility of left upper limb and complete recovery of facial palsy. Laboratory results were normalized and after

Table 1 – PSOM-SNE measurements in month 0, 3, 6 and 18

Month	Pediatric Stroke Outcome Measure Subscales and Outcomes					Total score
	left sensorimotor domain	right sensorimotor domain	language production	language comprehension	cognition-behaviour	
0	2.0	0.5	1.0	1	0.5	5.0/10
3	1.0	0.0	0.5	0	0.0	1.5/10
6	0.5	0.0	0.0	0	0.5	1.0/10
18	0.0	0.0	0.0	0	0.0	0.0/10

PSOM-SNE – Pediatric Stroke Outcome Measure Short Neuro Exam

Table 2 – Hand Grip Strength measurements in month 0, 3, 6 and 18

Month	
0	Application failure
3	29 kg (high risk)
6	33.5 (some risk)
18	42

a 10-day hospitalization the patient was discharged, receiving a physiotherapy and speech-language therapy referral. Over the following 3 months, the patient was administered 5 cyclophosphamide infusions, as per protocol, and oral prednisolone was tapered to 20 mg per day. His neurological condition presented further improvement and laboratory results remained normal. The reliable and objective disease-specific measure of neurological recovery after childhood stroke, the Pediatric Stroke Outcome Measure Short Neuro Exam (PSOM-SNE) and the Hand Grip Strength (HGS) were applied and confirmed the gradual clinical improvement of the patient from month 0 to month 18 (Table 1). A significant degree of deviation in PSOM-SNE is recorded in detail in the sectors: hemiparesis, hemifacial weakness, other motor deficit, difficulty with drinking, dysarthria, language deficit and comprehension, cognitive or behavioural deficit. In the HGS test the impossibility of its implementation was presented in the initial assessment with first application in month 3 and clinical improvement in all the tests until month 18 (Table 2). A follow-up MRA of brain and neck was performed 6 months later, showing additionally a narrowing of the A2 segment of right anterior cerebral artery and the MRI of the abdominal aorta revealed stenosis of the inferior mesenteric artery, leading to further imaging of the lower extremities which also revealed stenosis in the right posterior tibial artery. At that point, the patient was infected with SARS-CoV-2, presenting a short course of the disease and a complete and rapid clinical recovery. The dose of oral prednisolone was increased to 40 mg/day and the biological agent Tocilizumab was added in his treatment at the dose of 162 mg/week subcutaneously. Furthermore, the patient's serum creatinine increased (Cr: 1.25 mg/dl) and his GFR (glomerular filtration rate) was reduced below normal ranges, while the 24-hour urine protein test result was normal. An abdominal ultrasound and a CTA of renal arteries was performed, revealing no abnormalities, followed by a renal biopsy confirming the above result. To this day, 18 months from the disease onset, patient's neurological condition and neuroimaging remains stable, manifestations have majorly subsided and laboratory results are normal (Cr: 0.95 mg/dl). Methotrexate was added in his treatment, as he presented arthritis in his right elbow. He is closely monitored by the paediatric neurologist, rheumatologist, nephrologist and rehabilitation team.

Discussion

Takayasu arteritis is a large vessel vasculitis, characterized by granulomatous inflammation of arterial vessels. Several previous studies reported a prevalence of Takayasu arteritis that ranges from 4.7 to 33 cases per million in European population (Onen and Akkoc, 2017). The prevalence of stroke in Takayasu is estimated to be around 5–20%, based on small studies (Duarte et al., 2016; Kim and Barra, 2018). Diagnosis of Takayasu arteritis remains a challenge for the clinical

doctor, as it usually presents with non-specific symptoms at the early stages such as fever, headaches, weight loss and rash. As inflammation progresses, organ specific symptoms emerge, due to stenosis, occlusion and ischaemia. Takayasu patients have a variable clinical presentation with the most common clinical symptom in children being hypertension secondary to renal artery stenosis (Mathew et al., 2016). Although neurological manifestations are not common as the initial presentation of Takayasu arteritis in children, stroke should be a red flag and further investigation should be carried out (Zhou et al., 2011). Stroke occurrence at any point along the progression of Takayasu arteritis could result in neurological sequelae, including neurological impairment, recurrence of stroke and epilepsy (Couture et al., 2018). Fan et al. (2019) reported that patients who present with stroke, heart failure or coronary artery involvement at early stages of the disease, usually lead to worse progression of the disease and require close monitoring. Induction therapy of Takayasu arteritis requires high dose of glucocorticoids combined with non-biologic disease modifying agents, based on the EULAR treatment guidelines for Takayasu arteritis in adults. In cases of major relapse, such as the one presented in this report, biological agents such as Tocilizumab should be considered (Hellmich et al., 2020). In our case, a 16-year-old patient, with a history of reactive arthritis and a recent cerebral infarct, presented with a recurrent ischaemic stroke. Strong clinical suspicion led to prompt diagnosis of Takayasu arteritis and aggressive treatment with high doses of methylprednisolone and cyclophosphamide was administered. The initial good clinical response was followed by COVID-19 infection. Follow-up imaging revealed new-onset angiographic abnormalities, while the patient presented increased serum creatinine, with normal renal biopsy findings. Treatment with Tocilizumab was initiated and methotrexate was added and the clinical condition of the patient, 18 months later, remains stable. In our case, acute ischemic stroke was the first clinical presentation of Takayasu arteritis. We highlight the significance of early diagnosis of systemic vasculitis in children that defines the disease course and prognosis of the disease. Takayasu arteritis is a rare and potentially life-threatening condition in children and requires high suspicion from the clinical doctor. All paediatric patient with suspected systemic vasculitis should be directed to a referral center, as the diagnosis and treatment is challenging and requires multidisciplinary monitoring.

Conclusion

Takayasu arteritis should be included in the differential diagnosis of stroke in all young patients. Even though ischaemic stoke is rare as the first presentation of Takayasu arteritis, clinical suspicion can lead to early diagnosis and to prompt treatment, in order to minimize the disease progression and improve the prognosis.

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Gorlin-Goltz Syndrome – A Rare Case Entity in Young Child

Shovita Mondal[1], Nikil Kumar Jain[1], Abhishek Dutta[1], Nishant[1], Abhijit Dutta[2], Moushumi Shil[1], Suman Sen[3]

[1] Department of Oral and Maxillofacial Surgery, Awadh Dental College, Jamshedpur, Jharkhand, India;
[2] Department of Oral and Maxillofacial Pathology and Microbiology, Awadh Dental College, Jamshedpur, Jharkhand, India;
[3] Department of Oral Medicine and Radiology, Haldia Institute of Dental Science and Research, Haldia, West Bengal, India

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Key words: Nevoid basal cell carcinoma – Odontogenic keratocyst – Carnoy's solution – "En-bloc" resection – Marsupialization – Bifid rib

Abstract: Gorlin-Goltz syndrome (GGS) is an infrequent multisystemic disease with an autosomal dominant trait, which depicted presence of numerous basal cell carcinoma in conjunction with multiorgan abnormalities. This syndrome may be diagnosed early by a dentist by routine radiographic exams in the first decade of life, since the keratocystic odontogenic tumour are usually one of the first manifestations of the syndrome. This article includes a case report of the GGS with regard to its history, incidence, etiology, features, investigations, diagnostic criteria, keratocystic odontogenic tumour and treatment modalities.

