

# Foreign Bodies in the Abdominal Area: Review of the Literature

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## ABSTRACT

The aim of this paper is to update and summarize the relevant literature on the anatomical localization, incidence, and diagnostic and therapeutic approaches to abdominal foreign bodies. A comprehensive review was carried out on recorded cases related to the presence of foreign bodies in the abdominal area throughout the literature. Moreover, the phenomenon was discussed in relation to different patient categories associated with childhood, mental or neurological illness, incarceration, and drug trafficking as well as sexual accident or abuse. Particular importance is ascribed to the underlying psychopathology and motivation of foreign body ingestion in each category of patients. The surgical, psychiatric and legal implications of the issue are discussed in detail.

## KEYWORDS

foreign bodies; intra-abdominal; ingestion; surgery; endoscopy; pica; body packers; gossypiboma; forensic pathology; legal medicine

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## INTRODUCTION

The presence of foreign objects (FB) in the abdomen may be due to involuntary or deliberate input orally or rectally. In addition, their misplacement can also be a result of iatrogenic complications during therapeutic interventions and surgical procedures. Furthermore, in some cases, foreign bodies, such as bezoars, can be gradually created in the lumen of the gastrointestinal tract.

Foreign objects that pass through the esophagus into the stomach and pylorus are automatically excreted in 80–98% of the cases, while only 1% of the aforementioned cases will cause perforation or other serious complications.

The presence of foreign bodies in the abdomen is a medical condition that employs multiple specialties, as, depending on the case, it constitutes a subject of general surgery, gastroenterology, gynecology, pediatrics, and psychiatry as well as forensic medicine and pathology.

In the present paper, the presence of foreign bodies is distinguished according to their characteristics (size, shape, type), anatomical localization, and differences in the diagnostic and therapeutic procedure associated with these parameters. Furthermore, the main population groups with an increased incidence of foreign bodies in the abdomen are reported (children, elderly, neurological patients, psychiatric patients, people with neurodevelopmental or neurodegenerative disorders, prisoners, and drug addicts), while particular incidents are also noted due to special clinical and forensic interest. The present thesis does not mention medical implants (foreign objects) placed in the abdominal area for therapeutic purposes. Incidents related to retained (forgotten) surgical items revealed after abdominal surgical procedures, however, are examined as well as risk factors, complications, prevention strategies, and forensic extensions related to the subject.

## METHODOLOGY

A comprehensive review was carried out on recorded cases of the presence of foreign bodies in the abdominal area by searching the electronic data bases of PubMed, Google search, Google Scholar, Heal Link, EMBASE, Scopus, and Cochrane Library up to June 2018. The search terms were: “intra abdominal foreign bodies,” “bezoars,” “allotriophagy,” “pica disorder,” “retained surgical items,” “gossypiboma,” “body packers,” and “foreign body ingestion.” No language restriction was applied. All the articles have been evaluated and supplemented by searches of the bibliographies of key papers.

## RESULTS

### 1. ANATOMICAL LOCALIZATION OF FOREIGN BODIES

The most common anatomical sites of foreign body obstruction along the digestive tract are normal constrictions of the lumen, anatomical sphincters, acute angulations, curvatures, congenital deformities, tumors, or sites of previous surgical procedures that alter the anatomy of the area (e.g., adhesions or gastric rings).

In cases of foreign body ingestion, patients tend to seek help originally from the otorhinolaryngologist, as these patients tend to believe that the foreign body may be wedged in the hypopharynx or esophagus. The hypopharynx is a frequent area of bolus and foreign body retention. The situation is extremely urgent, as phenomena of asphyxiation often occur. After excluding the above, otorhinolaryngologists guide patients to general surgeons, so that the foreign body can be detected and surgically removed if this procedure is assessed as appropriate (1).

#### 1.1 Foreign bodies in the gastric cavity

Gastric foreign bodies usually do not induce any symptomatology. The presence of symptoms involving abdominal pain, fever, bleeding, or vomiting is indicative of complications such as pyloric stenosis, perforation, or rupture of the mucosa by a sharp foreign object (2).

The diagnosis is mainly based on history, x-ray, and gastroscopy. Over 90% of swallowed objects progress along the entire digestive tract without complication within four to five days, while the majority of them pass through within 72 hours. Unless there are signs of obstruction, bleeding or perforation, an abdominal x-ray has to be performed daily so that the course of the subject can be monitored (1).

If the object is within the stomach, it can be removed endoscopically. The administration of laxatives is prohibited due to the high risk of intestinal perforation. In obstruction, bleeding, or perforation, surgical treatment is necessary (3).

Roughly, objects with a diameter of over 2.5 cm have a lower probability of passing through the pyloric canal, whereas objects of a length of 5–10 cm are unlikely to progress through the duodenal flexure, and therefore endoscopic or surgical removal is necessary within a week (1–3).

#### 1.2 Foreign bodies in the small intestine

As mentioned above, foreign bodies with a length of at least 5 cm can be trapped in the duodenal flexure, in the area of the ligament of Treitz and the ileocecal valve. Obstruction or perforation by sharp objects most frequently occurs in the ileum and ileocecal valve.

Plain abdominal X-rays at regular intervals are useful for monitoring the course of ingested foreign bodies into the small intestine (as long as the objects are radiopaque and can be imaged). The presence of fever, pain, vomiting, meteorism, or bleeding signifies the presence of a complication. Endoscopic removal of foreign bodies in the small intestine is extremely difficult, and often even impossible, and thus, the usual therapeutic option is surgical intervention (4–5).

#### 1.3 Foreign bodies in the large intestine

Foreign bodies in the colon and rectum may cause obstruction or perforation. Swallowed objects may be trapped in the caecum and ascending and sigmoid colon, while foreign bodies that have entered the intestinal tract through the anal canal are most frequently trapped in the rectum and sigmoid colon (6).

Rarely, foreign bodies can also progress into the lumen of the appendix and cause obstruction, resulting into acute appendicitis (7). It should be noted, however, that the presence of a foreign body in the lumen of the appendix does not always lead to obstruction and direct inflammation. It is known that smooth and spherical objects, such as bullets, seeds, and others can create endoluminal formations, enteroliths that grow progressively and cause delayed appearance of inflammation (8). In cases of a foreign-body presence in the lumen of the appendix, it is also possible that acute appendicitis never develops (9). Nowadays, the prevailing view is that, if a foreign body that would be unlikely to regress in the intestine from the lumen of the appendix is found during a radiological examination, an on-the-spot appendectomy should be recommended.

In the presence of peritonitis, surgery is essential. In most cases of foreign body presence in the colon, endoscopic removal is feasible usually by means of suppressive medication, but general anesthesia may also be required. Enemas and laxatives are prohibited. Surgery is indicated if endoscopic removal fails (10).

## 2. CLASSIFICATION OF FOREIGN BODIES BY MEDICAL IMAGING

- (a) Organic chemical materials, low atomic weight (<20): for example, plant and flower thorns, woods, plastic, polyurethane, rubber, and fibers. These materials are not visible in simple X-rays, and are not usually observed in Computed Tomography (CT) scans, while they can be visualized by ultrasound or Magnetic Resonance Imaging (MRI) scans. Medical judgment and prudence are essential, however, for proper diagnosis (11).
- (b) Low atomic weight metals (20–40): for example, aluminum (Al) and derivatives, such as aluminum nails, common glass, silicon (Si), calcareous rocks, dirt, fish and meat bones. These materials are not easily displayed in plain X-rays and Computed Tomography (CT) scans, except by radiation adjustments, while, again, the diagnosis is uncertain. The aforementioned materials can be visualized more easily with ultrasound and Magnetic Resonance Imaging (MRI) (11).
- (c) Metals of high atomic weight (>40): for example, iron (Fe), nickel (Ni), copper (Cu), lead (Pb), silver (Ag), and gold (Au). The most common objects containing the aforementioned materials are aluminum car sheets, specific glasses, other metal objects, steel pins, steel needles, remnants of metal processing, heavy metal soils (e.g. pyrite, and lead, etc.), and radiopaque construction materials. All of these materials can be observed in simple X-rays (11).

## 3. ENDOSCOPIC TECHNIQUES FOR THE REMOVAL OF FOREIGN BODIES: INDICATIONS - CONTRAINDICATIONS - COMPLICATIONS

Endoscopic removal of foreign bodies from the digestive tract must be performed by an experienced endoscopist along with adequate nursing support. Initially, it is significant that the endoscopist confirms the non-existence of

any evidence of blockage of the upper respiratory tract during the removal process of the foreign bodies. Respiratory tract obstruction is not a rare condition during the removal process.

The presence of foreign bodies in the upper digestive tract is a strong indication for endoscopic removal. The main complications of the endoscopic removal of foreign bodies from the gastrointestinal tract are mucosal ulcers, perforations, and bleeding (2). Contraindications to the endoscopic removal of foreign bodies are patients' inability to co-operate, acute esophagitis (e.g., due to ingestion of caustic substances), and stomach perforation as well as congenital or acquired gastrointestinal tract deformities.

## 4. SURGICAL REMOVAL OF FOREIGN BODIES

Ingestion of voluminous or sharp objects is a strong indication for surgical, rather than endoscopic, removal. In addition, if the foreign body has caused gastrointestinal perforation (signs of acute abdomen, abdominal wall contraction, fever, abscess, or pneumoperitoneum), the indication for surgical removal is absolute. Other surgical removal indications are the following: absence of an experienced endoscopist, failure of previously conducted endoscopic methods, constantly unaltered localization of the foreign body for 48–72 hours amidst the occurrence of abdominal symptomatology, or the ingestion of cocaine packs. Although the occurrence of gastrointestinal perforation by foreign bodies is rare, any foreign object after perforating the gastrointestinal tract could make its way to any nearby abdominal organ. Throughout international literature, cases have been reported of foreign body migration from the gastrointestinal tract to the liver, pancreas, spleen, greater omentum, and mesenteric vessels (12–13).

## DISCUSSION

### 1. FOREIGN BODY INGESTION IN CHILDREN

Infants and children have a natural propensity to assess and identify their surroundings, usually through touching, tasting and often swallowing new, unfamiliar, and bizarre items. This tendency of children to swallow anything they are able to put in the oral cavity renders swallowing of foreign bodies a common clinical condition in child populations, most frequently between the ages of six months and three years.

Among children with a history of gastrointestinal tract surgery due to congenital abnormalities (e.g., esophageal atresia or intestinal atresia), entrapment of the foreign body in areas of anastomosis and rupture is more likely to occur (14).

#### 1.1 Most frequently ingested objects in children

In a retrospective statistical study of medical cases of foreign body ingestion in children spanning a period of 33 years (1964–1997), from the Pediatric Surgery Department of the University Hospital of Hong Kong, Cheng and others studied 525 incidents. The age of the children ranged from six months to 16 years (mean age 5.2 years),

while pre-school age (mean age 3.8 years) was found most prone to foreign body swallowing. The most commonly confirmed cases of ingested objects from children (either through endoscopic or imaging procedures) were the following: coins 49%, fish bones 29%, other metal objects (batteries, sharp objects) 13%, other bones 2.7%, items of plastic 1.6%, and glass 1.2% (15).

### 1.1.1 Metal coins

Metal coins contain 97.6% zinc and 2.4% copper, while they are the most commonly ingested foreign bodies among children. Zinc (atomic number 30) has corrosive properties, and its presence in a localized anatomic area for a long time could cause rupture. In addition, if a large quantity of coins is swallowed, severe poisoning may result, leading to death.

In children, coins with a diameter of less than 2 cm are usually propelled by peristalsis into the small intestine, while those with a diameter larger than 2.5 cm are unable to move further and need to be removed (14–15).

### 1.1.2 Metal disc-shaped batteries

The increased use of disc-shaped batteries in various household electronic devices, due to their small dimensions, has resulted in the increased incidence of their ingestion by children, and thus in the increased incidence of esophageal caustic injury in children. Batteries with a diameter of less than 20 mm are promoted along the gastrointestinal tract and are excreted naturally. Larger diameter batteries are usually trapped in the esophagus or gastric cavity. Their harmful effect on the tissues mainly in the esophagus is due to three mechanisms: a. pressure necrosis, b. low voltage electrical current, and c. release of their alkaline content causing necrosis and rupture (16). If batteries are not eliminated within five days' time or abdominal symptoms begin to develop (abdominal pain, peritoneal irritation), surgical removal is necessary (14, 16).

### 1.1.3. Sharp and long objects

Ingestion of sharp objects of a length greater than 5 cm, such as long needles, pins and toothpicks, is associated with increased morbidity and mortality, as it accounts for 15–35% of rupture occurrence due to foreign body ingestion. According to the relevant guidelines, in general, ingested objects longer than 5 cm (3 cm in small children) or greater than 2 cm in diameter, which are localized in the stomach, have to be surgically removed before being promoted into the intestine. The ingestion of smaller objects, such as small screws, small nails, and pins, can be conservatively treated with monitoring, as they are often excreted naturally (14).

## 2. FOREIGN BODY INGESTION IN PSYCHIATRIC AND NEUROLOGICAL PATIENTS

Among patients suffering from psychiatric and neurological disorders (myotonic dystrophies, Guillain-Barré syndrome, and Parkinson's disease), the presence of bezoars in the gastrointestinal tract is often revealed. Bezoars comprise foreign material concentrations in the di-

gestive system (especially in the gastric cavity). Bezoars are distinguished into the following types: a. phytobezoar; b. trichobezoar; c. lactobezoar (in premature infants); d. pharmacobezoar (due to medications with cholesteramine, sulfafate, nifedipine, or antacids); e. other bezoars (trichophytobezoar, diospyrobezoar, and bezoar due to dead parasitic worms – ascaris or *Toxocaracati*) (17).

In addition to mental or neurological disease, other predisposing factors for the formation of bezoars are the following: a. gastrectomy or vagotomy and pyloroplasty (5–12% in patients with a history of gastric operations); b. endocrinopathies (diabetes mellitus and hypothyroidism); c. other factors (cystic fibrosis, cholestasis, kidney failure, and inadequate chewing) (17).

Bezoars are a rare cause of occlusive ileus at a rate of 0.4–4%. Preoperatively, the diagnosis can be set via ultrasonography in 88% of cases, as an echogenic mass with acoustic shadowing. When bezoars are localized within the gastric cavity, the treatment is focused on enzymatic cleavage or endoscopic fragmentation. In the case of obstructive ileus, laparotomy is preferable for the promotion of the bezoar through the ileocecal valve into the large intestine, or removal of it by intestinal incision close to the obstructed site.

Rapunzel syndrome is a rare and unusual form of trichobezoar extending from the stomach to the small intestine. It usually occurs in young female individuals with a history of psychiatric disorders with signs and symptoms of gastrointestinal obstruction or malabsorption and requires surgery by laparotomy or laparoscopy (18).

Another trait of psychiatric patients is the ingestion of multiple objects simultaneously or repeatedly (19–20).

In addition, patients with mental disorders are likely to experience allotriophagy (Pica disorder). As allotriophagy (from allo- meaning “different” and -phagy meaning “eating”) is defined as the compulsive unrelenting desire of an individual to consume, that is, to eat or chew items that are not edible or non-nutritional. Such consumed items may be metal objects (metallophagia), pieces of wood or paper (xylophagia), soil or sand (geophagia), stones (lithophagia), glass (hyalophagia), ice (pagophagia), hair (trichophagia), feces (coprophagia), cigarette residues, ash, toothpaste, or soap (21).

This disorder is included in the category of “eating disorders” of ICD-10 and DSM, and is more common in individuals with mental disorders, impaired functioning (e.g., schizophrenia spectrum disorders or obsessive compulsive spectrum disorders), neurodevelopmental disorders (e.g., autism spectrum disorders), during medical conditions (e.g., pregnancy), and, in small children, due to lower developmental level. (21–22).

As the damage that this disorder can cause to an individual's health is often irreparable, patients that suffer from Pica disorder have to be examined by a psychiatrist for treatment and prevention (23).

A specific category of allotriophagy, as mentioned above, is xylophagia. Within international bibliography, a fatality was reported involving an individual who was suffering from schizophrenia, had been multiply hospitalized in psychiatric units, and was in treatment with antipsychotics (clozapine) and antidepressants (citalopram and mirtazapine) (24).