Mailing Address: Dr. Suman Sen, A-4/6, Purbasha, 160 manicktala main road, Kolkata, West Bengal, India; e-mail: sumansen20@yahoo.co.in

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Introduction

Gorlin-Goltz syndrome (GGS) is a genetic disorder with autosomal dominant inheritance, high penetrance, and variable expression. The syndrome also termed as nevoid basal cell carcinoma or basal call nevus syndrome. In this syndrome numerous basal cell carcinomas (BCCs), in conjunction with skeletal, ophthalmic, and neurological abnormalities are depicted. In 1987 Gorlin reviewed the syndrome history, tabulated the disease findings and their frequency, and commented on the naming of the syndrome. Historically, the syndrome was documented in two ancient Egyptian skeletons where multiple cysts, bifid ribs, relative shortening of the fourth metacarpal, and other skeletal findings were present.

In 1960 Gorlin and Goltz established the relationship of multiple basal cell epitheliomas, multiple jaw cysts, and bifid ribs, that the term nevoid basal cell carcinoma syndrome was coined (Rafiq et al., 2021). The syndrome also is frequently referred to as Gorlin-Goltz syndrome, Gorlin syndrome, and nevoid basal cell carcinoma syndrome (NBCCS) or the syndrome; dermatologists prefer the term nevoid basal cell carcinoma syndrome. In light of the risk of malignancy it is important to be aware of this syndrome and recognise the need for early referral for multidisciplinary management (Hasan and Akintola, 2018).

Case report

A 13-year-old male patient reported to the Department of Oral and Maxillofacial Surgery with a chief complaint of swelling and pain on right side of the face since

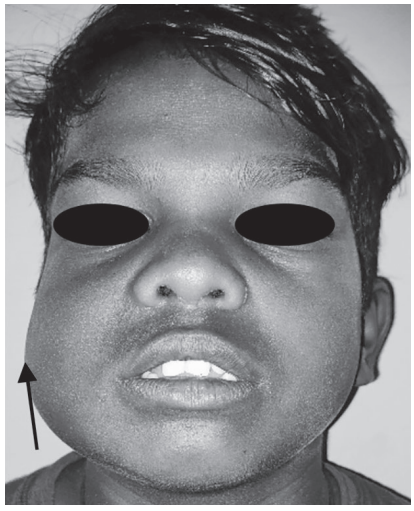

Figure 1 – Extra-oral clinical image shows swelling on right mid face region, hypertelorism.

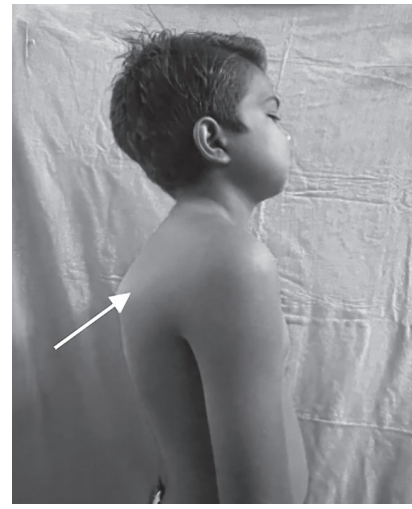

Figure 2 – Kyphosis.

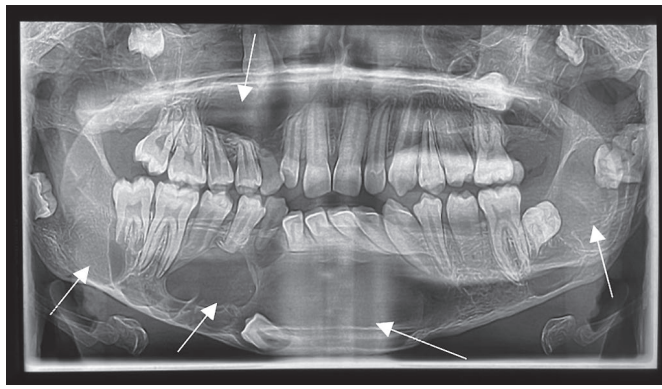

Figure 3 – Multiple radiolucent area.

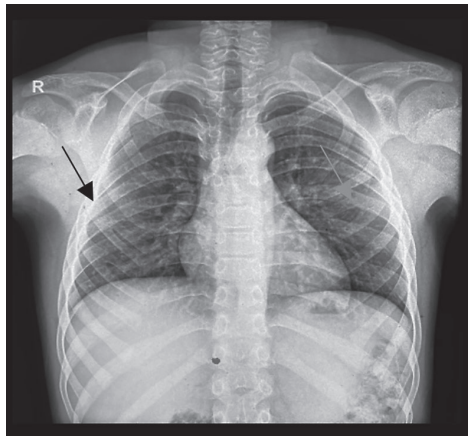

Figure 4 – Bifid rib.

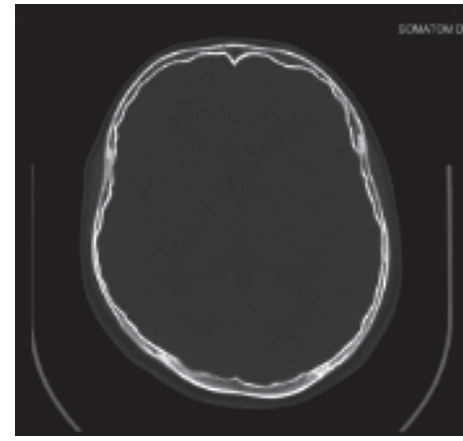

Figure 5 – Computed tomography scan of the skull.

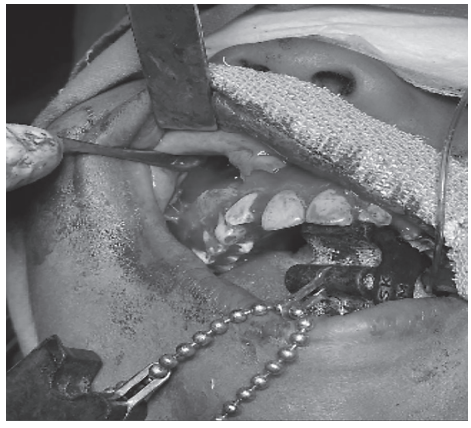

Figure 6 – Enucleated cavity on maxilla.