**Tab. 1** Studies examining cases of voluntary ingestion of objects among prisoners throughout literature.

Country	Period Studied	Patients Number	Epi-sodes	Age (range / mean value)	Common Localization	Number of FB	Common Type of FB	Size of FB	Intervention required	Psychiatric Diagnoses	Reference
Ireland	1989–1992	36	52	15–69 years, mean age 25.8	Not mentioned	52	Nail: 11, Battery: 7, Razor blades: 6, Coin: 6, Crucifix: 4, Glass: 4, Pin: 4, Metal Objects: 3, Spring: 2, Cutlery: 2, Plastic Objects: 2, Needle: 2, Wood particle: 1	<5 cm: 30, 5–10 cm: 4, 10–18 cm: 2	Endoscopic: 4, Surgical: 2	Psychiatric history: 10 (27.8%) Undefined diagnoses	(28)
USA (Tennessee)	5 weeks	8	14	14–45 years, mean age 24.8	Not mentioned	14	Razor blades, metal objects	Not mentioned	Conservative (all)	Psychiatric history: 5 (62.5%) Undefined diagnoses	(29)
USA (Wisconsin)	10 years	22	75	19–47 years, mean age 28	Not mentioned	256	Broken glasses, toothbrushes, pencils, razor blades	Not mentioned	Endoscopic: 75, Surgical: 71	Not mentioned	(27)
Korea	1998–2004	33	52	25–50 years, mean age 35	Esophagus 42%, Stomach 42%	52	Metal wires: 26, Tooth brushes: 8	Range (4–20 cm), mean size 11.9 cm	Endoscopic: 46, Surgical: 6	Psychiatric history: 7 (21.2%), Personality disorder: 5, Schizophrenia: 1, Peri-ictal psychosis: 1	(30)
Northern Ireland	1998–2007	11	26	21–48 years, mean age 28.1	Small bowel: 8 (30.8%)	26	Razor blades: 6, Batteries: 3	Not mentioned	Endoscopic: 2, Surgical: 3	Psychiatric history: 6 (54.5%), Alcohol abuse disorder: 6	(26)
Morocco	1 year	9	9	mean age 29	Not mentioned	9	Not mentioned	Not mentioned	Surgical: 4	Not mentioned	(31)
Romania	2003–2010	45	105	30–40 y.o.: 20, 40–50 y.o.: 17, >50 y.o.: 3	Duodenum: 16 (15.2%), Intestine: 14 (13.3%), Stomach: 8 (7.6%)	105	Not mentioned	<5 cm: 17, 5–10 cm: 35, 10–15 cm: 40	Endoscopic: 4, Surgical: 8	Not mentioned	(32)
Italy	2005–2015	15	21	19–48 years, mean age 33.9	Not mentioned	34	Sharp: 23, Flat: 6, Undefined: 5	Not mentioned	Endoscopic: 10, Surgical: 1	Psychiatric history: 8 (53.3%), Mixed anxiety–depressive disorder: 4, Conduct disorder: 4	(33)

### 3. FOREIGN BODY INGESTION IN PRISONERS

The voluntary ingestion of objects is a common tactic among prisoners, but with varying underlying motivations. The most common aim of prisoners is to avoid incarceration or be transferred to nursing homes or psychiatric units. Sometimes, however, this may be due to undiagnosed mental disorders (schizophrenia spectrum disorders, depressive disorder, or suicidal actions), and, in other cases, as a way of trafficking drugs or other objects (25). In a study conducted at a hospital in Northern Ireland that treated emergency cases from the Belfast prison during the period 1998–2007, 11 cases of foreign body ingestion by prisoners were recorded (26). A characteristic observation is that while, in the general population, foreign bodies are usually automatically excreted, and only a very small percentage of the cases require medical intervention, in prisoners, the portion of cases requiring medical intervention is relatively higher. In the above-mentioned study, statistical results indicated that 36% of the prisoners needed surgical or endoscopic intervention (18% laparotomy, 9% endoscopy, and 9% both endoscopic and surgical procedures). The same conclusion was reached by another study by Weiland and his colleagues in Wisconsin, USA, in which 30% of foreign bodies were removed by surgery (27). The following table depicts all studies examining cases of voluntary ingestion of objects among prisoners throughout the literature (Table 1). A history of foreign-body ingestion prior to incarceration is very rare. The phenomenon usually begins during imprisonment and is often recurrent, followed by considerably high costs for the national health system. The psychological examination of prisoners with a history of intentional swallowing of foreign objects reveals high levels of psychopathology as well as antisocial and impulsive behavior, usually within the spectrum of personality disorders. High levels of suicidal ideation, however, have been identified among prisoners who engage in voluntary ingestion of objects (28).

### 4. FOREIGN BODIES IN DRUG ADDICTS – “BODY PACKERS”

Throughout the literature, men, women, even pregnant women, and a minor have been recorded as body packers. In particular, a 12-year-old boy in the USA had been found to be carrying 84 packets of heroin in his gastrointestinal tract. Professional body packers most often swallow drug packs, sometimes import them rectally into the gastrointestinal tract, and, in other cases, carry them in the vagina (34). When they are admitted to the hospital, in cases of package rupture, the following clinical symptoms are usually present: hyperthermia, hypertension, tachycardia, mydriasis, epileptic seizures, or status epilepticus, delirium, lethargy or coma. In some cases, even fatalities have been reported as a result of acute poisoning by the transported narcotics (35). In a study by Schaper and colleagues, the research sample comprised 4660 individuals who were identified as body packers and were arrested at the airports in Frankfurt and Paris between 1985 and 2002. Of the aforementioned subjects, 1.4% (64 individuals) developed symptoms of acute poisoning from cocaine induced by package rupture (36).

### 5. FOREIGN BODIES IN THE ABDOMINAL AREA AS A RESULT OF SEXUAL ABUSE OR SEXUAL ACCIDENT

Foreign body insertion and retention in the rectum may occur in adults during sexual activity or among sexually abused children (37). Throughout international literature, fatalities have even been recorded due to foreign body insertion into the rectum. In a literature review of autoerotic deaths by Sauvageau and colleagues, two deaths were reported due to abdominal foreign bodies (38). The first case involved a 56-year-old male who rectally inserted the heel of a woman's shoe, resulting in rectal perforation and profuse bleeding. In the second case, a 40-year-old male died as a consequence of peritonitis after the insertion of a pencil into the urinary bladder.

### 6. PRESENCE OF NON-RECOLLECTED SURGICAL TOOLS / ITEMS IN THE ABDOMINAL AREA

In the relevant literature, retained surgical items are defined by specific terms such as gossypiboma, textiloma, gauzoma, or muslinoma. The most widespread term is “gossypiboma,” which derives from the Latin word “gossypium” meaning cotton, and the word “boma,” which in Swahili means a “hiding point,” and is used in the case where the forgotten object is a pad of absorbent material; a surgical gauze or compress (39).

Regarding the prevalence of this phenomenon, Lincourt and colleagues indicated that the majority of incidents associated with retained surgical items have been recorded in abdominal surgical procedures (46%) (40). The incidence of retained, surgical-item cases ranged from 1 in 1,000–1500 intra-abdominal surgical interventions according to reports during the 1980s, while results of modern epidemiological studies indicate a considerable drop in the incidence of the phenomenon to 1 in 5,500–18,760 surgical procedures (41–42). The aforementioned absolute figures may be underestimated, however, as they relate only to cases that eventually led to the court of justice (43).

Recent research has highlighted that the urgent nature of surgeries in specific cases comprises a key risk-factor for the retention of a surgical foreign body in the abdominal area, highlighting the fact that the relative risk increases almost nine-fold in comparison to regular surgeries (44). Other risk factors are summarized in Table 2.

**Tab. 2** Risk factors for retained surgical items in the abdomen.

Emergency nature of incident and surgery
Complex surgical procedures
Profuse bleeding
Change of surgical strategy (unscheduled)
Participation of various surgical subspecialties – groups
High body mass index of the patient
Prolonged operative duration
High number of individuals in the operating room
Shift or personnel changes
Insufficient training
Usage of an unusually large number of surgical gauzes
Usage of small-sized surgical gauzes

Tab. 3 Studies on retained surgical item cases throughout literature.

Country	Period Studied	Number of cases (Patients)	Age (range / mean value)	Type of Surgery: Number of cases	Retained Foreign Body	Emergency nature of admission and original operation	Obesity	Range of interval between operation and symptomatology	Mortality (Deaths)	Reference
Spain	1983–1993	10	25–75 years, mean age 48	General: 6, Gynecological: 2, Urological: 2	10 sponges	Not mentioned	Not mentioned	11 days – 28 years	None	(45)
Belgium	1978–1995	5	31–61 years	General: 3, Gynecological: 2	5 sponges	Not mentioned	Not mentioned	2 days – 7 years	2	(46)
Turkey	1995–2000	10	Not mentioned	General: 5, Gynecological: 5	8 sponges, 2 clamps	6	Not mentioned	15 days – 20 months	None	(47)
Jordan	1990–2003	11	20–64 years, mean age 45	General and urological: 7, Gynecological: 4	11 sponges	7	5	2–108 months, median time: 12 months	None	(48)
Turkey	1999–2004	14	25–79 years	General: 9, Gynecological: 2, Urological: 3	14 sponges	Not mentioned	7	3 days – 40 years	None	(49)
Turkey	1994–2009	12	25–75 years, mean age 52.2	General: 6, Gynecological: 4, Urological: 2	12 sponges	5	Not mentioned	10 days – 84 months, median time: 28.25 months	1	(43)
Togo (West Africa)	2006–2015	15	21–55 years, mean age 27	General: 3, Gynecological: 11	3 sponges 100 cm <sup>2</sup> , 12 sponges 900 cm <sup>2</sup>	5	Not mentioned	7 days – 4 years, median time: 9 months	2	(50)
Total	-	77	20–75 years	General: 37 (48.1%), Gynecological: 30 (39%), Urological: 9 (11.7%)	Sponges: 75 (97.4%), Clamps: 2 (2.6%)	23 (29.9%)	12 (15.6%)	2 days – 28 years	5 (6.5%)	-

In a minor portion of the cases, retained surgical items remain asymptomatic without any complications and are usually identified randomly during postliminary medical examinations. Therefore, a retained surgical object may be detected in the ensuing several months or even years. The health condition of the affected patients can be significantly burdened, leading to adverse outcomes and even death in some cases (44) (Table 3).

According to statistics drawn from studies on cases of retained surgical items reported in detail throughout literature, approximately half (48.1%) of the cases occurred in the context of general surgery procedures followed by gynecological – obstetrics surgeries (39%). Urological surgeries were less frequent and tabulated at 11.7% of retained surgical item cases in the literature. Sponges (surgical gauzes) were the most frequently retained surgical items, accounting for 97.4% of the cases. In approximately one-third of the cases (29.9%), the admission of the patient to the operating room was urgent (non-scheduled surgery), and 15.6% of the patients faced obesity issues. The onset of abdominal symptoms due to the retained surgical items was observed to range between two days (minimum) to twenty-eight years (maximum); 6.5% of patients did not survive the complications.

The most common symptomatology due to retained surgical items includes pain (42%), palpable mass, and fever. Other complications are reported in Table 4.

**Tab. 4** Complications due to retained surgical items in the abdominal area.

Abdominal pain not otherwise explained
Presence of abdominal mass
Intra-abdominal bleeding
Abdominal organ perforation
Intestinal obstruction
Fistula formation
Translocation to adjacent organ
Sepsis
Multiple complications (combination of the above)
Other (weight reduction, ileus, urinary retention, abdominal distension, nausea, vomiting, fever of unknown origin)

In an article by Tumer and colleagues, ten cases were studied which were associated with retained surgical items after abdominal surgery having occurred in hospitals in Turkey during the period 1995–2000. All of the patients survived. In all patients, a second surgical procedure was performed so the retained surgical items could be successfully removed. All cases were taken to court, and, in all cases, there was a legal conviction. In seven cases, the surgeons who were found responsible were convicted, while in three cases the legal conviction concerned the entire surgical team (47).

The cost of compensation related to the retention of surgical items is high even if there is minimal or non-existent current risk to the patient. The cost varies between \$37,041 and \$2,350,000 (US Dollars) (approximately €31,754 – €2,014,805 euros) per incident, which is an average of \$ 95,000 or € 81,453 per incident (42). Furthermore,

in the majority of cases, there is conviction of the surgeon-in-charge or the whole surgical team by court decision for medical error or malpractice, as retained instruments subsequent to surgery is a fully preventable and predictable cause of morbidity and mortality (43).

## CONCLUSION

The presence of foreign objects in the abdomen may be due to their gradual formation in the lumen of the gastrointestinal tract, voluntary ingestion, rectal input, or iatrogenic input as a complication after surgical interventions. The presence of foreign bodies in the abdominal area is a medical condition that employs multiple specialties and requires high medical costs at a health system level, although the minority of cases do exhibit complications and require intervention.

The present review aims to update and summarize international literature on the presence, occurrence, anatomical localization, diagnosis, and treatment of gastrointestinal foreign bodies. The phenomenon is examined and discussed with respect to different groups of patients: children, psychiatric and neurological patients, prisoners, body packers, and postoperative patients (retained surgical items) as well as individuals subjected to sexual abuse or accident. To the authors' knowledge, there is no other multidisciplinary comprehensive review on the subject of foreign bodies in the abdominal area throughout international literature.

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# Rhinogenic Orbital Inflammation – What Has Changed over the Past 50 Years?

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## ABSTRACT

**Introduction:** The purpose of the study was to compare the incidence, diagnostics, and treatment of rhinogenic inflammatory complications over the past 50 years.

**Material and methods:** Retrospective study of 292 patients of ENT department, University hospital: Group A treated from 1966 to 1995, Group B treated from 1996 to 2015.

**Results:** Preseptal inflammation was the most common type (73% vs. 74%), followed by subperiosteal abscess (21% vs. 20%). Surgery was indicated in 35% vs. 37% of the patients ( $p = 0.434$ ). The most commonly used surgical approach was the external route (80%) in Group A and endoscopic endonasal surgery (60%) or a combination of endoscopic surgery of the paranasal sinuses and external orbitotomy (30%) in Group B ( $p < 0.001$ ). The percentage of reoperations was 13% vs. 14%. In cases of revision surgery, the orbit was always treated using the external surgical approach. Complete recovery was achieved in 92% and 98.5% of the patients belonging to Group A and B, respectively ( $p = 0.622$ ).

**Conclusion:** Nowadays, the endoscopic endonasal approach is the most frequently used surgical technique for paranasal sinuses.

The technique used to treat the orbital complication itself depends on several factors. Nowadays, the endoscopic approach is preferred. The external approach can be considered in the case of recurrent or persistent abscesses, especially if they are located in the upper or the lateral part of the orbit.

## KEYWORDS

sinusitis; endoscopic surgery; orbital inflammation

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## INTRODUCTION

Inflammatory conditions affecting the orbit (orbital cellulitis) represent – from the aetiological point of view – a relatively broad spectrum of conditions. Primary orbital inflammation is rare; it is presumably preceded by an injury that penetrates orbital tissues. Secondary orbital inflammation is more common; it develops due to spread of infection from the surrounding structures. In particular, this concerns cases of rhinosinusitis. However, inflammation of the lacrimal pathways or the teeth (i.e. odontogenic inflammation) or inflammation arising from the surrounding skin (erysipelas or a furuncle involving the nose or lip), i.e. dermatogenic infection, can spread to the orbit as well. Only rarely does orbital inflammation spread from a distal infection site, via the intracranial or the haematogenous route.

Rhinogenic orbital complications are the most frequent. Pansinusitis predominates in comparison with isolated inflammation of the paranasal sinuses (60–80%). In descending order, the following types of inflammation can be listed: inflammation of the ethmoidal, frontal, maxillary, and sphenoid sinuses. Rhinogenic orbital inflammation mainly affects children aged 5 to 10 years. In older children or adults, complications arising from inflammation of the frontal sinuses occur relatively more frequently (1). Typically, the clinical course is more favourable in children (preseptal inflammation predominates). In contrast, adults have a higher risk of developing postseptal inflammatory complications and persistent vision handicap (2). Furthermore, aetiological agents in children are different than in adults. In childhood, the principal causative agents are *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae*, and *Moraxella catarrhalis*, whereas in adults, polymicrobial inflammation characterized by the presence of anaerobes is the most common type. The microbiology of infections of the paranasal sinuses can be anticipated according to the patient's age, clinical presentation, nasal patency and immunocompetence (3, 4). Although patients seek medical assistance when experiencing acute symptoms, histological findings in the mucous membranes of the paranasal sinuses in surgically treated patients indicate that in these cases, chronic inflammatory infiltration prevails. Therefore, from the aetiological point of view, acute exacerbation of chronic rhinosinusitis is frequently involved in the development of orbital cellulitis.

The classification of rhinogenic inflammatory complications involving the orbit is based on Chandler's classification (5–7) (Table 1).

The objective of our study was to compare the incidence, diagnosis, and treatment of rhinogenic inflammatory complications over the past 50 years.

**Tab. 1** Classification of orbital inflammations according to Chandler.

Stage 1	Preseptal cellulitis
Stage 2	Orbital cellulitis
Stage 3	Subperiosteal abscess
Stage 4	Orbital abscess
Stage 5	Cavernous sinus thrombosis

## MATERIALS AND METHODS

This was a retrospective study of 292 patients with rhinogenic orbital inflammation, hospitalized in the Department of Otorhinolaryngology and Head and Neck Surgery of the University Hospital in Hradec Kralove over the past 50 years (between January 1, 1966 and December 31, 2015). The study patients were divided into two groups. The first group (Group A) consisted of patients treated between 1966 and 1995, i.e. when functional endonasal surgery was not yet a standard treatment in the above-mentioned workplace. The second group (Group B) consisted of patients treated between 1996 and 2015; functional endonasal surgery was a standard surgical method. Table 2 presents patient characteristics of both groups. In addition to basic demographic data, the following factors were evaluated in both groups: the type (stage) of inflammatory complication involving the orbit, the type of treatment (conservative or surgical), the selected surgical approach (endoscopic or external), the treatment outcome, and other complications.

**Statistical analysis:** For the statistical analysis the Statistica (data analysis software system), version 13 was used. Fisher exact test was used for estimation of statistical significance between the groups and *p*-values less than 0.05 were considered as statistically significant.

**Tab. 2** Patient characteristics.

Item	Group A (1966–1995)	Group B (1996–2015)
Number of patients	159	133
Cases per year	5.3	6.7
Male : female	2 : 1	3 : 1
Children and Teenagers (≤ 20 years old)	121 (76%)	81 (61%)

## RESULTS

### THE TYPE OF ORBITAL COMPLICATION

In both groups, the proportion of individual types of orbital complications was similar. Preseptal inflammation prevailed (73% vs. 74%); subperiosteal abscess was the second most common type (21% vs. 20%). In both groups, only rarely were patients diagnosed as having postseptal complications (orbital cellulitis, abscess – Table 3). As for unusual associated intracranial complications, we observed three cases of cavernous sinus thrombosis in patients belonging to Group A, and two cases of epidural abscess in Group B.

**Tab. 3** Distribution of orbital complications.

Orbital complication	Group A	Group B
Preseptal cellulitis, abscess	119 (75%)	98 (74%)
Subperiosteal abscess	33 (21%)	27 (20%)
Orbital cellulitis, abscess	6 (4%)	5 (4%)
Subperiosteal and orbital abscess	1 (0.6%)	3 (2%)
Associated complications	3 (cavernous sinus thrombosis)	2 (epidural abscess)

### CONSERVATIVE TREATMENT VERSUS SURGERY

In both groups, most patients were treated conservatively; surgery was indicated in approximately one-third of the patients (35% vs. 37%). Statistically, there was no difference in treatment strategy between the Group A and Group B ( $p = 0.434$ ).