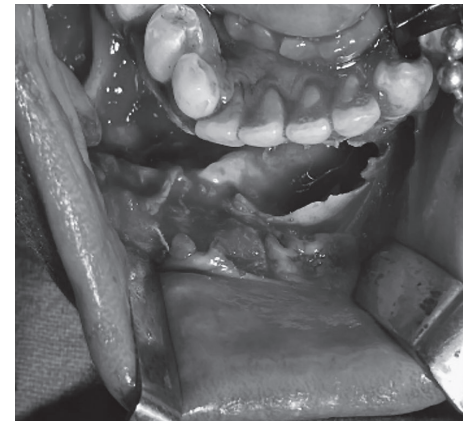

Figure 7 – Enucleated cavity on mandible.

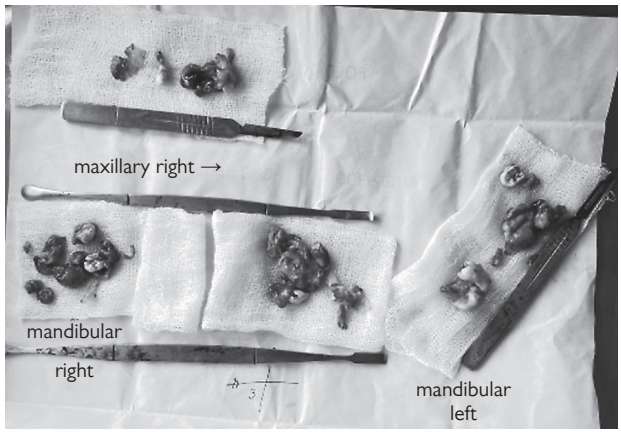

Figure 8 – Enucleated tissues.

last six months. Patient gave history of gradual increased in swelling with pain along with restricted mouth opening. Extraoral swellings were found to be firm and tender (Figure 1). On general physical examination he also had hypertelorism (Figure 1) and kyphosis (Figure 2).

Radiological investigations play a fundamental role in the diagnosis of GGS. An orthopantomograph (Figure 3) revealed multiple radiolucent lesions on both maxilla and mandible along with impacted teeth that are displaced by the cyst. Chest radiograph (Figure 4) showed the presence of a bifid rib on both sides raised suspicion of GGS. Computed tomography scan of the skull showing absence of calcification of the falx cerebri (Figure 5) raised suspicion of GGS.

Routine biochemical and haematological evaluations were carried out and the patient was hospitalized. Under all aseptic precautions, general anaesthesia was administered. Local anesthesia with adrenaline was injected and flap was raised intra orally in all quadrants one after another except maxillary left quadrant. No vital

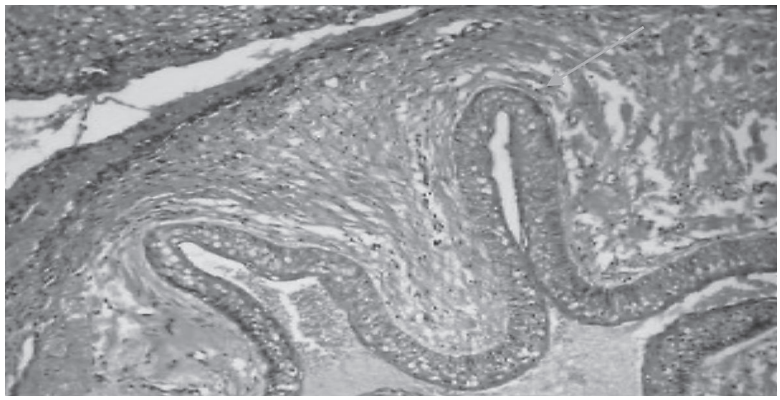

Figure 9 – Para-keratinized stratified squamous epithelium.

structures were seen near the lesions. A surgical curette was used for enucleating the cysts. Curettage was done using a curette and a round bur. The remnants of the cysts were removed using chemical cautery with Carnoy's solution (2.5%) for 3 min without chloroform followed by irrigation with saline (Figures 6 and 7).

The cysts were enucleated from all three quadrants followed by extraction of impacted teeth (Figure 8). The tissues removed were put in separate bottles containing formalin and enucleated tissues were sent for histopathological evaluation. As bone regeneration in children is faster, bone graft was not used.

The histopathology report showed a cystic lumen lined by corrugated, parakeratinized stratified squamous epithelium of 6–8 cell thickness. The epithelium is thrown into folds, with basal palisading nuclei and tomb-stone appearance (Figure 9) (hematoxylin and eosin stain 100×).

Discussion

The name Gorlin syndrome refers to the American oral pathologist and human geneticist Robert J. Gorlin (1923–2006). The American dermatologist Robert W. Goltz (1923–2014) was his co-author, which is the basis for the term "Gorlin-Gotz syndrome" (Al-Jarboua et al., 2019). The prevalence varies from 1/57,000 to 1/256,000 in general population along with regional variations and there is no gender predilection with a male:female ratio of 1:1 (Wadde et al., 2022). The syndrome occurs with equal frequency in both sexes. It has both a sporadic and familial incidence. Although detected in very young children they are commonly expressed between the ages 17 years and 35 years. The pathogenesis of Gorlin-Goltz syndrome is characterized by consequence of abnormalities in the PTCH1 gene. The deprivation of human patched gene, a tumour suppressor gene, forms the molecular basis of the syndrome (Acocella et al., 2009). This gene plays a most important role in embryonic structuring and cellular cycle result in mutation which accelerate further development of the disease including neoplasms.

Features of nevoid basal cell carcinoma syndrome

Clinical manifestations of the syndrome can be grouped into the following nine categories.

Cutaneous anomalies

Basal cell nevus/carcinoma (50–97%), other benign dermal cysts and tumours (21%), palmar/plantar pitting (90%), palmar and plantar keratosis and dermal calcinosis.

Dental anomalies

Multiple odontogenic keratocysts (75–100%), maxillary hypoplasia, mandibular prognathism, high arched palate or prominent palatine ridges (40%), cleft lip/palate (4%), impacted teeth and/or agenesis (3%), ectopic teeth and malocclusion.

Craniofacial anomalies
Calcification of falx (37–79%), tentorium cerebellum calcification (3%), bridged sella turcica (21%), macrocephaly (40%), brachycephaly, frontal bossing (25%), parietal and temporal bossing and coarse face (50%).

Skeletal anomalies
Polydactyly (3%), syndactyly, scoliosis (15%), hemivertebrae or other vertebral defects, flame-shaped lucencies of hand/feet, spina bifida (3%), osteoporosis (3%), cervical/bifurcated/fused/splayed/absent/rudimentary ribs (26%), brachymetacarpalism and shortened fourth metacarpal (12%).

Cardiac
Cardiac fibroma (3%).

Ophthalmic anomalies
Hypertelorism (40%), dystopia canthorum, congenital blindness (15%), internal strabismus (15%), congenital amaurosis, exotropia, glaucoma (3%), ptosis and coloboma (3%).

Neurological anomalies
Mental retardation (6%), dural calcification, bridging of sella, agenesis of corpus callosum, congenital hydrocephalus (3%), medulloblastoma (3–5%), agenesis/disgenesis of corpus callosum, meningioma (1% or less) and schizoid personality.

Table 1 – Diagnostic criteria

Major criteria	More than 2 BCCs, one BCC in patients younger than 30 years of age or more than 10 basal cell nevi
	Any odontogenic keratocyst (proven by histology) or polyostotic bone cyst
	Three or more palmar or plantar pits
	Ectopic calcification in patients younger than 20 years of age (lamellar or early falx cerebri calcification)
	A positive family history of NBCC
Minor criteria	Congenital skeletal anomaly (e.g., bifid, splayed, fused or missing rib, or bifid wedged or fused vertebra)
	Occipital-frontal circumference greater than the ninety-seventh percentile, with frontal bossing
	Cardiac or ovarian fibromas
	Medulloblastoma
	Lymphomesenteric cysts
	Congenital malformations such as cleft lip/palate, polydactylism or eye anomaly (e.g., cataract, coloboma or microphthalmos)

BCCs – basal cell carcinomas; NBCC – nevoid basal cell carcinoma

Table 2 – Modified diagnostic criteria by Kimonis et al. (1997)

Major criteria	More than 2 BCCs or one BCC in patients younger than 20 years of age Odontogenic keratocysts of the jaw (proven by histologic analysis) Three or more palmar or plantar pits Bilamellar calcification of the falx cerebri Bifid, fused or markedly splayed ribs A first degree relative with NBCCs
Minor criteria	Macrocephaly Congenital malformations (e.g., cleft lip or palate, frontal bossing, coarse faces and moderate or severe hypertelorism) Other skeletal abnormalities (e.g., sprengel deformity, marked pectus deformity and marked syndactyly of the digits) Radiological abnormalities (e.g., bridging of the sella turcica, vertebral anomalies, modelling defects of the hands and feet, or flame-shaped lucencies of the hands and the feet)

BCCs – basal cell carcinomas; NBCCs – nevoid basal cell carcinomas

Sexual anomalies
Hypogonadism (3%), uterine and ovarian fibromas (15%), calcified ovarian cysts (3%) and supernumerary nipple.

Laboratory findings
Increased serum uric acid level (3%), increased levels of alkaline phosphate and cyclic adenosine monophosphate.

The diagnostic criteria for nevoid BCC were established by Evans et al. (1993) and modified by Kimonis et al. (1997). According to them diagnosis of Gorlin-Goltz syndrome can be established when two major or one major and two minor are present (Tables 1 and 2).

Keratocystic odontogenic tumour (KCOT) have a greater predilection for the mandible 69% than the maxilla 31% (Maroto et al., 1999). In the mandible, 43% of KCOT occur in the molar-ramus region, followed by 18% in the incisor-canine region and 7% in the premolar region. In the maxilla, 14% occur in the incisor-canine region, followed by 12% in the molar tuberosity region and 3% in the premolar region.

In case of Gorlin-Goltz syndrome, parakeratotic KCOTs are seen. The major differences between OKCs associated with Gorlin-Goltz syndrome and solitary isolated OKCs are listed in Table 3. Our case showed a cystic lining that is thin and has stratified squamous cell lining with pinkish areas of keratinous material. Cystic wall has focal hyperplasia of lining cells and keratinous material with foci of chronic inflammatory cells mainly lymphocytes were seen.

Cone beam computed tomography (CBCT) provides a high-spatial resolution of all anatomic structures and accurate understanding of the relationship with the lesion. Three-dimensional representation of the scan images helps to assess the

Table 3 – Differences between syndromic KCOTs and solitary KCOTs

Feature	Syndromic KCOTs	Solitary KCOTs
Age	young individuals	middle or older aged
Cyst	multiple in layer	single
Site	maxillary posterior region commonly	mandibular posterior region
Recurrence rate	higher (82%)	lower (61%)
Epithelium	less thickness	more thickness
Odontogenic islands	most frequent	less

KCOTs – keratocystic odontogenic tumours

location, size, extent, and expansion of the lesion. CBCT enables us to remove OKC more precisely during surgery, reducing the rate of recurrence (Patel et al., 2022).

Patients should be given special attention and therefore should be monetarized and need multidisciplinary treatments continued in time, even a trivial change of signs and symptoms may be an important indicator of a precipitating event which puts the patient's life under threat. Various surgical procedures for odontogenic lesions that develop in the cortex of the jaw bones are simple enucleation, enucleation along with bone curettage and topical use of cytotoxic chemicals, "En bloc" resection, marsupialization, cryotherapy.

In this simple enucleation procedure, the cyst is completely removed in a single operating session. The bone cavity is spontaneously healed by bone regeneration. Enucleation with Carnoy's solution involves removal of the lesions followed by application Carnoy's solution within the surgical field (Abdoola et al., 2020).

Enucleating with peripheral osteotomy refers to enucleation of the lesion is followed by a peripheral ostectomy. The procedure includes of a rotary instruments for the removal of affected bone, ensuring the elimination of all the residual lining epithelium. Block resection of a jaw bone can be done either as marginal resection procedure or segmental resection procedure. In marginal resection procedure, the lesion is smaller, hence, it is possible to maintain the continuity of the jaw bone by preserving the portion of the uninvolved bone. When the lesion is aggressive it affects the complete or partial portion of the jaw (maxilla and mandible), in these cases the treatment is surgical resection of the affected portion followed by reconstruction of the jaw for rehabilitating the patient functionally and aesthetically. The main disadvantage of a conservative treatment is prolonged therapeutic time.

In marsupialization procedure the cystic sac is converted into pouch by deroofing. After locally anesthetizing the buccal/labial area, an oval/elliptical incision is taken to make a surgical window spanning 1 cm into a cyst; removal of cystic lining is done by creating a window which consist of oral mucosa and thinned out bony cortex and the boundaries of the cystic lining around the surgical opening are sutured to the surrounding oral mucosa.

Cryotherapy technique consist of use of a nitric oxide cryo-probe at a temperature of about –20 °C or lower on the bone walls after enucleation or on the soft tissues. The application is applied for 1 min and is repeated twice with an interval of 5 min. In addition, an increased risk of subsequent spontaneous fracture was also observed.

Hence, a surgeon has a pivot role in treating this type of syndromic cases as he will be the first person to detect such oral findings and predict occurrence of syndrome in future. Comprehensive treatment of this syndrome requires a teamwork with dental and medical opinion as well as genetic counselling.

Conclusion

Gorlin-Goltz syndrome is rare autosomal dominant genetic process. This case illustrates the need for awareness of the syndrome. Proper evaluation and characterization of clinical features are essential for diagnosis and management. In above case, two major criteria (OKC of the jaw and bifid rib) and 2 minor criteria (scoliosis and hypertelorism) were detected, suggesting that the patient had GGS. An appropriate long-term follow-up must be done after surgical treatment, follow-up should involve performing an orthopantomography every 6 months in young patients and a CBCT in case of doubt, or to evaluate contiguity with anatomical structures such as neurovascular bundles, teeth, and maxillary sinuses.