### SURGICAL APPROACH

A comparison of the surgical approaches revealed noteworthy differences (Table 4). In Group A, the external route constituted the most commonly used surgical approach (80%) whereas most patients belonging to Group B underwent endoscopic endonasal surgery (60%) or a combination of endoscopic surgery of the paranasal sinuses and external orbitotomy (30%). This difference in number of endoscopical and external surgical approaches was statistically significant ( $p < 0.001$ ). In both groups, the percentage of reoperations was similar (13% vs. 14%). In most cases, reoperation was indicated for persistent orbital abscesses, localized mainly in the superolateral part of the orbit. In cases of revision surgery, the orbit was always treated using the external surgical approach.

Tab. 4 Treatment.

Treatment	Group A	Group B
Conservative	103 (65%)	84 (63%)
Surgical	56 (35%)	49 (37%)
– external approach	44 (80%)	5 (10%)
– endoscopic	6 (10%)	29 (60%)
– combination	6 (10%)	15 (30%)
– reoperation	7 (13%)	7 (14%)

### COMPLICATIONS

Complete recovery was achieved in 92% of the patients belonging to Group A and 98.5% of the patients belonging to Group B (Table 5). Diplopia was the most common permanent consequence following resolution of inflammation (3.8% vs. 1.5%). In Group B, there were no cases of residual visual impairment or death, whereas in Group A, mild visual impairment, unilateral blindness, and death were noted in 2.5%, 1.3%, and 0.6% of the patients, respectively. In spite of this fact, there was no difference in complications rates between the Group A and Group B on statistical analysis ( $p = 0.622$ ). Complications as visual impairment, blindness and death were typical just only for adults.

Tab. 5 Results of treatment.

Treatment Result	Group A	Group B
Healed	146 (91.8%)	131 (98.5%)
Impaired vision	4 (2.5%)	0
Blindness	2 (1.3%)	0
Diplopia	6 (3.8%)	2 (1.5%)
Death	1 (0.6%)	0

### DISCUSSION

#### CLINICAL TERMINOLOGY OF RHINOGENIC ORBITAL INFLAMMATION

Differences between inflammation forms are expressed through the terms abscess, phlegmon, and cellulitis. Abscess is a collection of purulent material, typically due to inflammation, that is contained in a pathological cavity. Phlegmon is a diffuse, purulent inflammatory process spreading through sparse tissues. Cellulitis is a well-demarcated inflammatory response in the soft tissue with an accumulation of inflammatory cells. In the case of orbital cellulitis, there is a well-demarcated response in the soft tissue with an accumulation of inflammatory cells in a certain zone from the periorbita, towards the inside of the orbital cavity. Orbital phlegmon is an inflammation of the soft tissues of the entire eye socket. It is a diffuse bacterial inflammation that can be associated with orbital venous thrombosis together with septic signs and symptoms (8–11). The terms preseptal, epiperiorbital (subperiosteal), and the orbital space itself are used to describe the location of the inflammation in the orbit. The term preseptal cellulitis is preferred over the term inflammatory eyelid oedema because it is a better way of conveying the location of the inflammation, anterior to the orbital septum. The term subperiosteal is the most frequently used term for an abscess that is located between the bone and the periosteum. However, the term epiperiorbital is more comprehensible as it clearly describes the location, outside the periorbita. The terminology is similar to that used to describe intracranial complications, where the term epidural represents the space outside the cranial dura mater, i.e. between the dura mater and the bone. The anatomic boundary of the periorbita enables classification of inflammatory orbital complications into phase I and phase II complications (again, the terminology is analogous to that used to describe intracranial complications, with the cranial dura mater being the analogous boundary). Phase I complications include preseptal cellulitis and subperiosteal abscess; phase II complications include orbital cellulitis and orbital abscess (12).

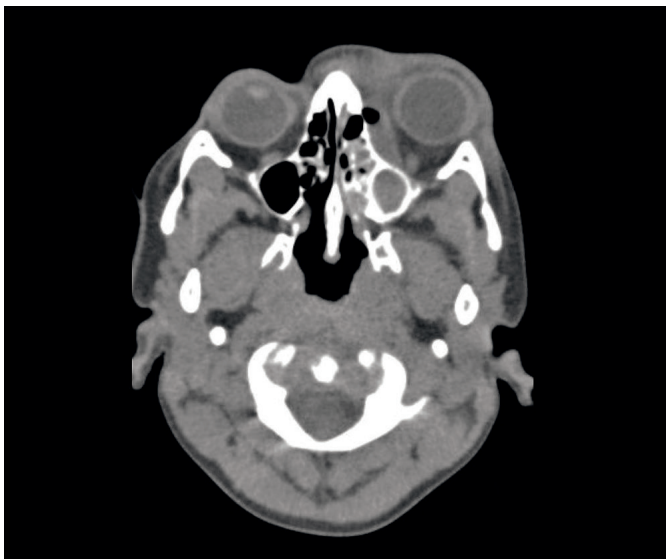
As for our study sample, the proportion of individual types of orbital complications remained essentially the same in both groups during the study period. Preseptal inflammation prevailed; subperiosteal abscess was the second most common type, and postseptal complications were rare. It is interesting to compare our study sample with that from Graz (13), which, by contrast, consisted of a high percentage of patients with postseptal complications (orbital abscess in 43%). This finding could probably be explained by a higher concentration of patients with serious postseptal complications in the workplace in Graz.

#### DIAGNOSTICS OF RHINOGENIC ORBITAL INFLAMMATION

The diagnosis of inflammatory orbital complications includes medical history taking, an ENT examination using nasal endoscopy, and an eye examination. Imaging-based examination is an indispensable method that enables a de-

tailed assessment of the condition of the paranasal sinuses as well as the orbital inflammation itself.

If the patient has preseptal cellulitis and his or her clinical condition is good, it is possible to wait and see whether imaging-based examination will be needed. Currently, plain X-rays are not indicated; they are not beneficial in orbital complications of sinusitis. In adult patients, a CT scan (ideally, using a contrast agent) is the method of first choice (Fig. 1). Noncontrast or contrast-agent enhanced MR imaging is preferred in paediatric patients (Fig. 2) as well as if postseptal orbital complication, cavernous sinus thrombosis, or another current intracranial complication is suspected (14). Unequivocal indications for imaging-based examination include the following conditions: inability to accurately evaluate vision, marked protrusion of the eyeball, ophthalmoplegia, bilateral oedema, visual



**Fig. 1** CT, horizontal section: left-sided pansinusitis, defect of lamina papyracea, inflammatory infiltration with air bubble along medial orbital wall.



**Fig. 2** MR, horizontal section: inflammatory infiltration of ethmoids and medial part of orbit on the right with dislocation of the course of medial rectus muscle.

disturbances, no improvement in health status after 24 hours of intensive conservative treatment using intravenous antibiotics, and CNS signs and symptoms.

## TREATMENT OF RHINOGENIC ORBITAL INFLAMMATION

### Conservative management

Antibiotic therapy is the mainstay of conservative management of orbital inflammation; broad-spectrum antibiotics are used. Monotherapy (aminopenicillin or cephalosporin) is sufficient for the treatment of preseptal inflammation, especially in children. By contrast, postseptal inflammation and orbital cellulitis in adults may require the use of combination therapy with two antibiotics (the addition of metronidazole or clindamycin) as in such cases, anaerobic strains are more frequently represented. In the first phase, empirical antibiotic therapy is instituted, and based on the culture and sensitivity results, the therapy can be adjusted according to further progress. Nasal mucosa toilet facilitates unobstructed passage through the nasal cavity as well as the unblocking of the exit points of the paranasal sinuses. Systemic or local corticosteroids can be used (9) although there are conflicting opinions regarding the question whether or not they are indicated for treatment of acute inflammation. As part of conservative management, maxillary sinus puncture can help in orbital cellulitis that develops due to maxillary sinusitis of odontogenic origin.

### Surgical management

Surgical management – without any delay – is undeniably indicated for the treatment of subperiosteal and orbital abscess. In some patients (especially in those presenting with anaerobic infections), an imaging-based examination reveals air bubbles in the subperiosteal space, or in rare cases, in the intraconal space. This finding frequently precedes the development of a well-demarcated abscess area, which is an indication for surgical intervention as well (the surgical procedure frequently shows purulent collections in the corresponding area). Furthermore, a CT scan may not reveal orbital abscess in all patients, especially if it is performed without a contrast agent. Therefore, even if an imaging-based examination does not reveal any abscess area, it is necessary to consider a surgical intervention (so that, at the very least, the primary inflammation site in the paranasal sinuses could be treated and the epiperiorbital space could be explored) in the following conditions: impaired visual acuity, progression of local and systemic signs and symptoms despite adequate conservative management for more than 24 hours, or no local improvement after 48–72 hours of antibiotic therapy.

Currently, it is possible to use the following surgical approaches:

The endoscopic endonasal approach – treatment of inflammation in the area of the affected paranasal sinuses; ethmoidectomy is used the most frequently (15). Following entry via the lamina papyracea, it is possible to drain subperiosteal abscess, which is in close contact with the medial wall of the orbit. All abscesses that do not meet the

mentioned requirement and that are located deeper within the orbit are less suitable for the endonasal approach. Treatment of orbital abscess entails making an incision in the periorbita. The endonasal approach requires excellent surgical skilfulness. The endonasal approach is affected by bleeding from the inflamed mucosa of the paranasal sinuses and by small spaces in paediatric patients.

The external approach – it consists of orbitotomy (usually medial), external ethmoidectomy and drainage of subperiosteal or orbital abscess. Treatment of orbital abscess entails making an incision in the periorbita. The external approach is recommended if an abscess is located in the eyelid or in the upper, the lower, and the lateral part of the orbit as well as in patients undergoing revision surgery following endoscopic endonasal treatment.

The combined approach – endoscopic endonasal approach for paranasal sinuses and external approach for inflammatory orbital complications.

In our study sample, a large proportion of the patients received conservative treatment; surgical treatment was indicated in approximately one-third of the patients. In Group A, the external surgical approach was the most common technique, whereas Group B was treated mainly endoscopically or by using a combination of endoscopic surgery of the paranasal sinuses and external orbitotomy. The percentage of reoperations was similar in both groups; the advantages of the endoscopic approach include reduced perioperative morbidity and no scars in the facial area. Nowadays, the endoscopic approach is preferred. The external approach can be considered in the case of recurrent or persistent abscesses, especially if they are located in the upper or the lateral part of the orbit.

## CONCLUSIONS

Nowadays, the endoscopic endonasal approach is the most frequently used surgical technique for paranasal sinuses. The technique used to treat the orbital complication itself depends on several factors. Nowadays, the endoscopic approach is preferred. The external approach can be considered in the case of recurrent or persistent abscesses, especially if they are located in the upper or the lateral part of the orbit.

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## DISCLOSURE STATEMENT

The authors declare that they have no competing interest.

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# Monocyte to Lymphocyte Ratio, Neutrophil to Lymphocyte Ratio, and Red Cell Distribution Width are the Associates with Gouty Arthritis

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## ABSTRACT

**Background:** Neutrophils, monocytes, and macrophages activations are associated with a gout attack. Monocyte to lymphocyte ratio (MLR), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), red cell distribution width (RDW), and mean platelet volume (MPV) are well-known inflammation markers. In this study, we aimed to investigate whether they could be a predictive marker to the gout attack.

**Material and Methods:** A hundred and ten gout patients (male/female, 86/24) and 90 (male/female, 64/26) age-, gender-, and body mass index-matched volunteer controls were included in the study. Blood samples were obtained in the intercritical and attack period of the patients. Hemogram, serum uric acid (SUA), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) values were studied.

**Results:** In the attack period NLR ( $p < 0.001$ ), PLR ( $p < 0.05$ ), MLR ( $p < 0.001$ ), RDW ( $p < 0.05$ ), MPV ( $p < 0.05$ ), ESR ( $p < 0.001$ ), CRP ( $p < 0.001$ ) and SUA ( $p < 0.001$ ) values were significantly higher than intercritical period values. According to the results of regression analysis; There was an independent strong relationship between the gout attack and SUA, (Beta [ $\beta$ ] = 0.352,  $p < 0.001$ ), ESR ( $\beta = 0.329$ ,  $p < 0.001$ ), CRP ( $\beta = 0.286$ ,  $p < 0.001$ ), MLR ( $\beta = 0.126$ ,  $p < 0.001$ ), RDW ( $\beta = 0.100$ ,  $p = 0.003$ ) and NLR ( $\beta = 0.082$ ,  $p = 0.014$ ).

**Conclusions:** MLR, RDW, and NLR may be a strong predictive marker for a gout attack. MPV and PLR values in the gout attack may be associated with systemic inflammation.

## KEYWORDS

gouty arthritis; monocyte to lymphocyte ratio; neutrophil to lymphocyte ratio; red cell distribution width; mean platelet volume

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## INTRODUCTION

Gout is a common type of inflammatory arthritis and its prevalence is increasing. It is common in men compared to women. Acute episodes usually begin with sudden pain and swelling in the joints of the toe or the lower extremities (1–3). There are many mechanisms that initiate a gout attack that is one of the reasons for hyperuricemia (4). Generally, when serum uric acid (SUA) level exceeds 6.4 mg/dl, the inflammatory attack starts with accumulation in small joints in the form of monosodium urate (MSU) crystals (5). The crystals accumulate in the joint range and synovium where areas have excessive macrophage and neutrophil migration. MSU crystals cause the release of pro-inflammatory cytokines, such as interleukin (IL)-1beta, IL-6, tumor necrosis factor (TNF), and some chemokines (6). These pro-inflammatory cytokines and lysosomal enzymes are released from macrophages and neutrophils (7). Colchicine inhibits the inflammatory effect of MSU crystals and the release of IL-1beta (6). As a result of phagocytizing of MSU crystals by treatment or spontaneously by macrophages, the acute attack ends and the asymptomatic period begins (5, 8). Gout patients are exposed to local and systemic inflammation, especially during the acute attack.

The presence and prognosis of systemic inflammation are increased by elevated C-reactive protein (CRP), leukocyte, platelet, ferritin and decreased albumin levels (9). Neutrophils, monocytes, and macrophages migrate to the site of inflammation, causing inflammation by releasing some cytokines and chemokines (8). Also, lysosomal enzymes released from neutrophils and cytokines cause reactive oxygen species (ROS) (4). Increased ROS and cytokines cause the release of young and immature erythrocytes and platelets from the bone marrow to the periphery (10). There is a relationship between the inflammation level and the hemogram parameters such as neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), monocyte to lymphocyte ratio (MLR), red cell distribution width (RDW), and mean platelet volume (MPV), therefore; recently, researchers focused on investigating the reliability of these inexpensive hematological parameters. Many studies have reported that the parameters may be a reliable and inexpensive marker as a prognostic factor for predisposition to thrombosis, cancer patients, cerebrovascular and cardiovascular diseases (11–15).

In this study, we aimed to investigate whether NLR, PLR, MLR, MPV, and RDW were higher in gout patients compared to the control group. Secondly, we aimed to investigate whether these parameters increase in gout attack and which markers may be the predictive marker for the gout attacks.

## METHODS

### SAMPLES SELECTION

The study included 110 outpatients with gout (male/female: 86/24) who were admitted to our hospital, Department of Rheumatology and Internal Medicine polyclinics, and 90 volunteer controls (male/female: 64/26) were

included in the study. Patients' Gout was diagnosed according to the American College of Rheumatology (ACR) 2012 criteria (16). Blood samples were taken between 24 and 48 hours after the onset of pain, redness swelling complaints in patients with gout attack according to ACR 2012 criteria. Blood samples for hemogram, erythrocyte sedimentation rate (ESR), CRP, SUA, ALT, blood urea nitrogen (BUN) and creatinine values were taken in the intercritical period (17, 18). Patients with any of the following conditions were not included in the study; other rheumatologic diseases, uncontrolled diabetes, uncontrolled hypertension, uncontrolled hyperlipidemia, hematological and solid malignancy, acute and chronic infections, septic arthritis, hemodialysis or peritoneal dialysis patients with renal failure, and smokers.

### RADIOLOGIC EXAMINATION

X-ray and radiological imaging of the patients were performed during attacks and non-attacks period. In the intercritical period, bone erosions were differentiated from other erosive arthritis according to the shape of gout, and the diagnosis of gout was confirmed by clinical, laboratory and radiological findings. The presence of soft tissue swelling in the joint region of acute gout arthritis was evaluated radiologically by direct X-ray.

### BLOOD PRESSURE (BP) MEASUREMENTS

After both gout patients and control group at least 15 minutes relaxing, systolic and diastolic BP measurements obtained from their right and left arm by using sphygmomanometer. According to their elevated arm measurements, the dominant side was determined for blood pressure measurement and that the arm was used for subsequent BP measurements. After two additional measures which were obtained from the dominant arm, waiting 5 minutes between two measures, mean systolic and diastolic BP were calculated in order to get a proper measurement.

### BIOCHEMICAL PARAMETERS

BUN, creatinine, ALT, and SUA were performed with the photometric assays of the Abbott Architect C16000 analyzer, CRP test was performed with the nephelometric method of the Coulter Immage 800 device. The hematologic tests were performed by the Abbott Cell Dyn Ruby analyzer and ESR were studied in automatized Westergren device (Eventus VacuPlus ESR 100, Ankara, Turkey). NLR was obtained by dividing the neutrophil count by lymphocyte count. MLR was calculated by dividing the number of monocytes by lymphocytes. PLR was obtained by dividing the platelet count by the number of lymphocytes. The RDW reported on all standard hemograms, is an automated measure of the variation in red blood cell (RBC) size or volume. The RDW is represented as a coefficient of variation and is mathematically calculated as the standard deviation of RBC volume/mean corpuscular volume (MCV)  $\times$  100 (18). MPV value was obtained from direct hemogram results.