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An Unusual Etiology of Fluorodeoxyglucose Avid Intrathoracic Lymph Nodes

Alyssa Bonnier[1], Santu Saha[2], Adam Austin[3], Biplab K. Saha[3]

[1] Department of Critical Care Nursing, Goldfarb School of Nursing, Barnes Jewish College, Saint Louis, USA;
[2] Department of Medicine, Saha Clinic, Bangladesh;
[3] Department of Pulmonary, Critical Care and Sleep Medicine, University of Florida, Gainesville, USA

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Abstract: A middle-aged man in his 50s, active smoker, presented to the pulmonary office for lung cancer evaluation. On a low-dose computed tomography for lung cancer screening, he was found to have an 8 mm endobronchial lesion in the right main stem bronchus. A PET-CT revealed no endobronchial lesion, but incidentally, fluorodeoxyglucose (FDG) avidity was present in the right hilar (SUV 13.2) and paratracheal lymph nodes (LNs). He underwent bronchoscopy and EBUS-TBNA of station 7 and 10 R LNs. The fine needle aspiration (FNA) revealed necrotizing epithelioid granuloma. The acid-fast bacilli (AFB) and Grocott methenamine silver (GMS) stains were negative. He had suffered from pneumonic tularemia 13 months ago and immunohistochemical staining for *Francisella tularensis* on FNA samples at Center for Disease Control and Prevention was negative. The intense positron emission tomography (PET) avidity was attributed to prior tularemic intrathoracic lymphadenitis without active tularemia, a rare occurrence. To the best of our knowledge, PET-positive intrathoracic lymph node beyond one year without evidence of active tularemia has not been previously reported.

Mailing Address: Biplab K. Saha, MD., FCCP, Department of Pulmonary, Critical Care and Sleep Medicine, Medical Sciences Building, Room #M-452, University of Florida, 1600 SW Archer Road, Gainesville, FL 32608, USA; e-mails: spanophiliac@yahoo.com, biplab.saha@medicine.ufl.edu

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Introduction

Tularemia is an uncommon and potentially fatal zoonotic disease caused by *Francisella tularensis*. Less than 200 cases are reported in the United States (US) each year (Centers for Disease Control and Prevention, 2009). Tularemia is endemic in the northern hemisphere affecting North America, Europe, Asia, and northern Africa. *F. tularensis* is a facultative gram negative aerobic intracellular coccobacillus that can be transmitted incidentally to humans from direct contact with the infected animals or via an invertebrate vector, such as tick or deer fly (most prevalent mode of transmission). The common animal reservoirs are small mammals such as rabbits, hares, and rodents, but natural infection has been reported in more than 250 wild species (Golovliov et al., 2021). As *F. tularensis* can survive in soil, water, and vegetation for up to 3 to 4 months, contact with contaminated environment or ingestion of contaminated food could also cause disease (Matyas et al., 2007; Golovliov et al., 2021).

Depending on the mode of transmission, there are several distinct ways tularemia can present in clinical practice. Although pneumonic tularemia is considered an uncommon presentation, it could be due to the lack of consideration of tularemia as a potential etiology for pneumonia. The incidence of pneumonic tularemia has varied between 25 to 64% in the United States (Centers for Disease Control and Prevention, 2009; Thomas and Schaffner, 2010). In contrast, the incidence of pneumonic tularemia appears to be less in Europe (Väyrynen et al., 2017). This could be because the *F. tularensis* subspecies *tularensis*, the most virulent strain, is the most common infecting agent in the USA. In contrast, nearly all cases in Europe are caused by *F. tularensis* subspecies *holarctica,* a less virulent organism. Pulmonary involvement can occur in two ways: 1) inhalation of the pathogen, or 2) hematogenous spread from a non-pulmonary source, generally from ulceroglandular or typhoidal forms of tularemia (Tärnvik and Berglund, 2003).

In addition to pulmonary parenchymal involvement, pneumonic tularemia is often associated with hilar and mediastinal lymphadenitis (Väyrynen et al., 2017; Martinet et al., 2021). The pulmonary parenchymal involvement may radiologically manifest as consolidation, pulmonary nodule, or masses. The combination of parenchymal lesions and intrathoracic lymphadenopathy often raises concerns for lung cancer (Kravdal et al., 2020). Patients also report constitutional symptoms, including high fever, fatigue, and significant weight loss raising the suspicion of malignancy even higher. During the acute and subacute phases of the disease, the pulmonary infiltrate and thoracic lymph nodes have been reported to have intense positron emission tomography (PET) positivity, making the distinction nearly impossible (Martinet et al., 2021). However, isolated fluorodeoxyglucose (FDG) avid mediastinal and hilar lymph nodes in patients with a remote history of tularemic pneumonia have never been reported.

Case report

A middle-aged man in his 50s was seen in the pulmonary office for lung cancer evaluation. The patient was an active smoker and had more than a 45-pack-year history of smoking. He underwent a low-dose computed tomography (LDCT) scan for lung cancer screening by his primary care provider and was found to have an 8 mm endobronchial lesion in the right main stem bronchus (Figure 1). No significant intrathoracic lymphadenopathy was noted. He was referred to the oncology team. A PET-CT was obtained at the oncologist's request, which revealed no endobronchial lesion, but incidentally, FDG avidity was present in the right hilar (SUV 13.2) and right paratracheal lymph nodes (Figure 2). The patient was then referred to the pulmonary service.

In the office, the patient complained of mild chronic cough with sputum production throughout the year, occasional episodes of wheezing, and exertional shortness of breath while walking uphill, needing to rest. He denied any fever, night sweats, chills, or loss of appetite. He reported nearly 6.8 kg weight loss over the past two months. He did not have any personal history of tuberculosis, exposure to patients with tuberculosis, or other sick contacts. The patient had worked as a car mechanic his whole life and regularly participated in hunting activities. He lived in a mid-western state and did not travel outside the US or to the south-western US. The patient suffered from pneumonic tularemia 13 months ago. Computed tomography (CT) of the chest at that time revealed dense bronchocentric consolidation in the right upper lobe with hilar and mediastinal lymphadenopathy (Figure 3). The diagnosis was made from bronchoscopy and bronchoalveolar lavage.

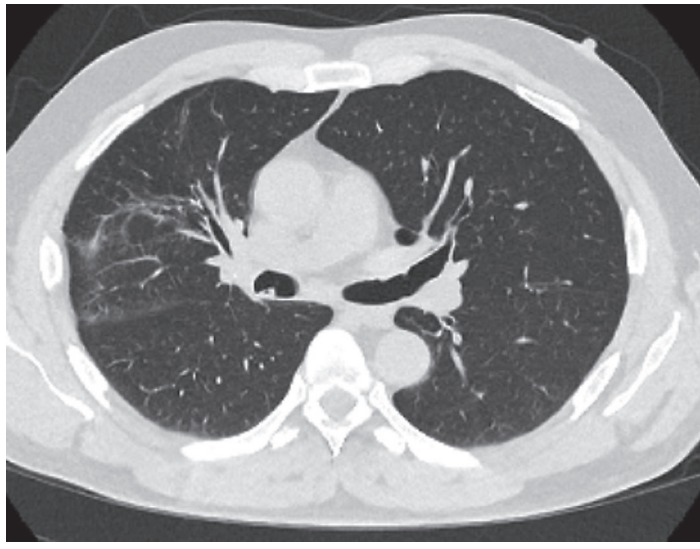

Figure 1 – Axial computed tomography (CT) of the chest demonstrated an 8 mm endobronchial lesion in the right main stem bronchus.