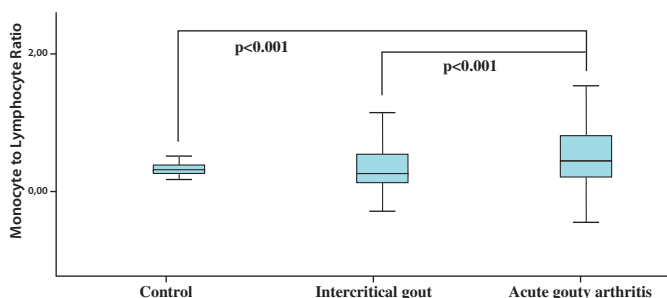


**STATISTICAL ANALYSIS AND ETHIC ISSUE**

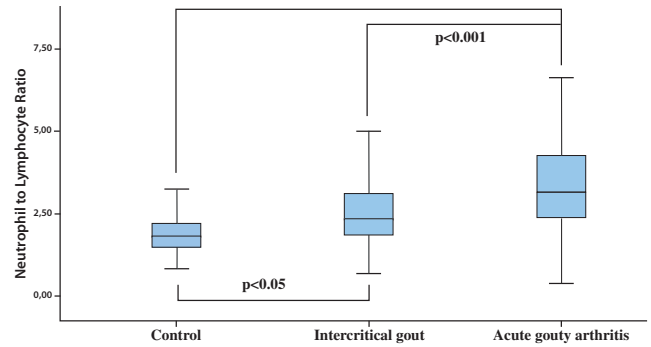
The results were given as mean ± standard deviation, median (range), and n (%). Statistical evaluation was performed with SPSS (version 18) package program. Kolmogorov Smirnov test was used to determine whether the data were homogenous. Homogeneous distribution data were compared with the Independent Student Test. Non-homogeneous distribution data such as CRP, ESR, NLR, PLR, and MLR were compared with the Mann Whitney U test. The Chi-square test was used to evaluate categorical data. The Pearson correlation test was used for correlation analysis. Stepwise linear regression analysis was performed to determine the independent variables associated with attack formation in patients with gout. Regression analysis was performed as an independent variable such as NLR, MLR, PLR, MPV, hemoglobin, SUA, creatinine, BUN, CRP, and ESR. A p value of < 0.05 was considered significant. The study was approved by the local ethics committees, and informed consent from each participant was obtained.

**RESULTS**

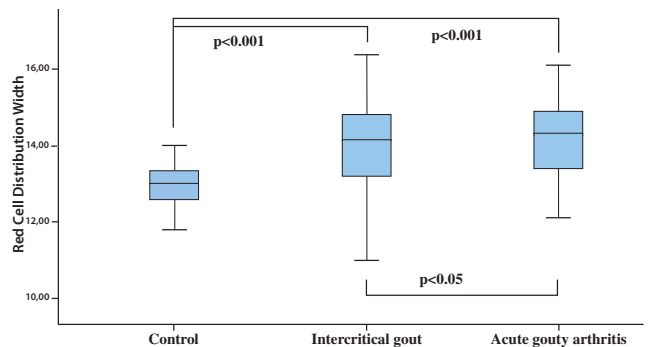
Age (58.1 ± 11.8 vs. 58.5 ± 14.9 years, p = 0.810), gender (M/F: 86/24 vs 64/26, p = 0.415), and body mass index (26.3 ± 3.6 vs. 26.7 ± 4.2 kg/m<sup>2</sup>, p = 0.454) of patients with gout were similar to those of control patients. The comorbid conditions of these patients were similar to the control group. Age, sex, disease duration, co-morbidity and medications of the patients were given in table 1. The intercritical period NLR (p < 0.05), RDW (p < 0.001), ESR (p < 0.05), SUA (p < 0.001) systolic BP (p < 0.05), and diastolic BP (p < 0.05) values of the gout patients were significantly higher than the control group values. In the attack period of the gout patients: NLR (p < 0.001), PLR (p < 0.001), MLR (p < 0.001), RDW (p < 0.001), MPV (p < 0.05), ESR (p < 0.001), CRP (p < 0.001) and SUA (p < 0.001) were higher than the values of the control group. When the attack period and intercritical period of gout patients were compared, the attack period was NLR (p < 0.001), PLR (p < 0.05), MLR (p < 0.001), RDW (p < 0.05), MPV (p < 0.05), ESR (p < 0.001), CRP (p < 0.001) and SUA (p < 0.001) values were significantly higher than intercritical period values. MLR, NLR and RDW values of the patients were given in figures 1, 2 and 3, respectively. All values of attack period, non-attack period and control group are shown in Table 1.



**Fig. 1** Monocyte to lymphocyte ratio in attack and intercritical period of gouty arthritis.



**Fig. 2** Neutrophil to lymphocyte ratio in attack and intercritical period of gouty arthritis.



**Fig. 3** Red cell distribution width in attack and intercritical period of gouty arthritis.

**Tab. 1** Age, gender, duration comorbidities, and medications for Patients.

	Gout Patients (n = 110)	Control (n = 90)	P value
Age (years)	58.1 ± 11.8	58.5 ± 14.9	0.810
Gender (M/F)	86/24	64/26	0.415
BMI (kg/m <sup>2</sup> )	26.3 ± 3.6	26.7 ± 4.2	0.454
Duration of disease (months)	14.0 (1.0–96.0)		
SBP (mmHg)	125.9 ± 11.0	122.0 ± 11.5	0.016
DBP (mmHg)	80.1 ± 8.7	76.1 ± 9.5	0.002
Hypertension	26 (23.6)	22 (24.4)	0.512
Coronary artery disease	12 (10.9)	10 (11.1)	0.570
Diabetes mellitus	9 (8.1)	11 (12.2)	0.391
Hyperlipidemia	25 (22.7)	25 (27.7)	0.255
Chronic renal failure	3 (2.7)	2 (2.2)	0.594
Thyroid disease	0 (0.0)	1 (0.9)	0.450
Colchicine	97 (88.1)	0 (0.0)	0.001
Allopurinol	102 (92.7)	0 (0.0)	0.001
NSAIDs	14 (12.7)	0 (0.0)	0.001
Steroids	6 (5.4)	0 (0.0)	0.001
Acetyl salicylic acid	20 (18.1)	18 (20)	0.441
Diuretic (single or combination)	5 (4.5)	9 (10)	0.110

The results were given as mean ± SD, median (range) or n (%). M, male; F, female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NSAIDs, non-steroidal anti-inflammatory drugs.

**Tab. 2** Hematological and biochemical parameters in attack and attack free period in patients with gout.

Variables	Attack period (n = 110)	Attack free period (n = 110)	Control (n = 90)
WBC ( $\times 10^9/L$ )	9.6 $\pm$ 2.0 * $\ddagger$	8.4 $\pm$ 1.9	8.8 $\pm$ 3.0
Neutrophil ( $\times 10^9/L$ )	6.4 $\pm$ 2.0** $\ddagger$	5.4 $\pm$ 1.9	5.3 $\pm$ 2.5
Lymphocyte ( $\times 10^9/L$ )	2.1 $\pm$ 0.7**	2.3 $\pm$ 0.9**	2.7 $\pm$ 0.7
Monocyte ( $\times 10^9/L$ )	0.8 (0.1–5.3)* $\ddagger$	0.7 (0.1–2.3)	0.8 (0.2–8.5)
Platelet ( $\times 10^9/L$ )	270.3 $\pm$ 93.7 $\ddagger$	239.3 $\pm$ 80.5*	266.3 $\pm$ 63.5
Hb (g/dL)	13.9 $\pm$ 1.3**	13.8 $\pm$ 1.1**	14.6 $\pm$ 1.7
MPV (fL)	10.3 $\pm$ 1.0* $\ddagger$	9.9 $\pm$ 1.0	9.9 $\pm$ 0.8
NLR	3.1 (0.4–8.8)** $\ddagger$	2.3 (0.6–13.1)*	1.8 (0.8–6.4)
PLR	128.8 (28.8–360.0)** $\ddagger$	104.1 (28.9–362.5)	95.0 (44.1–204.0)
MLR	0.4 (0.1–3.7)** $\ddagger$	0.3 (0.1–1.9)	0.3 (0.1–2.1)
RDW	14.2 $\pm$ 0.9** $\ddagger$	13.8 $\pm$ 1.3**	13.1 $\pm$ 1.2
ESR (mm/h)	36.0 (11.0–100.0)** $\ddagger$	12.0 (2.0–53.0)*	8.0 (2.0–37.0)
CRP (mg/dL)	29.5 (5.6–120.0)** $\ddagger$	3.5 (1.3–32.0)	2.2 (0.3–15.9)
Uric acid (mg/dL)	8.4 $\pm$ 1.5** $\ddagger$	7.3 $\pm$ 2.3**	4.7 $\pm$ 1.1
BUN (mg/dL)	33.6 $\pm$ 17.6**	30.8 $\pm$ 15.3**	15.3 $\pm$ 4.7
Creatinine (mg/dL)	1.0 $\pm$ 0.4*	1.0 $\pm$ 0.4*	0.9 $\pm$ 0.2
ALT (IU/L)	36.5 $\pm$ 20.6*	32.1 $\pm$ 22.5	27.9 $\pm$ 19.8

The results were given as mean  $\pm$  SD and median (range).

**WBC**, white blood cell; **HB**, hemoglobin; **MPV**, mean platelet volume; **NLR**, neutrophil to lymphocyte ratio; **PLR**, platelet to lymphocyte ratio; **MLR**, monocyte to lymphocyte ratio; **RDW**, red cell distribution width; **ESR**, erythrocyte sedimentation rate; **CRP**, C-reactive protein; **BUN**, blood urea nitrogen; **ALT**, alanine aminotransferase.

\*  $p < 0.05$ , \*\*  $p < 0.001$  vs control group

$\ddagger p < 0.05$ ,  $\ddagger\ddagger p < 0.001$  vs Attack free period

**Tab. 3** NLR, RDW, MLR, uric acid, and correlation analysis of other factors in patients with gout arthritis.

Variable	r value	SUA		CRP	
		p value	r value	p value	r value
Intercritical period	NLR	0.100	0.138	0.275	0.001
	RDW	0.215	0.001	0.070	0.340
	MLR	0.115	0.089	0.155	0.022
	MPV	0.006	0.924	0.141	0.037
	PLR	0.063	0.355	0.168	0.013
	SUA			0.118	0.081
	CRP	0.118	0.081		
	SBP	0.250	0.009	0.164	0.088
	DBP	0.216	0.023	0.234	0.014
Attack period	NLR	0.202	0.001	0.332	0.001
	RDW	0.319	0.001	0.173	0.002
	MLR	0.045	0.435	0.092	0.109
	MPV	0.032	0.577	0.142	0.012
	PLR	0.068	0.232	0.221	0.001
	SUA			0.300	0.001
	CRP	0.300	0.001		

**SUA**, serum uric acid; **CRP**, C-reactive protein; **NLR**, neutrophil to lymphocyte ratio; **RDW**, red cell distribution width; **MLR**, monocyte to lymphocyte ratio; **MPV**, mean platelet volume; **PLR**, platelet to lymphocyte ratio; **SBP**, systolic blood pressure; **DBP**, diastolic blood pressure.

**Tab. 4** Stepwise Linear Regression Analyses, determinants of attack period in patients with Gout Arthritis.

Independent Variables	Gout attack as Dependent Variable	
	Beta coefficients	p value
<b>SUA</b>	0.352	0.001
<b>ESR</b>	0.329	0.001
<b>CRP</b>	0.286	0.001
<b>MLR</b>	0.126	0.001
<b>RDW</b>	0.100	0.003
<b>NLR</b>	0.082	0.014

**SUA**, uric acid; **ESR**, erythrocyte sedimentation rate; **CRP**, C-reactive protein; **MLR**, monocyte to lymphocyte ratio; **RDW**, red cell distribution width; **NLR**, neutrophil to lymphocyte ratio.

In the correlation analysis for intercritical period, there was a positive correlation between SUA and RDW ( $p < 0.001$ ). We found a positive association between CRP and NLR ( $p < 0.001$ ), MLR ( $p = 0.022$ ), and PLR ( $p = 0.013$ ). In addition, we determined a positive association between MLR and systolic BP ( $r = 0.199$ ,  $p = 0.039$ ). In the correlation analysis for attack period; we found a positive association between SUA and NLR ( $p < 0.001$ ), RDW ( $p < 0.001$ ), and CRP ( $p < 0.001$ ). We determined a positive relation between CRP and NLR ( $p < 0.001$ ), RDW ( $p = 0.002$ ), MPV ( $p = 0.012$ ), and PLR ( $p < 0.001$ ). Correlation analysis results are shown in Table 3.

According to the results of stepwise linear regression analysis; There was an independent strong relationship between gout attack and SUA ( $p < 0.001$ ), ESR ( $p < 0.001$ ), CRP ( $p < 0.001$ ), MLR ( $p < 0.001$ ), RDW ( $p = 0.003$ ) and NLR ( $p = 0.014$ ). Regression analysis results are shown in Table 4.

## DISCUSSION

In this study, NLR and RDW values were significantly higher in gout patients compared to the control group. In the attack period, NLR, PLR, MLR, RDW, and MPV values were significantly higher than the intercritical period and control group. In the regression analysis, we found that SUA, CRP, ESR, MLR, NLR and RDW values could be predictive markers for a gout attack.

Even if the SUA concentration is normal, a gout attack may develop (19). However, there is a high correlation between SUA concentration and gout attack (20). In our study, we found a very high relationship between gout attack and SUA level. Also, according to our findings, the SUA level is the most important predictive marker for a gout attack. Hyperuricemia leads to ROS formation and endothelial dysfunction (21). Hyperuricemia is associated with obesity, type 2 diabetes mellitus, atherosclerosis and hypertension (22, 23). We found the systolic and diastolic BP values in gout patients to be higher than the control group. Also, we found a high relation between both systolic and diastolic BP and SUA levels in the correlation analysis. Gout patients may be prone to cardiovascular disease because hypertension is one of the major factors of atherosclerosis.

Although SUA inhibits the formation of ROS, it causes ROS formation due to autophagy defects (24). SUA shows the pro-inflammatory effect by direct stimulation of the monocytes (24, 25). Monocyte stimulation causes inflammation by releasing pro-inflammatory cytokines (26). Monocytes play an important role both at the beginning and end of the gout attack (27). MSU crystals provide transformation of monocytes into macrophages by stimulating transforming growth factor beta (8, 27). In addition, monocytes release pro-inflammatory cytokines in the peripheral circulation. Monocytes accumulate in the arterial wall and turn into macrophages and they cause local damage by releasing pro-inflammatory cytokines from macrophages (28). Low lymphocyte counts have been reported to be related to inflammation, atherosclerosis and plaque development (29). Increased monocyte count and decreased lymphocyte count cause to raise MLR. There has been a strong relationship between MLR and systemic inflammation, cardiovascular disease and myocardial infarction (30, 31). In our study, we found MLR as a strong predictive marker in the gout attack. Also, there was also a relationship between MLR and systolic BP. The relationship between MLR values and subclinical atherosclerosis in gout patients may be investigated in the novel studies.

Neutrophils are the major responsible cells in gout inflammation and attack development (32). The deposition of MSU crystals in tissues leads to massive leukocyte inflammation (33). The release of pro-inflammatory cy-

tokines and lysosomal enzymes from leukocytes causes ROS to increase in the environment. Increasing the number of neutrophils also leads to an enhancement in blood viscosity and hypercoagulability by causing interaction in the platelets and endothelium (34). Neutrophils show nonspecific inflammation and the decrease in the number of lymphocytes reflects the inflammation (35). NLR, therefore; shows more effective inflammation than the neutrophil count (34). NLR is a powerful independent indicator of prognosis in systemic inflammation, atherosclerotic heart disease, and cancer patients (11, 12). We determined a high relationship between NLR and SUA, especially during the attack episode of gout. In our study, we found NLR as a strong independent predictive marker for a gout attack in regression analysis.

Inflammation and increased ROS suppress erythropoiesis. As a result of this suppression, erythrocytes in the bone marrow pass into peripheral blood before they reach adequate maturation, and complete immature erythrocytes increase in peripheral blood (36). Pro-inflammatory cytokines increase the synthesis of hepcidin in the liver by IL-6 mediated inhibition of iron reaching normoblasts and these cytokines suppress normoblast proliferation and increase the phagocytosis of erythrocytes (37, 38). As a result of these events, the non-functional juvenile erythrocytes increase in the peripheral blood. RDW is the best test that shown this increase in the laboratory. RDW has been found to be an independent predictive marker for inflammation and atherosclerotic heart disease (39). We found a high relationship between RDW and SUA, particularly during the gout attack. In our study, we found RDW as a strong predictive marker for a gout attack.

Platelets adhere to endothelial cells and increase inflammation by causing leukocytes to migrate to this area and cling to it. On the other hand, increased cytokines and ROS affect the thrombopoiesis and increase the output of immature, larger than normal and active platelets to the peripheral blood (10). These active platelets release serotonin, thromboxane A<sub>2</sub>, ADP and clotting factors to the environment. MPV is a laboratory test that reflects these platelets in the blood (10, 14). Studies have reported that releasing the juvenile, dysformic erythrocytes and young, large platelets to peripheral blood circulation; increase in the risk of thrombosis (14, 39). Conway et al. reported that platelet activity was significantly increased during the attack, particularly with a predisposition to thrombosis (40). Increased MPV is associated with systemic inflammation and atherosclerotic heart disease (10). However, in our study, we found that PLR and MPV values for gout attack were not predictive markers.

## CONCLUSION

NLR and RDW values were higher in gout patients with an attack-free period than controls. Inflammation continues in gout patients during attack-free periods. Increased NLR, PLR, MLR, RDW, and MPV values during the acute attack period indicate that inflammation is exacerbated. MLR, RDW, and NLR may be a strong predictive marker for a gout attack and systemic inflammation. Both attack

period and intercritical period, increased inflammation and platelet activation may cause various organ and system diseases especially cardiovascular disease. Whether the inexpensive hematological parameters are a marker for cardiovascular disease in gout patients can be investigated in novel studies.

## CONFLICT OF INTEREST STATEMENT

None to declare.

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# Validation of the Slovakian version of Boston Carpal Tunnel Syndrome Questionnaire (BCTSQ)

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## ABSTRACT

**Introduction:** The most common occupational disease is carpal tunnel syndrome. The aim of this study was to translate and validate the Slovakian version of BCTSQ.