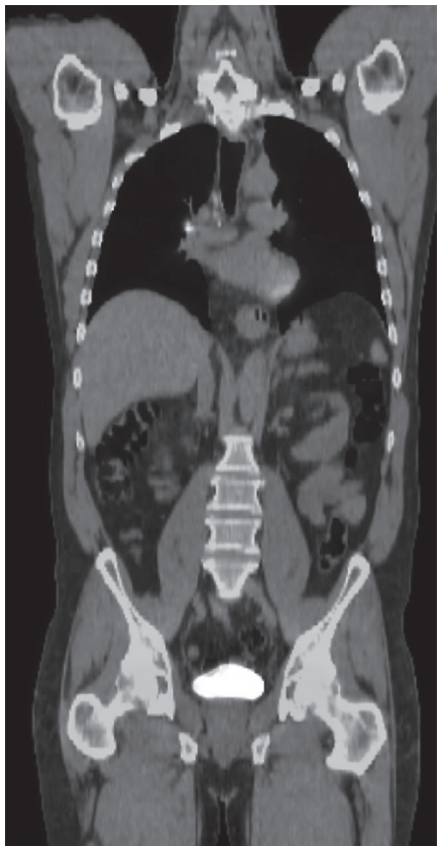

Figure 2 – Coronal view of the positron emission tomography (PET) scan revealed fluorodeoxyglucose (FDG) avid right hilar and paratracheal lymph nodes. The endobronchial lesion was not present on the PET-CT scan.

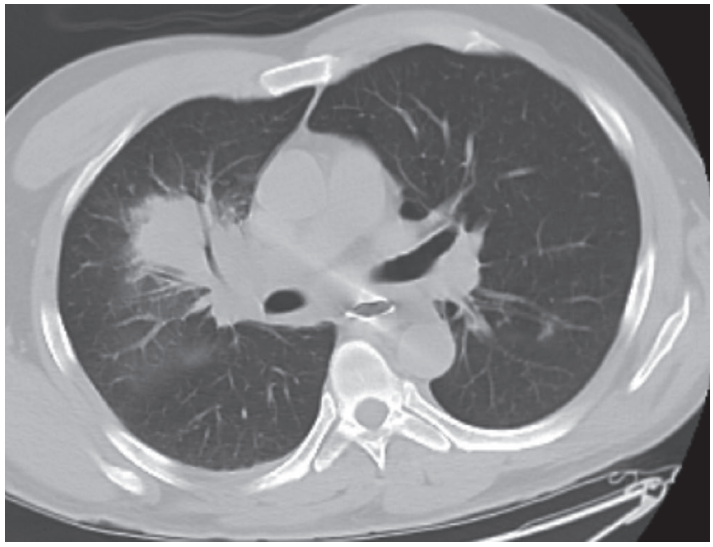

Figure 3 – Axial computed tomography (CT) of the chest 13 months ago showed dense bronchocentric right upper lobe consolidation and right hilar lymphadenopathy.

On physical examination, the patient appeared to be tired and without any distress. Chest examination was unremarkable other than bilateral reduced breath sound. There was no skin rash, joint pain, or swelling.

Given his extensive history of smoking and high FDG avidity in the intrathoracic lymph nodes, the patient underwent bronchoscopy and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) of station 7 and 10 R lymph nodes. The fine needle aspiration (FNA) revealed necrotizing epithelioid granuloma without any evidence of malignancy from both lymph node biopsies (Figure 4). The acid-fast bacilli (AFB) and Grocott methenamine silver (GMS) stains were negative for mycobacteria and fungi, respectively. As the patient was a heavy smoker for years, the primary concern was for metastatic lung malignancy. Small cell carcinoma (SCC) of the lung can present with hilar and mediastinal involvement without a parenchymal lesion. The other concern was lymphoma. However, it was very unusual that the patient had FDG avid intrathoracic lymph nodes but without lymphadenopathy. The list of etiologies for FDG avid intrathoracic lymph nodes is extensive; however, his clinical presentation was not suggestive of any particular etiology. As intrathoracic lymphadenitis in the setting of acute pneumonic tularemia has been previously reported to have FDG avidity, given his past history, we also considered that to be a potential etiology. The immunohistochemical staining (IHC) for *F. tularensis* on FNA samples at the Center for Disease Control and Prevention (CDC) was negative, suggesting the absence of active infection.

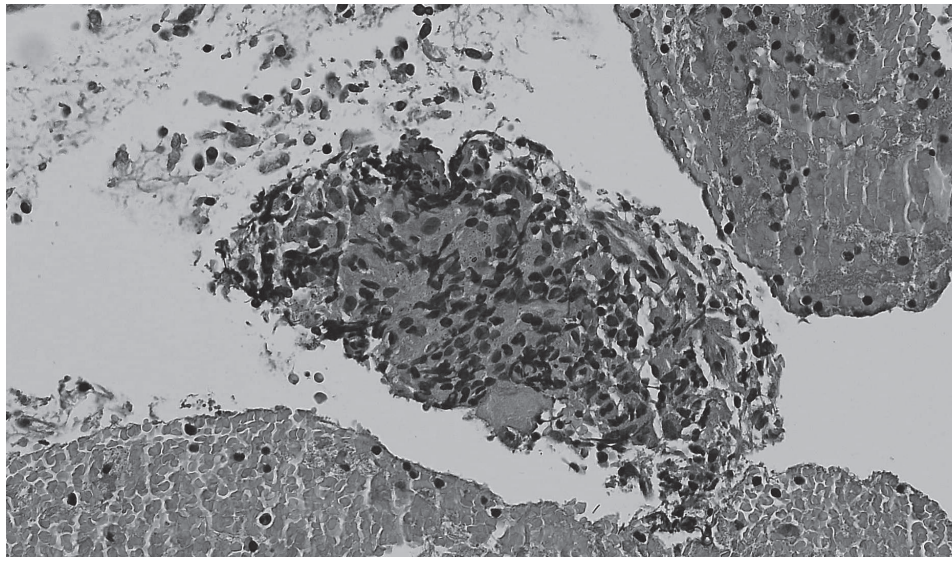

Figure 4 – Histopathologic analysis of fine needle aspiration from the right hilar lymph node revealed necrotizing epithelioid granuloma.