**Methods:** The original questionnaire was translated into the Slovakian language by two expert translators. Cronbach's alpha coefficient was used to analyze the internal consistency of the questionnaire. Construction validity was evaluated by using Pearson's correlation coefficient and Spearman's rank correlation. The results were processed in the statistical program SPSS 24. The level of significance  $p > 0.05$  was considered significant. To analyze the validity, a factor analysis of the BCTSQ and the correlation between BCTSQ and SF-36 were obtained. Results: A total of 32 employees at risk of local muscular overload completed the BCTSQ and Health Questionnaire SF-36. Cronbach's alpha for SSS was  $>0.8$ . The alpha coefficient for FSS was  $>0.9$ . The Pearson's coefficient and Spearman's rank correlation was  $>0.9$  for each domain. Cronbach's alpha for SF-36 was  $>0.7$  and the Pearson's coefficient and Spearman's rank correlation was  $>0.6$  and  $p$ -values  $<0.001$ .

**Conclusions:** The questionnaire has sufficient reliability, validity and can be a suitable tool for the evaluation of subjective response of employees at the risk of local muscular overload, as well as of patients with carpal tunnel syndrome.

## KEYWORDS

Boston Carpal Tunnel Syndrome Questionnaire; SF-36; carpal tunnel syndrome; subjective evaluation; validation

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## INTRODUCTION

Health measurement scales are important not only for patient outcomes tools to evaluate health status. It is necessary for evaluation of subjective response of employees at risk of local muscular overload and also carpal tunnel syndrome (CTS). These scales are an important step in prevention to reduce the overload of musculoskeletal system. CTS is the most expensive musculoskeletal disorder. The costs to employers, employees and insurance companies from carpal tunnel syndrome can rack into the billions (1-4). While the total economic costs of workplace carpal tunnel syndrome in the Slovak Republic are not fully known, its pervasive effect throughout various work industries is apparent. Among the specific measures, the 36-Item Short-Form Health Survey (SF-36) is the most recently devised scales. It fields an 8-scale profile of scores, as well as summary measures. The questionnaire has a wide use in the clinic and also prevention to evaluate health conditions (5, 6). As one of the specific disease measures for the evaluation of severity of symptoms and functional status in CTS is Boston Carpal Tunnel Syndrome Questionnaire (BCTSQ) (7). The BCTSQ is one of the available tools for evaluation of the functional and symptomatic aspects of CTS. This questionnaire has been called by several names, e.g. Carpal Tunnel Questionnaire, Brigham and Woman's Carpal Tunnel Questionnaire and Levine Questionnaire (8). The Carpal Tunnel Syndrome Questionnaire is valid and available in few languages (e.g. Polish, Spanish, and Persian).

## MATERIAL AND METHODS

The original BCTSQ is in English. The first step was author's agreement to translate questionnaire into the Slovakian language in accordance with standard methods. The both parts of the original questionnaire (Symptom Severity Scale and Functional Status Scale) were translated into the Slovakian language by two English expert translators. The confirmed questionnaire was interpreted back into the original language to check for any possible content inequality between the original questionnaire and its final translated version.

BCTSQ consists of 2 parts. First part/scale is Symptom severity scale (SSS). This part consists of 11 items classified in 6 domains: pain (number 3-5), paresthesia (number 8), numbness (number 6), weakness (number 7), nocturnal symptoms (number 1, 2, 9, 10) and overall functional status (number 11). The answers are rated from 1 point (no symptoms) to 5 point (most severe symptoms). The overall score for functional status is calculated as the mean of all 11 items (Table 1).

The second part/scale is Functional status scale (FSS) consists of 8 items. The answers are rated from 1 point (no difficulty with the activity) to 5 point (cannot perform the activity). The overall score for functional status is calculated as the mean of all 8 items (Table 2) (7, 9).

Translated versions have been submitted to the author. After author's agreement the final Slovak questionnaire

**Tab. 1** Symptom severity scale.

Number	Items
1	Severity of nocturnal pain
2	Frequency of nocturnal awakening due to pain
3	Severity of daytime pain
4	Frequency of daytime pain
5	Duration of daytime pain
6	Severity of numbness
7	Severity of weakness
8	Severity of tingling
9	Severity of nocturnal numbness or tingling
10	Frequency of nocturnal awakening due to numbness or tingling
11	Difficulty with grasping and use of small object (keys, pens)

**Tab. 2** Functional status scale.

Number	Items
1	Writing
2	Buttoning of clothes
3	Holding a book while reading
4	Gripping of a mobile phone
5	Opening of jars
6	Household chores
7	Carrying of grocery basket
8	Bathing and dressing

was answered by 10 employees at risk of local muscular load and CTS to determine its comprehensibility. The next step was the validation of the Slovak version of the questionnaire. The data collection was carried out in November in 2018 in the concrete structures factory. The BCTSQ was filled (test) by 32 participants along with the Health Questionnaire SF-36. After 12 days, the translated questionnaire was refilled by some of the previous employees (retest). The questionnaire was anonymous and participants were aware of the purpose of the study. Employees responded to demographic questions (gender, age, height, weight, BMI, laterality, problematic hand, profession, duration of exposure). The return of the questionnaire was 100%.

Reliability was investigated by looking at the reproducibility and internal consistency based on the test-retest method. The following analyses were conducted to examine validity. A factor analyses was conducted to examine the construct validity and the unidimensionality of the SSS and FSS. The completeness of item responses for the BCTSQ was examined.

Correlation coefficients between the BCTSQ and SF-36 were obtained, and the following hypotheses were examined to investigate concurrent validity: SSS would exhibit the largest (strongest) association with "bodily pain" (SF-36 BP) among the SF-36 subscales. FSS would exhibit the largest (strongest) association with "physical functioning" (SF-36 PF) or "role-physical" (SF-36 RP).

The responsiveness of BCTSQ and SF-36 were examined by calculating the standardized response mean and the effect size.

To determine the internal consistency of the questionnaire we used the Cronbach's alpha coefficient. Values in the range of 0.9 and above indicate an excellent internal consistency; values between 0.8 and 0.9 indicate a good internal consistency. Values between 0.7 and 0.8 indicate an acceptable internal consistency, values between 0.6 and 0.7 indicate a questionable internal consistency, a value between 0.5 and 0.6 indicate a poor internal consistency and values in the range 0.5 and below indicates an unacceptable internal consistency. The instrument test-retest reliability of BCTSQ (SSS, FSS) was assessed with the intra-class correlation coefficient. All correlation coefficients among BCTSQ and SF-36 results were calculated with use of Pearson's correlation coefficient and Spearman's rank correlation coefficient. Results between 0.1–0.3 indicate small strength of association; between 0.3–0.5 indicate medium strength of association and values in the range of above 0.5 being considered large (strong). The statistical processing of the results was performed in SPSS 24. For statistically significant we considered  $p < 0.05$ .

## RESULTS

There were 32 participants (28 males, 4 females) - employees in the concrete structures factory at risk of local muscular overload of upper extremities and CTS included in this study. Average age of employees in the tracked group was  $38.13 \pm 11.39$  years with the average duration of exposure  $9.78 \pm 7.23$  years. The youngest employee was 21 years old and the oldest 63 years old. The longest occupational exposure was 30 years and the shortest was one year. Average height was  $176.06 \pm 7.48$  cm. Average weight was  $82.16 \pm 13.45$  kg and BMI  $26.52 \pm 3.69$ . Among 32 employees, 30 were right-handed, 2 employees were left-handed and 15 employees reported problems with both hands during work.

The mean, standard deviation (SD), median and range of the BCTSQ (SSS, FSS) and SF-36 (PF, RP, BP) are shown in Table 3.

**Tab. 3** Mean scores for BCTSQ and SF-36 ( $n = 32$ ).

Instrument scale	Mean	SD	Median	Minimum	Maximum
SSS	2.06	0.59	2.00	1.00 <sup>a</sup>	3.50
FSS	1.56	0.78	1.30	1.00 <sup>a</sup>	3.30
SF-36 PF	77.50	20.98	80.0	5.00 <sup>b</sup>	100 <sup>a</sup>
SF-36 RP	43.75	45.35	37.50	0 <sup>b</sup>	100 <sup>a</sup>
SF-36 BP	26.88	20.07	20.00	0 <sup>b</sup>	60 <sup>a</sup>

SD – Standard Deviation; SSS – Status Severity scale; FSS – Functional Status Scale; SF-36 PF – physical functioning; SF-36 RP – role-physical; SF-36 BP – bodily pain of the 36-Item Short Health Survey

<sup>a</sup> Maximum health status score

<sup>b</sup> Minimum health status score

There were 32 employees involved in the assessment of test-retest reliability. The period between the first and sec-

ond tests was 12 days. Internal consistency was assessed by use of Cronbach's alpha coefficient. Cronbach's alpha (test) for 11 items (SSS) was 0.86 ( $n = 32$ ), which means good internal consistency. The alpha coefficient for 8 items (FSS) was 0.94 ( $n = 32$ ), which means an excellent internal consistency. Instrument test-retest reliability was assessed with the interclass correlation coefficient. Cronbach's alpha (retest) for 11 items (SSS) was 0.86 ( $n = 32$ ). The alpha coefficient for 8 items (FSS) was 0.94 ( $n = 32$ ). Before verifying the design validity of the questionnaire, we validated the normality of data by the Kolmogorov-Smirnov test, which confirmed the normal data layout. We used the test-retest method to verify the structural validity of the questionnaire with a Pearson's coefficient correlation. A large level of match between values in all domains has been demonstrated. The Pearson's coefficient was more than 0.9 for each domain.

The results of the Pearson's coefficient above 0.5 for each domain and the overall score point to large reliability of the questionnaire in the Slovak language.

The Pearson's correlation coefficient (Table 4) between the SSS and the subscales of the SF-36 ranged from 0.63 to  $-0.61$ . The strongest correlation was observed for "bodily pain" followed the correlation between the SSS and "role-physical" and "physical functioning." The correlation between FSS and the subscale SF-36 ranged from 0.55 to  $-0.57$ . The strongest correlation was observed for "physical functioning" followed "bodily pain" and "role-physical".

**Tab. 4** Person's correlation coefficient ( $n = 32$ ).

Pearson's correlation with		
Instrument scale	BCTSQ-SSS	BCTSQ-FSS
BCTSQ-FSS	0.57**	–
SF-36 PF	$-0.60^{**}$	$-0.57^{**}$
SF-36 RP	$-0.61^{**}$	$-0.39^*$
SF-36 BP	0.63**	0.55**

SD – Standard Deviation; SSS – Status Severity scale; FSS – Functional Status Scale; SF-36 PF – physical functioning; SF-36 RP – role-physical; SF-36 BP – bodily pain of the 36-Item Short Health Survey

\*  $p < 0.05$

\*\*  $p < 0.01$

**Tab. 5** Spearman's rank correlation ( $n = 32$ ).

Spearman's rank correlation with		
Instrument scale	BCTSQ-SSS	BCTSQ-FSS
BCTSQ-FSS	0.57**	–
SF-36 PF	$-0.72^{**}$	$-0.55^{**}$
SF-36 RP	$-0.63^{**}$	$-0.42^*$
SF-36 BP	0.69**	0.58**

SD – Standard Deviation; SSS – Status Severity scale; FSS – Functional Status Scale; SF-36 PF – physical functioning; SF-36 RP – role-physical; SF-36 BP – bodily pain of the 36-Item Short Health Survey

\*  $p < 0.05$

\*\*  $p < 0.01$

The Spearman's rank correlation (Table 5) between the SSS and the subscales of the SF-36 ranged from 0.69 to  $-0.72$ . The strongest correlation was observed for "bodily pain" followed the correlation between the SSS and

“role-physical” and “physical functioning.” The correlation between FSS and the subscale SF-36 ranged from 0.58 to -0.55. The strongest correlation was observed for “physical functioning” followed “bodily pain” and “role-physical”.

## DISCUSSION

As a result of constant modernization, automatization and robotization in the past few years, the number of works in which small muscle groups of hands and forearms are loaded is increasing. The work is monotonous and repetitive with excessive number of movements. For this reason, the number of occupational diseases due to the long-term excessive unilateral load of upper extremities is increasing every year.

The questionnaire is predominantly used among patients with a diagnosed CTS. On the other hand, based on studies of available literature, we evaluated the questionnaire as a suitable tool for subjective analysis of local muscular overload as a potential development of CTS. The necessity of assessment of subjective work-related difficulties with musculoskeletal system in the working environment is evidenced by the increasing trend of CTS as the most common occupational diseases. In the Slovak Republic, 308 cases of occupational diseases (187 males and 121 females) were reported to the National Health Information Centre in 2018. In 2017, 354 cases of occupational diseases and professional poisonings were reported in which females accounted for 41.52% (147 cases) of the total. The most frequently reported occupational diseases in 2018 was the long-term excessive unilateral load of upper extremities (CTS) was reported in 147 employees, i. e. 47.72% of all reported occupational diseases in the Slovak Republic (13).

The BCTSQ was validated in a several languages and nowadays is available in few countries (9–12). As for internal consistency, the Cronbach's alpha for first part (SSS) of BCTSQ (0.86) and for the FSS (0.94) were equivalent to those of the original version of BCTSQ (SSS:0.89, FSS:0.91) (7). For intra-class correlation coefficient of the SSS (0.98) and the FSS (0.99) were equivalent to the Pearson correlation coefficient of the original version of BCTSQ (SSS:0.85, FSS:0.87) (7). Likewise, the Polish version of the questionnaire showed high test-retest reliability (SSS:0.9, FSS:0.92) (12). The Persian version showed acceptable but lower correlation than the study mentioned previously (SSS:0.58, FSS:0.77) and The Cronbach's alpha coefficients was 0.86 for SSS and 0.88 for FSS (10). The Cronbach's alpha coefficients of the Polish-BCTSQ (0.91 for the SSS and 0.92 for the FSS) are indicative of the high internal consistency of the questionnaire. Our study revealed high internal consistency of BCTSQ implying good coherence among all questions. In this study, our data showed good correlation in terms of the test-retest reliability. There are many types of validity processes of quality of life (QOL) questionnaires. In our study we adopted correlation between BCTSQ and SF-36. The moderate correlation between BCTSQ and SF-36 subscales (bodily pain and physical functioning) supports this validity. These results demonstrate the importance of BCTSQ measures that make up health-related QOL.

## CONCLUSIONS

We can conclude that the Slovakian version of BCTSQ has sufficient reliability and validity to assess the severity of symptoms and the functional status. The questionnaire can serve as a suitable tool for evaluate of subjective response of patients with CTS, as well as of employees at the risk of local muscular overload of upper extremities.

## ETHICAL APPROVAL

The study protocol was approved by the Ethics Committee at the Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, reference number EK 138/2018, and it conforms to the provisions of the Declaration of Helsinki in 1995.

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# Effect of Non-Surgical Periodontal Therapy on the Serum Sialic Acid Levels in Diabetic Patients with Periodontitis

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## ABSTRACT

Sialic acid (SA), a family of acetylated derivatives of neuraminic acid, an acute phase reactant by itself. It usually occurs as a terminal component at the non-reducing end of carbohydrate chains of glycoproteins and glycolipids. SA participates in multiple physiological functions, such as cell-to-cell interactions, cell migration and proliferation. Diabetes mellitus (DM) is a chronic metabolic disorder characterized by rise in blood glucose level. Periodontitis is a chronic inflammatory disease of the periodontal tissue, leading to destruction of bone surrounding the tooth and ultimately tooth loss. There is a two way relationship between diabetes mellitus and periodontitis. Periodontitis is the sixth complication of diabetes along with retinopathy, nephropathy, neuropathy, macrovascular disease, and altered wound healing. Inflammatory mediators like interleukin-6 and tumor necrosis factor-alpha produced during periodontal inflammation can interfere with the actions of insulin receptors and worsen the glycemic control of diabetic patients.

Periodontitis is a major cause of tooth loss, affecting over 300 million people and bacteria associated with periodontitis are also linked with systemic problems like endocarditis, atherosclerosis. Recent work has highlighted a major role for the host sugar sialic acid in the biofilm physiology and host-pathogen interactions of *T. forsythia*, a key periodontal pathogen.

There exists a need for a biomarker, for early detection of disease evolution and more robust therapy efficacy measurements. Serum sialic acids were estimated in Indian population by diphenylamine method and Thiobarbituric acid method. The average values were  $68 \pm 2.6$  mg percent by DPA method and  $56 \pm 5$  mg percent by TBA (thiobarbituric acid assay) method. Age and sex showed no influence on serum sialic acid level. Objectives of the present study was to compare (TSSA) level in healthy subjects, subjects with (CMP) with and without (NIDDM) and its effect on non-surgical periodontal therapy.

In the present study, the participants were divided into three groups: Group A, B and C. Group A consists of systemically healthy subjects, Group B consists of subjects with (CMP) while Group C consists of subjects with (CMP) with (NIDDM) and results of this study indicated that, at baseline, there were significant differences between Group A, B and Group C with respect to all the clinical parameters, including (GI), (OHI-S), (PPD), (CAL), (TSSA) and (HbA1c) levels. Thus (TSSA) level could be considered as novel biomarker in the progression of periodontal disease and diabetic status. Periodontitis could be considered as a potential, modifiable, and independent risk factor for the development of diabetes. Early detection of elevated (TSSA) level may help in interpreting the progression of periodontitis, risk of development of diabetes mellitus in future and also to prevent complications.

## KEYWORDS

total serum sialic acid; chronic periodontitis; diabetes mellitus; biomarker; non-surgical periodontal therapy; acute phase proteins

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## INTRODUCTION

Periodontitis is a chronic inflammatory disease affecting the periodontium and resulting in progressive attachment and alveolar bone loss (1). During the disease process, there is an increase in production of proinflammatory mediators like tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ], interleukin-6 [IL-6], interleukin-1beta [IL-1 $\beta$ ], and interferon gamma [IF- $\gamma$ ]; and elevated levels of acute phase proteins like capsular reactive protein, C-reactive protein (CRP) and thus causing insulin resistance and apoptosis of pancreatic  $\beta$  cells.

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is characterized by a cascade of events like activation of acute phase proteins, release of proinflammatory cytokines. In 2010, it was estimated that 285 million people had been diagnosed with diabetes mellitus worldwide, with a prevalence of 6.4%. This is predicted to reach up to 439 million, a prevalence of 7.7%, by 2030 (2). Diabetes mellitus and periodontitis are chronic diseases which are highly prevalent in the world population and its association have been proved by many investigators. Periodontitis is considered as the sixth complication of diabetes mellitus (3). There exists a bidirectional relationship between diabetes mellitus and periodontitis and this fact is confirmed by many authors (4). Sialic acid (SA) belongs to a family of acetylated derivatives of neuraminic acid, an acute phase protein by itself, a nine carbon acidic monosaccharide that occur naturally at the end of sugar chains attached to the surface of cells and soluble proteins (5). An important function of host SA is to regulate innate immunity (6). The concentration of SA in human serum is abnormally high during tissue destruction, tissue proliferation, depolymerization or inflammation. Elevated level of serum SA has been seen in malignancy, diabetic mellitus, and coronary artery disease (7).

Acute phase inflammation has been suggested to be associated with infectious diseases such as periodontal diseases. SA occupies the interface between the host and pathogenic microorganisms. Micro-organisms incorporate SA into their cell surface, which helps them evade the innate immune response of the host. Removal of terminal SA either by neuraminidase (sialidase) enzyme of virulent bacteria or by inherited disorder of host endogenous neuraminidase from sialylated glycoprotein, could incorporate onto the surface of developing plaque which may play a role in its formation and cause destruction of host tissue. There exists a need for a biomarker, for early detection of disease evolution and more robust therapy efficacy measurements.

## MATERIAL AND METHODS

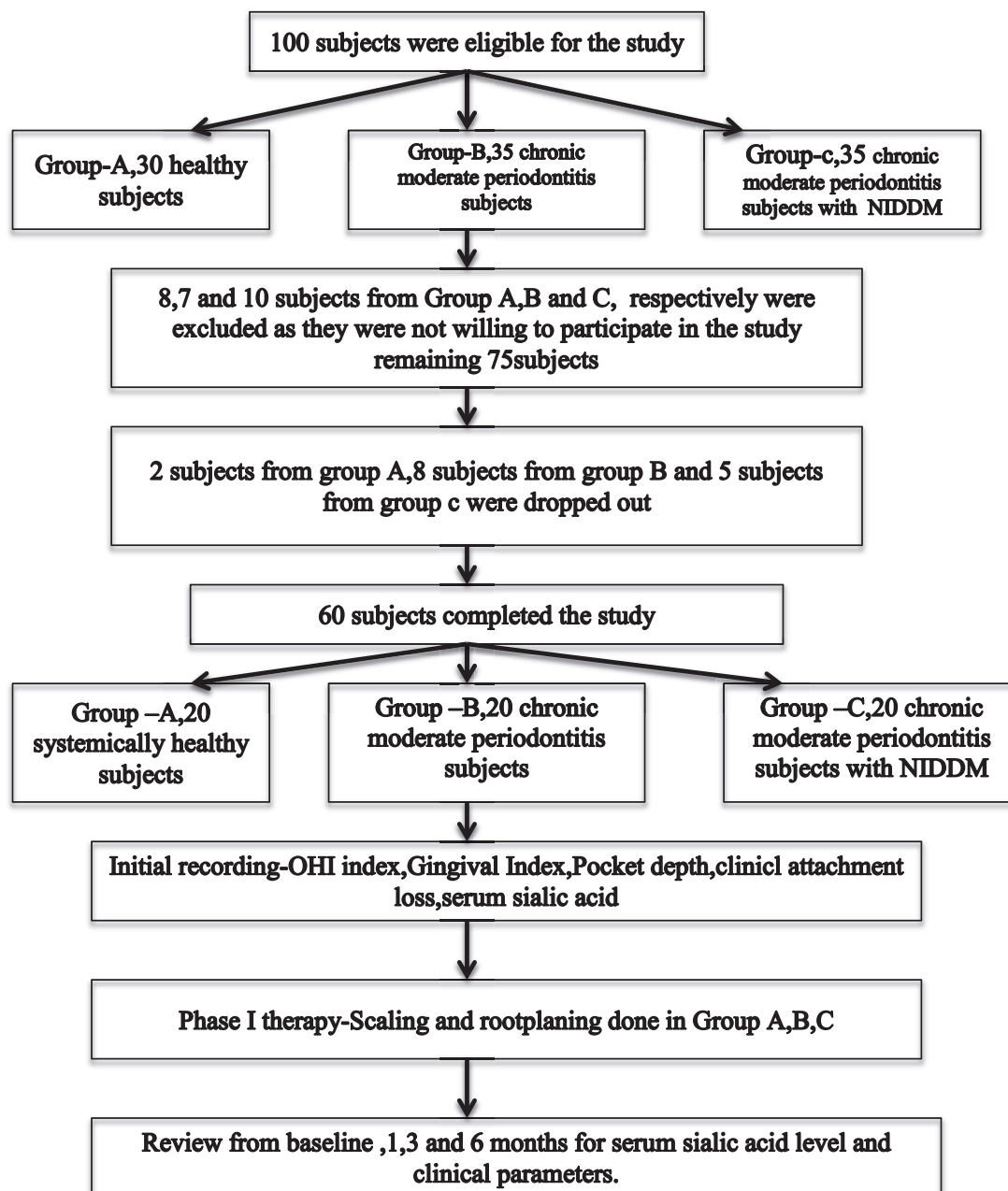
Systemically healthy subjects without periodontitis, subjects with chronic moderate periodontitis (CMP) with and without Non-insulin dependent diabetes mellitus (NIDDM), with (HbA1c)  $\leq 7$  reporting to Out Patient Department of Periodontics, were selected. After obtaining the written informed consent and the ethical committee approval, the selected subjects were divided into three

groups; Group A [Systemically healthy subjects without periodontitis, having Oral Hygiene Index - Simplified (OHI-S) score 0.8 to 1, Gingival Index (GI) score 0.1 to 1, with Probing pocket depth (PPD) not  $>3$  mm and with no Clinical Attachment Loss (CAL)], Group B [Systemically healthy subjects with CMP as defined by CAL 3–4 mm, having OHI-S score 1.3 to 3, GI score 1.1 to 2, with PPD  $\geq 3$  to  $\leq 5$  mm, CAL  $\geq 3$  to  $\leq 4$  mm in 5 or more teeth were selected]. The groups A and B had the Random blood sugar (RBS) levels  $<140$  mg/dl, and Group C [Subjects with CMP with NIDDM whose HbA1c levels were  $\leq 7$ , having OHI-S score 1.3 to 6, GI score 1.1 to 3, PPD  $\geq 3$  to  $\leq 5$  mm and CAL  $\geq 3$  to  $\leq 4$  mm in 5 or more teeth, and the RBS level was  $>140$  mg/dl]. All participants in the study were between 35 to 65 years with  $\geq 20$  natural teeth present at the time of study. Subjects with history of other systemic diseases, those who had used antibiotics in the preceding 6 months, pregnant ladies, lactating mothers, smokers, alcoholics, and those who had undergone periodontal therapy in the past six months were excluded from the study.

A total of 100 subjects were found to be eligible for participating in this comparative study. 30 subjects met the criteria for Group A, 35 subjects each, met the criteria for Group B and Group C. Of these 100 subjects, 60 subjects completed the study, 20 in each group. The attrition of subjects was compensated by the initial intake of extra samples. For all the selected subjects, the periodontal clinical parameters like OHI-S, GI PPD, CAL were recorded by a single examiner at baseline, one, three and six months. The PPD and CAL were assessed using Williams graduated probe markings at six sites per tooth (mesio-buccal/labial, mid-buccal/labial, disto-buccal/labial, mesio-lingual/palatal, mid-lingual/palatal, disto-lingual/palatal) in all teeth, excluding third molars. (Fig. 1) To ensure reproducibility during examinations, a customized acrylic stent was used as a reference to determine site and angle of insertion of periodontal probe. Total serum sialic acid (TSSA), HbA1c levels were also evaluated at baseline, one, three and six months.

Four ml and two ml of peripheral venous blood was collected from antecubital vein of the study subjects and stored at  $-20$  °C. Two ml blood in violet vacutainers was thawed for HbA1c level estimation whereas four ml blood stored in red vacutainers was centrifuged to separate serum and given for TSSA estimation using Ehrlich's method by trained nurses using a commercially available ion-exchange (HPLC) device (BIO-RAD D10) at Department of Nano medicine and Biochemistry.

All the selected subjects in the study had confirmed their glycemic level status by measuring their RBS level using glucometer (ONETOUCH Select Simple Blood Glucose Monitoring System) and their systemic conditions were evaluated by thorough medical history. All subjects had their RBS  $< 140$  mg/dl except for subjects in group C. The weight of subjects in kilogram and height in meters were recorded and their Body Mass Index BMI was calculated. After recording the clinical parameters and collection of blood samples for TSSA and HbA1c estimation, all subjects received oral hygiene instructions and non-surgical periodontal therapy which included thorough supragingival scaling, performed using piezoelectric ultrasonic scaler, and root planing using gracey curettes. The clini-



**Fig. 1** Flowchart showing flow of patient selection and dropouts.

cal parameters which were recorded at the baseline were again evaluated during recall visits at one, three and six months intervals.

**ESTIMATION OF TSSA**

Preparation of standard solution: Sialic acid solution (standard):

The standard neuraminic acid (sigma) contained total of 25 mgs. Out of this, 12.5 mg was weighed and mixed with 5 ml of milli Q water to get 2.5 µg/µl of sialic acid. Samples of 200 µl were mixed with 400 µl of 0.2 N H<sub>2</sub>SO<sub>4</sub> and incubated in dry bath at 80 OC for 1 hour. Then 1 ml of 10% Trichloroacetic acid (TCA) was added and mixed, after which the solution was centrifuged at 3000 rpm for 5 minutes. The supernatant (250 µl) was collected for analysis. It was diluted with 1ml of milli Q water and a further

250 µl of Ehrlich’s reagent was added. The solution was boiled in the water bath for 30 minutes. The reaction was controlled by cooling the samples in an ice bath. Serum samples and standard samples were added in the range of 0, 40, 80 and 160 µl to the microtiter well and were subjected to Ehrlich’s method for TSSA. Absorbance of the color was measured by a spectrometer at 650 nm. The pure sialic acid (2.5 µg/µl of tube) was used as the standard and 0.2N H<sub>2</sub>SO<sub>4</sub> -10% (TCA) was used as a blank. 40 µl of serum collected from each subject was taken as the test sample and quantification of TSSA in individual samples was performed in triplicates.

**STATISTICAL ANALYSIS**

Statistical tests were performed using the software Statistical Package for Social Sciences (SPSS), version 20.

The mean BMI, PPD, CAL, GI, OHI-S, HbA1c and TSSA levels were calculated per group. Intergroup comparisons of age and (BMI) were performed using ANOVA. Chi-square test was used for finding the association of TSSA with gender. Inter group and intra group comparison of parameters at baseline, one, three and six months recall interval were performed using ANOVA test and paired *t*-test. Independent sample *t*-test was used for intergroup comparison in Group B and C for PPD and CAL. Post hoc analysis was done using Bonferroni for multiple comparisons. Karl Pearson correlation was used to test correlation of clinical parameters, HbA1c levels with TSSA levels. The results were considered statistically significant when *p*-value was  $\leq 0.05$ .

## RESULTS

Comparison of TSSA levels in systemically healthy subjects, subjects with CMP with and without NIDDM, and the effect of non-surgical periodontal therapy on TSSA levels in these groups and its influence on diabetic status were assessed in this study. Table 1 shows the inter group comparison of age, sex and BMI. There was no statistically significant difference between the groups with respect to age and sex, however, BMI showed a statistically significant difference between the groups ( $p < 0.05$ ).

Table 3 shows intergroup correlation of BMI with TSSA levels indicating no correlation between BMI with TSSA levels between the groups ( $p > 0.05$ ). Table 2 shows intergroup comparison of OHI-S and multivariate intergroup comparison of (OHI-S) at baseline and six months. At baseline, the mean OHI-S of Group A, B and Group C were

**Tab. 1** Comparison of Gender, Age and BMI between Group A, B and C.

		Groups			P value	
		A	B	C		
Gender	Males	count	10	16	14	0.201
		% within gender	50%	61.5%	49.5%	
	Females	count	10	14	16	
		% within gender	50%	38.5%	50.5%	
Age	Mean	48	44	47	0.132	
	Standard deviation	7.13	7.20	5.50		
BMI (kg/m <sup>2</sup> )	Mean	18.27	19.34	20.41	0.48	
	Standard deviation	3.1	3.2	3.11		
n		20	20	20		

Baseline comparison of general characteristics.

$1.13 \pm 0.15$ ,  $3.17 \pm 0.64$  and  $3.51 \pm 0.45$  respectively. At six months recall, for Group A, Group B and group C the mean OHI-S were  $0.47 \pm 0.03$ ,  $0.90 \pm 0.25$ ,  $0.96 \pm 0.16$  respectively. Inter group comparison using ANOVA test indicated that there was statistically significant difference in OHI-S between the groups at baseline ( $p < 0.05$ ), but at the end of six months recall there was no statistically significant difference between the groups ( $p > 0.05$ ) except between group C and the other two groups. Intra group comparison using paired *t*-test showed that for Group A there was no statistically significant difference in OHI-S at baseline and at the end of six months recall ( $p > 0.05$ ), Group B showed a statistically significant difference in OHI-S at baseline and at the end of six months recall ( $p < 0.05$ ). While Group

**Tab. 2** Comparison of clinical parameters between Group A, B and C.

		Baseline	1 month	3 months	6 months	P value
OHI-S	Group A	$1.13 \pm 0.15$	$0.90 \pm 0.10$	$0.63 \pm 0.07$	$0.47 \pm 0.03$	0.000
	Group B	$3.17 \pm 0.64$	$2.25 \pm 0.59$	$1.36 \pm 0.50$	$0.90 \pm 0.25$	0.000
	Group C	$3.51 \pm 0.45^{*}\ddagger$	$2.67 \pm 0.41$	$1.95 \pm 0.41$	$0.96 \pm 0.16^{*}$	0.000
Gingival index	Group A	$0.80 \pm 0.22$	$0.59 \pm 0.15$	$0.48 \pm 0.12$	$0.45 \pm 0.10$	0.000
	Group B	$2.61 \pm 0.36^{*}$	$1.97 \pm .30$	$1.23 \pm 0.23$	$0.81 \pm 0.22$	0.000
	Group C	$2.31 \pm 0.39$	$1.73 \pm 0.32$	$1.30 \pm 0.23$	$0.90 \pm 0.19^{*}$	0.000
Probing pocket depth	Group A	$2.12 \pm 0.78$	$2.76 \pm 0.67$	$2.56 \pm 0.64$	$2.49 \pm 0.64$	0.101
	Group B	$6.57 \pm 0.55$	$8.41 \pm 4.76$	$6.89 \pm 4.07$	$5.5 \pm 2.09^{*}$	0.000
	Group C	$6.99 \pm 0.46^{*}\ddagger$	$6.11 \pm 0.47$	$5.68 \pm 0.42$	$5.2 \pm 0.50^{*}$	0.000
Clinical attachment level	Group A	$2.12 \pm 0.78$	$2.76 \pm 0.67$	$2.56 \pm 0.64$	$2.49 \pm 0.64$	0.103
	Group B	$6.61 \pm 0.77$	$8.42 \pm 4.65$	$6.91 \pm 4.11$	$5.6 \pm 2.12^{*}$	0.000
	Group C	$6.98 \pm 0.48^{*}\ddagger$	$6.16 \pm 0.49$	$5.69 \pm 0.49$	$5.9 \pm 0.32^{*}$	0.000
Total serum sialic acid level	Group A	$66.85 \pm 2.00$	$65.20 \pm 1.82$	$63.55 \pm 1.39$	$62.64 \pm 1.29$	0.000
	Group B	$144.50 \pm 22.2$	$115.8 \pm 18.1$	$91.85 \pm 13.95$	$70.05 \pm 6.16^{*}$	0.000
	Group C	$212.5 \pm 29.9^{*}\ddagger$	$158.1 \pm 27.5$	$113.15 \pm 18.99$	$77.70 \pm 8.54^{*}$	0.000
HbA1c levels	Group A	$5.67 \pm 0.27$	-	$4.86 \pm 0.17$	$4.61 \pm 0.41$	0.000
	Group B	$5.74 \pm 0.19$	-	$5.34 \pm 0.17$	$4.50 \pm 0.30$	0.000
	Group C	$6.65 \pm 0.42^{*}$	-	$5.70 \pm 0.44$	$5.26 \pm 0.41$	0.000

\* Indicates highly significant when groups were compared.

\* $\ddagger$  Indicates highly significant when groups were compared (inter group).

**Tab. 3** Correlation between Age, Gender and BMI with Serum sialic acid level.

			Group A	Group B	Group C
Total serum sialic acid	Age	Pearson correlation	0.203	0.209	0.211
		P value	0.062	0.068	0.069
	Gender	Pearson correlation	0.030	0.122	0.290
		P value	0.856	0.600	0.294
	BMI	Pearson correlation	0.156	0.264	0.213
		P value	0.071	0.059	0.173
	OHI-S	Pearson correlation	0.042	-	-
		P value	0.458*	-	-
	Probing pocket depth	Pearson correlation	-	0.039	-
		P value	-	0.465*	-
	Clinical attachment	Pearson correlation	-	-	0.034
		P value	-	-	0.432*
	Gingival index	Pearson correlation	-	0.032	0.037
		P value	-	0.456*	0.474*
	HbA1c levels	Pearson correlation	-	-	0.029
		P value	-	-	0.457*
	n	-	20	20	20

C showed a highly significant difference at six months ( $p < 0.05$ ).