The intense PET avidity in the intrathoracic lymph nodes was determined to be secondary to prior tularemic intrathoracic lymphadenitis. Since the IHC for *F. tularensis* was negative, the patient was not treated with antibiotics. The patient did not require any further imaging or diagnostic study. He was asked to continue with yearly LDCT for lung cancer screening. He was advised to quit smoking.

Discussion

We have reported the case of an active smoker with PET-positive right hilar and paratracheal lymph nodes without lymphadenopathy. The patient had suffered from pneumonic tularemia about 13 months ago, and the FDG avidity was determined to be secondary to the prior intrathoracic lymphadenitis from tularemia. To the best of our knowledge, PET-positive intrathoracic lymph node beyond one year without evidence of active tularemia has not been previously reported.

F. tularensis is one of the most virulent bacteria known to man, with an infective dose of 10 cells (Dennis et al., 2001). As a result, it has been considered a potential bioterrorism agent. Once the bacteria gain access to the human body either by inoculation or inhalation, it proliferates locally and infects the macrophages and neutrophils (Pechous et al., 2009). *F. tularensis* is capable of evading the host defence mechanisms by escaping in the cytosol from the phagosome and causing death of the immune cells and release of pro-inflammatory cytokines and chemokines and thus propagating more tissue damage (Kinkead and Allen, 2016). *F. tularensis* can also infect the erythrocytes and survive there despite adequate antibiotic therapy causing recurrence of the disease (Horzempa et al., 2011). After the initial episode of nonspecific systemic illness, patients generally seek medical attention with symptoms consistent with one of the six distinct phenotypes, depending on the mode of transmission (Evans et al., 1985). These are: a) ulceroglandular disease (most common presentation, up to 80%), b) oculoglandular disease, c) glandular disease (refers to lymph node involvement), d) pharyngeal or oropharyngeal disease, e) typhoidal disease, and f) pneumonic disease.

Primary pneumonic tularemia due to inhalation of the bacteria is a rare occurrence. Most cases of pulmonary involvement are due to hematolymphogenous dissemination in the setting of typhoidal tularemia (Thomas and Schaffner, 2010). In case of primary pneumonic type, patients often present with high-grade fever, headache, cough, mild sputum production (which can progress over time), and occasionally retrosternal or pleuritic chest pain. However, a subacute presentation is also common. A history of potential exposure to an infected animal or tick bite may or may not be present. The differentiation from community-acquired pneumonia (CAP) may not be possible clinically. Acute respiratory distress syndrome occurs infrequently (Sunderrajan et al., 1985). Patients with pneumonic tularemia are more likely to be hospitalized and have a higher risk of death (Väyrynen et al., 2017).

The physical examination findings are nonspecific. Signs of consolidation, crackles, and pleural friction rub can be audible. Initial imaging with a chest X-ray could be unrevealing (Väyrynen et al., 2017). Unilobar or multilobar infiltrates are common. Hilar lymphadenopathy has been reported in approximately 30 to 45% of patients (Miller and Bates, 1969; Rubin, 1978). About one-third of patients suffer from pleural effusion (Thomas and Schaffner, 2010). In most cases, pneumonia and intrathoracic lymphadenopathy resolve with time. One study showed a median time of 14 weeks (range 6–28 weeks) for the resolution of radiographic abnormalities following treatment (Väyrynen et al., 2017). Although PET positivity has been consistently reported during the active phase of the disease, how long the FDG avidity persists is unknown. Perhaps, no study has ever evaluated this, as it would be a moot point to repeat a PET-CT in a patient with a known infection and no malignancy. In one case series where four patients with suspected lung cancer and positive PET-CT were followed longitudinally (all patients had tularemia), 3/4 patients had a complete resolution of the CT within three months. Only one patient did not have regression of the lesion (Fachinger et al., 2015). Other retrospective studies have also reported regression of lesions or complete resolution with short-term (3 months) follow-up (Martinet et al., 2021). PET positivity more than one year after the initial infection has never been reported in the literature. More interestingly, the FDG avidity could be present without any lymphadenopathy. The histopathologic analysis of the pulmonary lesions or lymph nodes may show necrotizing epithelioid granuloma mimicking tuberculosis and other fungal infections. Sometimes nonspecific lymphadenitis can be seen in EBUS-TBNA samples.

A definitive diagnosis of pulmonary tularemia can be made by isolating the bacteria in a culture medium from appropriate samples. Otherwise, a fourfold rise of the antibody titter in 2–3 weeks following initial presentation is diagnostic. Although rarely commercially available, a positive *F. tularensis* PCR or IHC from a clinical sample is also diagnostic. The first line of therapy for severe disease includes an aminoglycoside. Patients are generally treated for 7–10 days. Second-line medications are doxycycline and ciprofloxacin. The risk of relapse is higher with doxycycline (Meric et al., 2008).

Conclusion

Fluorodeoxyglucose avidity is common during active intrathoracic tularemia, which can persist beyond one year, raising suspicion of malignancy. Necrotizing epithelioid granulomas can be seen in patients with tularemia and may mimic tuberculosis and endemic fungal infections. Considering tularemia as a diagnostic possibility in the appropriate setting may assist in the accurate diagnosis and prevent more invasive lung interventions, such as lung resection.

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Iatrogenic Chronic Abdominal Pain in a Geriatric Patient: A Case Report

Apichai Wattanapisit[1,2], Natthawan Pankamnerd[2], Sanhapan Wattanapisit[3]

[1] School of Medicine, Walailak University, Nakhon Si Thammarat, Thailand;
[2] Walailak University Hospital, Nakhon Si Thammarat, Thailand;
[3] Thasala Hospital, Nakhon Si Thammarat, Thailand

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Key words: Abdominal pain – Chronic – Geriatric – Polypharmacy

Abstract: Chronic abdominal pain is a challenging problem in clinical practice, with several pathophysiological mechanisms underlying its aetiologies. This case report presents a geriatric patient with multiple comorbidities who had experienced intermittent abdominal pain for over 10 years. Alarming symptoms were ruled out, and a functional gastrointestinal disorder was determined as the most likely cause. The patient's medical history and previous treatments were thoroughly reviewed, revealing that long-term use of metformin and an oral iron supplement was the iatrogenic symptom triggers. The abdominal pain resolved upon discontinuation of these two medications. This case report highlights the significance of reviewing iatrogenic causes and periodically assessing chronic medical conditions to identify potential contributing factors of chronic abdominal pain.