Multivariate intergroup comparison of GI at baseline and at six months was performed using ANOVA and is shown in Table 2. The test indicated that there was a statistically significant difference in GI between the groups at baseline ( $p < 0.05$ ), but at the end of six months there was no statistically significant difference between the groups ( $p > 0.05$ ), except for Group C which showed significant difference ( $p < 0.05$ ). Intra group comparison using paired *t*-test showed that for Group A there was no statistically significant difference in GI at baseline and at the end of six months ( $p > 0.05$ ), while both Group B and C showed a statistically significant difference in GI at baseline and at the end of six months ( $p < 0.05$ ).

Table 2 shows intergroup comparison of PPD and CAL of Group B and Group C at baseline, one, and three and at the end of six months. At baseline, there was a statistically significant difference between the groups with respect to PPD and CAL ( $p < 0.05$ ) with group C showing a greater PPD reduction and CAL gain. At six months, there was no statistically significant difference between the groups with respect to both the parameters ( $p > 0.05$ ).

Intra group comparison of Group B using paired *t*-test indicated that there was statistically significant difference in (PPD) ( $p < 0.05$ ) and (CAL) ( $p < 0.05$ ) at baseline and at the end of six months. Intra group comparison of Group C using paired *t*-test indicated that there was statistically significant difference in (PPD) ( $p < 0.05$ ) and (CAL) ( $p < 0.05$ ) at baseline and at the end of six months.

Table 2 shows the comparison of TSSA at baseline, one, three and six months recall and the multivariate intergroup comparison of TSSA at baseline and at six months. Intra group comparison using paired *t*-test showed that all the groups had shown a significant reduction in the level of TSSA ( $p < 0.05$ ). And group C has shown a highly sig-

nificant difference from baseline to six months recall visit ( $p < 0.05$ ). Intergroup comparison of groups A, B and C using ANOVA test showed that group C had shown a significant difference in TSSA levels when compared to other groups at six months recall ( $p < 0.05$ ).

Table 2 shows the multivariate intergroup and intra-group comparison of (HbA1c) levels of groups A, B and C at baseline, one, three and six months recall. Intragroup analysis using paired *t* test showed that there was no significant difference in either of three groups ( $p > 0.05$ ). Intergroup analysis of HbA1c using ANOVA showed that Group C had shown the significant difference at baseline when compared to other groups ( $p < 0.05$ ). But there was no significant difference among the groups at six months recall visit ( $p > 0.05$ ). Table 3 showed the positive correlation of TSSA level with periodontal clinical parameters like GI, OHI-S, PPD from baseline to six months ( $p < 0.05$ ).

## DISCUSSION

Oral-systemic disease interrelationship has become a major concern because oral infections and conditions may contribute to pathologic processes elsewhere in the body (8–11). There are evidences suggestive, that periodontal diseases may trigger potential systemic inflammations (10) and a source of elevated proinflammatory markers. Interventional studies showed the effects of periodontal treatment on serum inflammatory markers (11).

Objectives of the present study were to compare TSSA level in healthy subjects, subjects with CMP[1]with and without NIDDM and its effect on non-surgical periodontal therapy. SA is a protein-bound carbohydrate considered to be monosaccharide and occurs in combination with other monosaccharides like galactose, mannose, glucosamine, galactosamine (12). It exists as a group of

acetylated neuraminic acid, N-glycolylneuraminic acid and diacetylneuraminic acid. Only N-acetyl neuraminic acid has been isolated from human serum. Total SA is the combination of free SA and bound SA. The bound SA can be either a protein or lipid. Lipid associated sialic acid (LASA), is a marker of the acute-phase response (12). It is a predictor of several systemic disorders, cardiovascular events, rheumatoid arthritis, diabetes (13), and head and neck cancer patients (14). Elevated serum and urinary SA concentrations were associated with several risk factors for diabetic vascular disease: diabetes duration, (HbA1c) levels, Triglycerides (TGLS) and cholesterol concentrations, waist to hip ratio and hypertension (15). Total SA is present in biological fluids like saliva, serum, Gingival Crevicular Fluid (GCF), cerebrospinal fluid etc. The reason why we opted for TSSA is that it gives more realistic evidence of the disease progression, enough sample volume rather than multiple attempts for adequate sample volume contamination with other constituents as seen in (GCF) and saliva.

Non-enzymatic glycosylation of hemoglobin is not induced by inflammation, but rather results from hyperglycemia caused by insulin resistance, low insulin levels and impaired wound healing. Thus this could explain why subjects with periodontitis have high (HbA1c) levels. Vascular endothelium carries high levels of SA (16) and vascular damage leads to its increase in its circulation. A relationship between serum SA levels and microvascular complications has been observed before for microalbuminuria and clinical proteinuria in type 1 and type 2 diabetes mellitus (17).

In the present study, the participants were divided into three groups: Group A, B and C. Group A consists of systemically healthy subjects, Group B consists of subjects with CMP while Group C consists of subjects with NIDDM and results of this study indicated that, at baseline, there were significant differences between Group A, B and Group C with respect to all the clinical parameters, including GI, OHI-S, PPD, CAL, TSSA and HbA1c levels. The above results were in accordance with results obtained, in various studies performed by Davis G. et al., Jawazaly J. et al., Usman M. S. et al., where they found a significant association between elevated TSSA levels in oral epithelial cells and gingivitis (19) and NIDDM (20). According to Jawazaly G. (21) there exists a significant association between elevated levels of salivary LASA levels and periodontal diseases. The elevation in TSSA concentration could be attributed to elevated levels of sialidase enzyme activity in periodontal diseases (22). TSSA concentration increases rapidly following the inflammatory and injury process. According to Crook M. (23) and Yokoyama et al. (24) SA concentrations in the blood may be a useful marker of diabetic complications, but there had been no large scale studies examining the link between SA and complications in type 1 diabetes. According to Syed et al. (25) increase in circulating serum SA is an early manifestation of diabetic renal disease.

The three groups were similar in terms of gender and age, whereas there was a slight variation in terms of BMI. These results were in accordance with the study done by Singh R. and Ramraju B. (26), Usman et al. (20), Shivanandanayak et al. (15) that showed age, sex and duration of di-

abetes, degree of metabolic control had no influence over the TSSA level. Other contradictory studies are as follows, Crook M et al. (27) showed that serum SA was significantly higher in men with diabetic complications than those without any complications. SA concentration increases with age in both men and women and the results may be due to young age of the subjects (mean 32.5 years in the men, 33.3 years in the women) included. While the present study was done in higher range of age groups could explain the positive results obtained with respect to the elevated level of TSSA in type 2 diabetic patients.

In the present study, HbA1c level was taken to know the glycemic status because it reflects on chronic exposure of hemoglobin to blood glucose. And is not affected by blood glucose fluctuations on the day of assay. (HbA1c) is considered as a beneficial indicator of long-term homeostasis, reflecting an average blood glucose concentration for the past two to three months (29). In the absence of diabetes, non-enzymatic glycosylation of proteins can occur which is considered as a major disadvantage of this assay. Diagnosis of diabetes based on elevated (HbA1c) levels is not dependable. Only those subjects with (RBS) level <140 mg/dl were included in our study. (HbA1c) levels were measured in laboratory using ion exchange (HPLC) and was evaluated after non-surgical periodontal therapy and smokers were excluded from our study. Wolf et al. (36) showed that periodontitis is associated with a slight elevation in glycosylated hemoglobin in non-diabetic subjects. According to Rajan p et al. (30), (HbA1c) levels were slightly higher and statistically significant in chronic periodontitis than in healthy controls. To detect any change in (HbA1c) levels there should be at least a three-month interval from the baseline estimation. In this study reexamination was performed at two months interval for six months and this may be one of the reasons for not obtaining a significant difference in groups A, B and C. Intergroup comparison between groups A, B and C showed a significant difference at baseline ( $p < 0.05$ ) (Table 2). The reason why there was no significant reduction in (HbA1c) levels after six months may be due to the fact that the subjects were under good diabetic control, and of moderate periodontitis (31). This is in contradictory to the results obtained by Guo H. (32) Jayachandran et al. (33) and Yun F. et al. (34) that showed a significant decrease in (HbA1c) levels after non-surgical periodontal therapy. According to Guo et al. (32) the selected subjects had no glycemic control during their reexamination and this may be reason for their results obtained.

The results of present study was in accordance with previous studies of Amitha et al. (30) Crook M. A. et al. (35) that showed elevation of TSSA level in chronic periodontitis and NIDDM subjects when compared to healthy subjects. According to previous authors serum SA could be considered for monitoring disease progression. Reevaluation of clinical parameters and SA level after giving periodontal therapy was not considered and exclusion of confounding factors demographic characteristics were its limitations.

In the present study after SRP, there was no significant change in GI, OHI-S, PPD, TSSA and (HbA1c) levels of healthy subjects without any systemic diseases Group A, while there was significant reduction in GI, OHI-S, PPD,

CAL and TSSA levels in CMP subjects with and without NIDDM Group B and C after non-surgical periodontal therapy. These findings were in accordance with the results of many previous studies (37, 38) which showed the effectiveness of SRP in improving the periodontal status of subjects with periodontitis. In Group C, the (HbA1c) levels of the subjects were not significantly reduced after SRP. These results indicate the possibility that the slight reduction in the HbA1c levels in Group C after six months could be due to an improvement in their periodontal status brought about by SRP. The mean TSSA levels at the end of six months for Group A, B and Group C were  $62.64 \pm 1.29$  mg/dl,  $70.05 \pm 6.16$  mg/dl and  $77.70 \pm 8.54$  mg/dl respectively and it was in accordance with previous studies (39, 40). At the end of six months, there was statistically significant difference in TSSA levels of Group B and Group C ( $p < 0.001$ ) (Table 2). The significance was more when Group C was compared with Group A ( $p < 0.05$ ). This indicated that even though there was a significant reduction in TSSA levels in subjects with periodontitis with and without NIDDM after SRP, the clinical parameters never came down to the level as that of healthy subjects.

Several studies have investigated the effect of periodontal therapy on the glycemic control of diabetes patients. Faria-Almedia R. et al. (41) reported that SRP alone significantly reduced (HbA1c) levels in diabetics. According to Crook M. (35) total (SA) concentration in the blood may be useful marker of diabetic complications, but the efficacy of non-surgical periodontal therapy was not considered. SRP did not result in the complete elimination of periodontal pockets and the complete gain in CAL and TSSA level in CMP subjects with and without NIDDM (Group B and Group C) and this is due to the fact that SRP alone cannot eliminate tissue-invading periodontal pathogens (42).

In our study, the significant reduction in clinical parameters, including OHI-S, GI, PPD, CAL and (HbA1c) levels, clearly indicates that periodontal inflammation was reduced after SRP, which could possibly lead to a decrease in TSSA levels. The role of TSSA in systemically healthy subjects, subjects with CMP with and without NIDDM is well understood from the present study. Proper oral hygiene measures also played a very important role in maintaining the periodontal status by reduction in periodontal clinical parameters, so as in TSSA level and glycemic status after non-surgical periodontal therapy.

Strength of this study include adhering to strict inclusion and exclusion criteria, use of ion-exchange (HPLC) for estimation of (HbA1c) levels, Ehrlich's method of estimation of TSSA levels and monitoring the RBS levels of all subjects for confirming their non-diabetic status instead of relying on medical history alone. Only those with RBS levels  $<140$  mg/dl were included in the study. Since subjects were recruited directly from Outpatient Department, we were not able to record the Fasting Blood Sugar (FBS) levels which would have been a more reliable measurement than RBS levels.

Limitations of this study includes lack of microbiological analysis and its correlation with TSSA level, possible laboratory errors due to manual method of estimation, small sample size, measuring only RBS to confirm the glycemic status of the participants, not evaluating serum

and/or GCF levels of pro-inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IF- $\gamma$  and CRP, and the failure to adjust for confounding factors like race/ethnicity. Future studies and clinical trials; better technique sensitive method for estimation of TSSA level has to be carried out to overcome these limitations.

## CONCLUSION

Based on the results obtained from this Non-Randomized Clinical Trial, it can be concluded that, TSSA levels do decrease after Non-surgical periodontal therapy in chronic moderate periodontitis patients with and without NIDDM, we may conclude that TSSA could be considered as a novel biomarker in progression of periodontal disease and diabetic status. Periodontitis could be considered as a potential, modifiable, and independent risk factor for the development of diabetes. Early detection of elevated TSSA level may help in interpreting the progression of periodontitis, risk of development of diabetes mellitus in future and also to prevent complications. In the future, more randomized controlled clinical trials with larger sample sizes, and with strict adjustments of confounding factors, such as race/ethnicity and smoking status, are needed to confirm the findings of this study. However, periodontology must explain a multitude of unclear or insufficiently clear phenomena which will be a priority of science in forthcoming years.

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# Fusiform Cervical Mass in a 6-Year Old Boy; Do not Forget the Thymic Cyst

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## ABSTRACT

Cervical thymic cyst is a rare clinical entity, with approximately one hundred cases reported in the literature so far. The purpose of this case report is to highlight some certain features, along with an extensive research of the relevant literature.

A 6-year-old boy was admitted to the Otorhinolaryngology Department due to the presence of a left-sided, painless, latero-cervical swelling, first observed by his parents 2 weeks ago. Physical examination revealed a painless, well-delineated mass, with no signs of inflammation. No enlarged cervical nodes were present. The mass extended from the mandibular angle, under the sternocleidomastoid muscle, in proximity with the ipsilateral neurovascular bundle. Ultrasound transverse gray-scale panoramic view detected a well-circumscribed lesion, with fine echogenic foci, appearing in close proximity with the upper pole of the left thyroid lobe and the ipsilateral common carotid artery. Elective surgical intervention with complete mass excision was performed. Histopathological examination confirmed the diagnosis of a cervical thymic cyst.

Cervical thymic remnants represent a group of neck masses that pediatricians and pediatric surgeons should consider in differential diagnosis of both cystic and solid neck masses. Most cystic cervical thymic masses are found in the lower third of the anterior neck, extending deep to the sternocleidomastoid muscle, featuring close anatomic relationship with the composites of the ipsilateral carotid sheath. Elective surgery is kept as the treatment of choice, after ruling out the possibility of subject immunologic disturbance.

## KEYWORDS

thymic cyst; cervical thymic remnant; child; Hassall's corpuscles; thymo-pharyngeal duct

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## INTRODUCTION

Thymus arises from the third and fourth pharyngeal pouches during the sixth gestational week (1, 2). Over the next few weeks, thymic tissue migrates caudally and medially along the thymo-pharyngeal duct to the superior and anterior mediastinum (1, 2). During childhood and adolescence, it features generally soft-tissue attenuation and undergoes fibro-fatty involution with aging (1, 2).

Thymus is organized into multiple lobules, arranged into an outer cortex and an inner medulla. The cortex is composed of immature T-lymphocytes and thymic epithelial cells; the medulla contains maturing lymphocytes and whorls of spindle-shaped epithelial cells, which create Hassall corpuscles with keratinized cores (3). Thymus is usually located in the anterior mediastinum; however, ectopic thymic tissue can be found anywhere, from the skull base to the diaphragm, along the thymo-pharyngeal duct (4, 5).

Cervical thymic cysts are rare and usually present during the first decade of life (6, 7). Heise et al have previously demonstrated that cervical thymic cysts constitute almost 0.3% of all congenital cervical cysts found in childhood (8). Cervical thymic cyst represents a rare entity, with only about one hundred cases reported in the literature so far (9). According to findings from patients who underwent neck or thyroid ultrasonography, the prevalence of ectopic thymus varies widely from 2.3 to 17.3% (10, 11, 12). Kim et al examined 3,195 patients, demonstrating a prevalence equal to 0.4% (13). Hsieh et al. demonstrated that among 331 patients under the age of 18 years presenting with cystic neck masses 181 (54.68%) had thyroglossal cysts, 83 had cystic hygromas (25.08%), 54 had branchial cleft cysts (16.31%), 3 had bronchogenic cysts (0.91%), while 9 cases (2.72%) remained unclassified. Interestingly, only one case was diagnosed as thymic cyst (0.30%) (8).

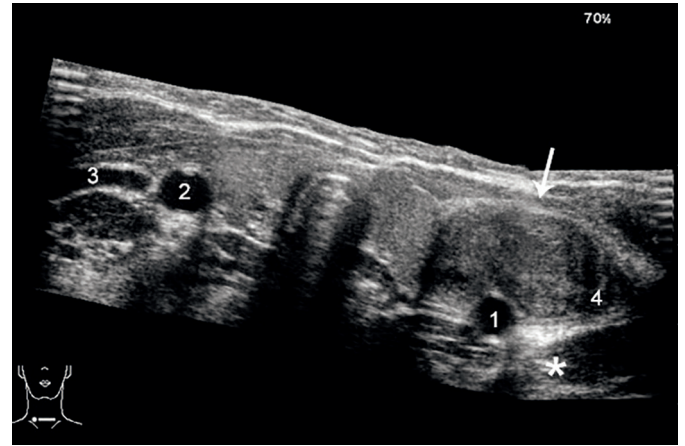
Despite the fact that the majority of ectopic thymic lesions are located in proximity with the thyroid gland, many pediatricians are not familiar with these entities (10). Through this case study, we aim at highlighting some certain features of thymic cysts, along with a comprehensive research of the relevant literature.

## CASE DESCRIPTION

A 6-year-old boy was admitted to the Otorhinolaryngology Department due to the presence of a left, painless, latero-cervical, fusiform mass, which was first observed by his parents 2 weeks ago. Mass volume did not alter, while it did not exert pressure phenomena on the adjacent anatomic structures. After a detailed medical history, it was reported that the patient had recurrent episodes of inspiratory stridor without respiratory distress, lasting a few minutes. Physical examination revealed the presence of a painless, well-delineated, soft mass, while no signs of inflammation or enlarged cervical lymph nodes were detected. The mass extended from the mandibular angle, under the sternocleidomastoid muscle, in proximity with the ipsilateral neurovascular bundle. Rest physical examination was unremarkable. Performance of the Valsalva

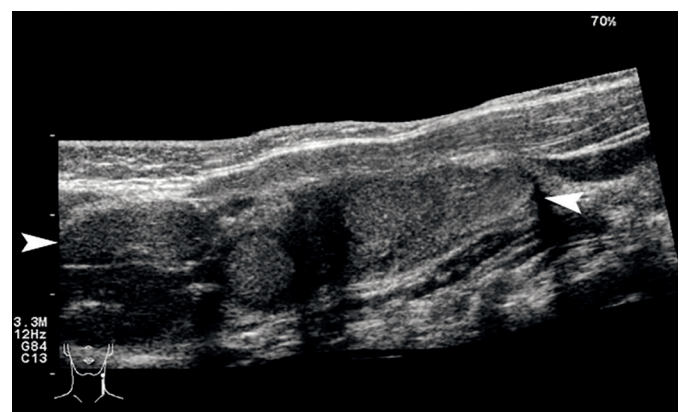
maneuver was not associated with a significant alteration in mass dimensions. Chest radiograph did not reveal any co-existing pathology.