Mailing Address: Apichai Wattanapisit, MD., School of Medicine, Walailak University, Floor 8, Building C, Walailak University Hospital, 222, Thaiburi, Thasala, Nakhon Si Thammarat, 80160, Thailand; Phone: +66 75 47 7401; Fax: +66 75 67 2807; e-mail: apichai.wa@wu.ac.th

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Introduction

Chronic abdominal pain, defined as a constant or intermittent pain lasting for at least 3 months, is a challenging problem in clinical practice (Sabo et al., 2021; Lukic et al., 2022). The aetiologies of chronic abdominal pain can be categorised as abdominal wall pain (e.g., postherpetic neuralgia, intercostal neuralgia), abdominal pain of visceral origin (e.g., peptic ulcer disease, renal cancer), abdominal pain syndromes of generalised diseases (e.g., abdominal migraine), functional gastrointestinal disorders (e.g., functional dyspepsia, irritable bowel syndrome) (Sabo et al., 2021). Unexplained or non-specific diagnoses are common among patients with abdominal pain (Viniol et al., 2014; Price et al., 2022). This article presents a geriatric patient who had suffered from chronic abdominal pain for more than 10 years and was referred to a geriatric clinic.

Case report

A 72-year-old male had been treated for type 2 diabetes mellitus, dyslipidaemia, benign prostate hyperplasia, and anaemia for more than 10 years. He had suffered from abdominal pain since the treatments of his chronic medical conditions began. The pain was intermittent and non-severe. He had regular visits with physicians for the underlying diseases, also, he visits general practitioners and specialists for the chronic abdominal pain. The most recent oesophagogastroduodenoscopy and colonoscopy, performed about 10 months prior to a referral to the geriatric clinic, revealed negative findings.

At the geriatric clinic, the medical history and laboratory tests were reviewed. Type 2 diabetes mellitus, dyslipidaemia, and benign prostate hyperplasia were under control. Fasting plasma glucose and glycated haemoglobin (HbA1c) were 119 mg/dl and 5.7%, respectively. Low-density lipoprotein cholesterol was 78 mg/dl. The complete blood count showed normal levels of white blood cells (7,000 cells/mm^3, neutrophil 61%, lymphocyte 26%, monocyte 6%, eosinophil 6%, and basophil 1%) and platelets (204,000 cells/mm^3). The red blood series showed hypochromic microcytic anaemia (red blood cell count 4.73×10^6 cells/mm^3, haemoglobin 10.0 g/dl, haematocrit 31.8%, mean corpuscular volume 67.2 fl, mean corpuscular haemoglobin 21.1 pg, mean corpuscular haemoglobin concentration 31.4 g/dl, poikiolocytosis 1+, hypochromia 1+, few target cells, and few ovalocytes).

His medications were metformin 500 mg daily, simvastatin 20 mg daily, dutasteride 0.5 mg + tamsulosin 0.4 mg before bedtime, ferrous fumarate 200 mg twice daily, and folic acid 5 mg daily. He had been taking metformin, simvastatin, ferrous fumarate, and folic acid for more than 10 years. His abdominal pain was non-localised, mild intensity, intermittent, dull, not related to meals. He had no nausea, vomiting, abnormal bleeding, anorexia, dysphagia, unintended weight loss,

constipation, or changes in bowel habit. Physical examination revealed a normal abdominal contour, no surgical scar, normoactive bowel sounds, no tenderness, no organomegaly, no palpable mass, normal rectal sphincter tone, and no melena upon testing by digital rectal exam.

The long-term use of metformin and ferrous fumarate was discussed with the patient. The two medications were discontinued after the first geriatric clinic visit. Blood tests for serum iron, ferritin, transferrin, total iron binding capacity, and haemoglobin typing were collected to differentiate the diagnosis of hypochromic microcytic anaemia (iron deficiency anaemia and thalassemia trait). Medications for symptomatic treatment for functional dyspepsia were prescribed (omeprazole, domperidone, simethicone, alginic acid), and a 4-week follow-up was scheduled.

At the second geriatric clinic visit, the values of serum iron, ferritin, transferrin, and total iron binding capacity were within the normal range. The patient had not experienced abdominal pain since the first few days after discontinuing metformin and ferrous fumarate. The haemoglobin typing showed a normal pattern (HbA 97.6% and HbA2 2.4%; not ruling out alpha thalassemia trait). The osmotic fragility test was positive. Other blood tests were within the normal range, including fasting plasma glucose (116 mg/dl) and HbA1c (5.8%). The patient was informed about controlled type 2 diabetes mellitus and the possibility of thalassemia trait. Six weeks after the second geriatric clinic visit, the patient had no abdominal pain.

Discussion

This case report presents a geriatric patient who had multiple comorbidities and suffered from chronic abdominal pain. The most likely aetiology was functional gastrointestinal disorders. Alarm symptoms or red flags indicating organic causes were not found in this patient (Black et al., 2020). Besides its pathophysiology, including alteration of gut motility; intestinal mucosal permeability; bile acids; immune-mediated conditions; visceral hypersensitivity; microbiome; central nervous system, medications should be reviewed (Duffy et al., 2023). Several medications are considered as iatrogenic symptom triggers, such as nonsteroidal anti-inflammatory drugs, antibiotics (e.g., doxycycline), opioids, dopaminergic agents, potassium chloride, metformin, and iron (Duffy et al., 2023).

After reviewing the medications, it was found that the patient had been on long-term use of metformin and iron supplement (ferrous fumarate). Metformin, a first-line medication for type 2 diabetes mellitus, affects the gastrointestinal tract through several mechanisms (McCreight et al., 2016). A systematic review revealed that patients using metformin had about a 50% higher risk of abdominal pain compared to controls (placebo or other diabetic medications) (Nabrdalik et al., 2022). Nausea and diarrhoea were also adverse effects of metformin (Nabrdalik et al., 2022). Oral iron supplementation, a first-line treatment for iron deficiency

anaemia, can lead to gastrointestinal side effects (e.g., abdominal pain, bloating, nausea, vomiting, constipation, diarrhoea) due to remaining non-absorbed iron in the intestines (Malesza et al., 2022). The two medications were identified as possible causes of chronic abdominal pain in this patient. This hypothesis was confirmed when the symptom of abdominal pain disappeared after discontinuing the medications.

The case report reflects three important lessons. First, iatrogenic causes of chronic abdominal pain should be reviewed for all patients, especially in older adults. Polypharmacy is a common issue among geriatric patients, which increases the risks of inappropriate medication use, drug interactions, and adverse drug reactions. Second, the assessment of chronic illnesses is recommended periodically. This case demonstrates the importance of continuity of care. Iron deficiency anaemia was a less likely diagnosis; however, long-term iron supplementation had been prescribed. The status of type 2 diabetes mellitus should be monitored. For this patient, diabetes remission, defined as HbA1c < 6.5% at least three months after the cessation of antidiabetic medications, was possible. Third, communication is very important. The blood test showed hypochromic microcytic anaemia, and oral iron supplementation had been prescribed to control his anaemia for over 10 years. Type 2 diabetes mellitus was his underlying disease, and a lifelong treatment had been expected. Discontinuing the long-term medications required a reasonable explanation.

Conclusion

This case report presents a geriatric patient with chronic abdominal pain, most likely attributed to a functional gastrointestinal disorder resulting from long-term medication use. It highlights the importance of reviewing iatrogenic causes, including medication use, and periodically assessing chronic illnesses to identify potential contributing factors.

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