Performance of transverse gray-scale panoramic view ultrasound showed the cystic lesion located on the left lateral neck region (Figure 1).



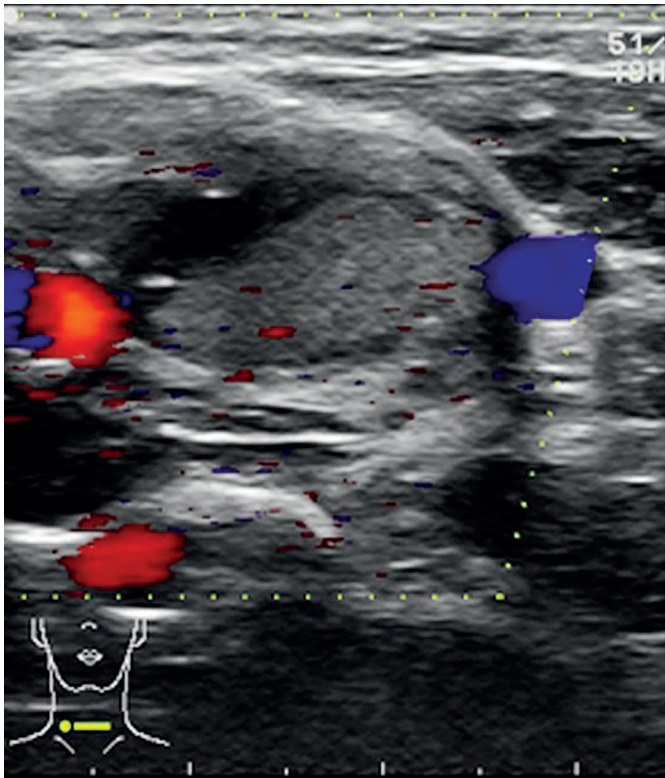
**Fig. 1** Ultrasound transverse gray-scale panoramic view showing the large cystic lesion situated on the left lateral neck region. The lesion (arrow) is well-circumscribed, contains fine echogenic foci and appears in close proximity with the left thyroid lobe and the common carotid artery. On pressure by the probe, the echogenic foci could be seen moving. Posterior acoustic enhancement (asterisk) suggests the cystic nature of the lesion. 1: left common carotid artery, 2: right common carotid artery, 3: right jugular vein, 4: displaced left internal jugular vein.

The lesion was well-circumscribed, containing fine echogenic foci and appearing in close proximity with the left thyroid lobe and the common carotid artery. On pressure by the probe, the echogenic foci could be seen moving (Figure 2).

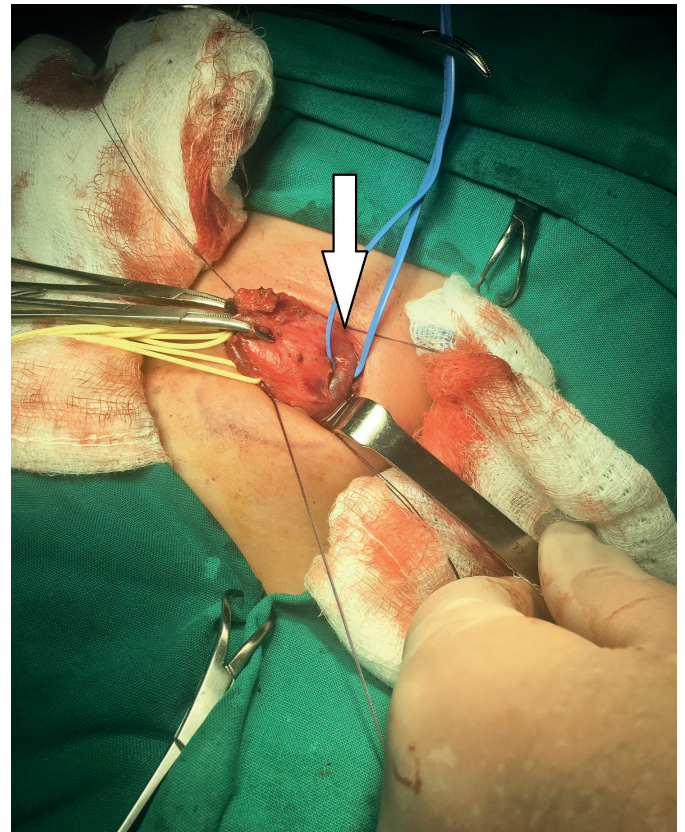


**Fig. 2** Ultrasound longitudinal gray-scale panoramic view showing the elongated cystic lesion (highlighted by the arrowheads). The multi-locular nature of the lesion, containing septations and the posterior acoustic enhancement can be readily appreciated in this image.

The multi-locular nature of the lesion, containing septations, was also documented. Color Doppler transverse view revealed the absence of vascularity within the lesion, along with the displacement of the internal jugular vein and the common carotid artery (Figure 3).



**Fig. 3** Color Doppler transverse view showing the absence of vascularity within the lesion, the displacement of the jugular vein (blue color) and the common carotid artery (red color). Note is made of a fluid-fluid level formed between an anechoic and an echogenic content.



**Fig. 4** The mass was bluntly dissected and completely removed, without being opened. Notice the close anatomic relationship with the internal jugular vein (white arrow).

We also performed a chest CT scan, which confirmed the normal configuration of the 2 lobes of the thymus gland. Laboratory exams, including thyroid function tests, were unremarkable.

Thus, we proceeded with the elective surgical intervention. Elective surgical excision was performed under general endotracheal anesthesia. A curved skin incision along the anterior surface of the mass was performed. A multi-cystic mass, extending between the left gonial angle of the mandible and the upper pole of the ipsilateral thyroid lobe was bluntly dissected and completely removed (Figures 4, 5).

Meticulous mass dissection from the left internal jugular vein and the left common carotid artery (carotid sheath) was performed. No extension of the mass to the mediastinum was documented (Figure 6).

Macroscopically, the surgical specimen consisted of a solid-cystic mass, which appeared multilocular, sizing  $14.5 \times 2.5 \times 2.9$  cm in dimensions (Figure 7).

Applied drain on the surgical site was removed 48 hours postoperatively and the patient was discharged home on the third postoperative day in excellent general condition.

Histopathological examination revealed that the solid part of the lesion consisted of thymic tissue with Hassall's corpuscles. In proximity to its solid part, a fibrous wall lined with stratified epithelium was also identified. Additionally, lymphocytic infiltration of the cystic wall and several cholesterol granulomas were found. Crystals

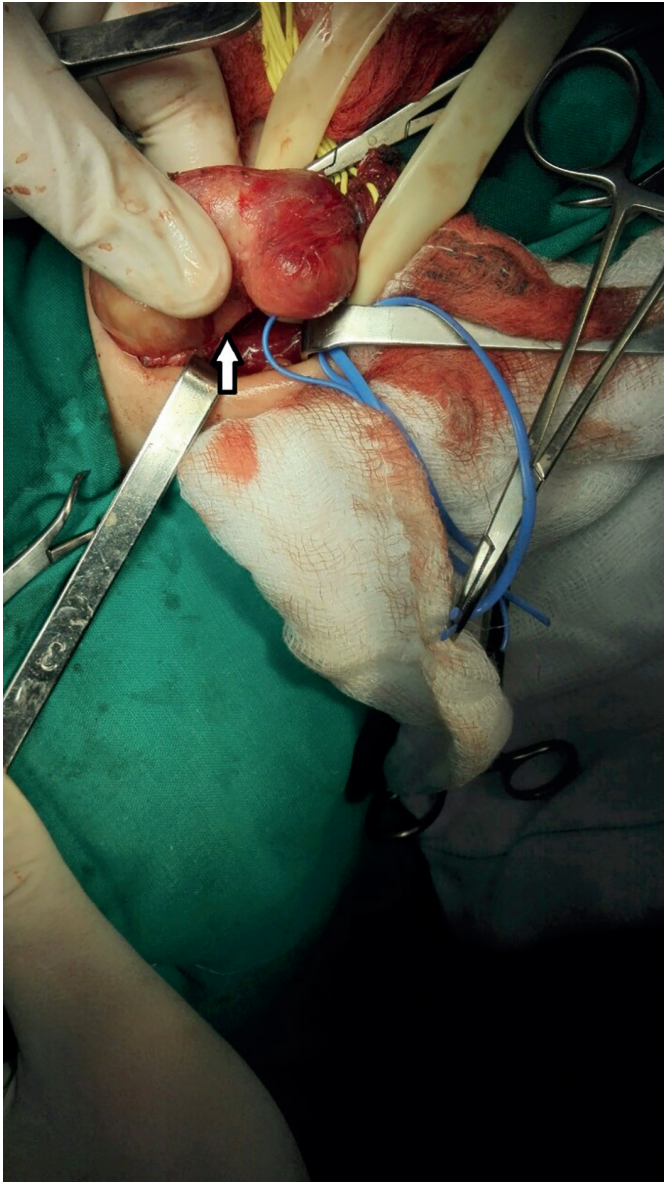
of cholesterol crystals were also present in the luminal fluid (Figures 8, 9).

Postoperative course was uneventful, while the patient remains asymptomatic, with no symptoms of recurrence during the follow up. The latter constituted of an ultrasonographic examination performed in the 6 and 12 months postoperatively, with no radiologic signs of thymic cyst's recurrence, along with meticulous physical examination.

## DISCUSSION

Differential diagnosis of neck masses during childhood includes thyroglossal duct cyst, cystic hygroma, bronchogenic cyst, branchial cyst, cervical lymphadenopathy with cystic degeneration, laryngocele, tumors and dermoid cyst. Rarely, it may also include a thyroid or parathyroid cyst (7, 14).

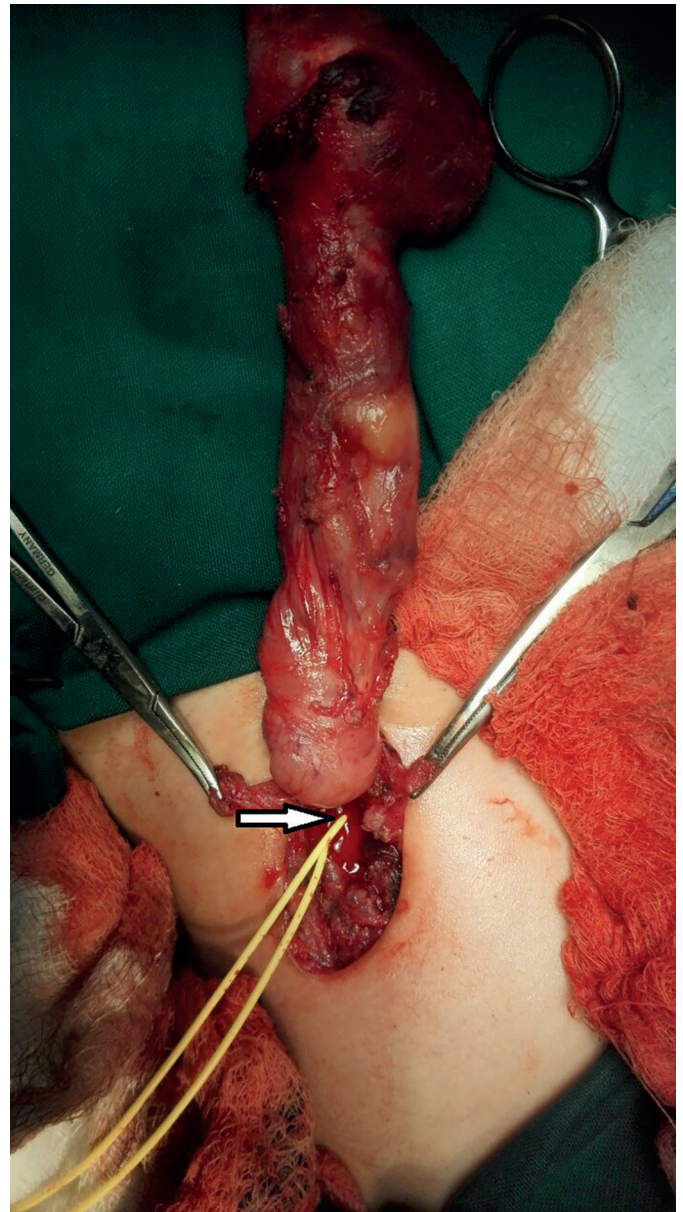
The increasing number of cervical thymic cysts reported in the literature during the last few years reflects probably the greater awareness of pediatricians and pediatric surgeons concerning this rare clinical entity. It cannot be excluded that in the past lots of cases of thymic cyst might have been misdiagnosed as branchial cleft cysts. Due to the gradual development of atrophy of the thymic remnants, tissue sampling from several different parts of the surgical specimen may be required, in order to be documented the right diagnosis of a thymic cyst (15).



**Fig. 5** The mass was bluntly dissected and completely removed, without being opened. Notice the close anatomic relationship with common carotid artery (white arrow).

Thymic cysts may be congenital; in such a case, they can be practically found anywhere along the thymo-pharyngeal duct (9). In their retrospective analysis, Bang et al. demonstrated that only 1 out of 16 cases examined in total corresponded to ectopic thymus located at the supraclavicular area (10). Rarely, thymic remnants are detected in atypical sites, such as the retropharynx (16). Alternatively, a thymic cyst may be acquired, developing after chemotherapy or thoracotomy, or even associated with thymic tumours distorting and compressing normal thymic tissue (17, 18). Overall, up to 70% of cervical thymic cysts are left-sided, while there is a slight predominance among males (19, 20).

The thymo-pharyngeal duct guides thymus descent towards the mediastinum (1, 2). Normally, the upper part of this canal regresses thereafter; any failure of its closure can lead to the formation of a congenital unilocular cervical thymic cyst. Degeneration of Hassall's corpuscles within remnants of ectopic thymic tissue can lead to genesis of



**Fig. 6** The lower end of the cystic mass was adjacent to the upper pole of the left thyroid lobe (white arrow).

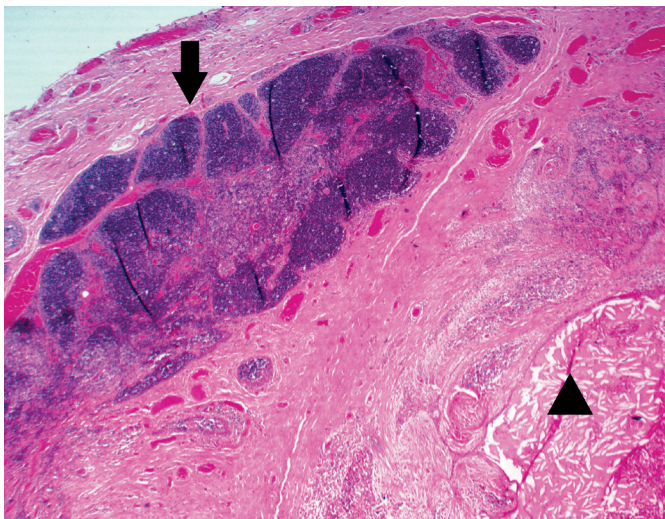
an acquired multilocular thymic cyst, as in our case. Thus, two main theories concerning cervical thymic cyst pathogenesis have been proposed by Speer; first, the persistence of the thymo-pharyngeal duct and second, the degeneration of Hassall's corpuscles (21, 22).

The size of a thymic cyst varies from 1 to 26 cm while in our case it was 14.5 cm (23). Most thymic cysts are asymptomatic, while in 6–13% of all cases, affected patient may present with stridor – as in our case –, dysphonia, dysphagia or hoarseness (9, 21, 22, 24). Thymic cyst may be complicated with infection or hemorrhage during its course, thus presenting in an acute manner (16). Recurrent upper respiratory tract infections and fluctuation in size are also commonly reported (25). Eifinger et al. describe a case of a ruptured thymic cyst in a neonate in the context of hemorrhage, leading to acute respiratory distress (26).

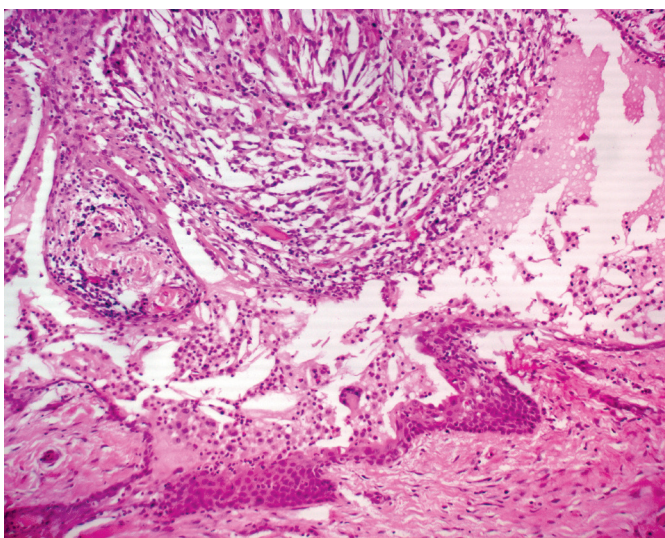
Valsalva maneuver may produce an apparent enlargement of the mass either due to the venous engorgement or via the increased intrathoracic pressure. Physical exami-



**Fig. 7** Macroscopic view of the excised mass.



**Fig. 8** Thymus gland cyst – Cystic area (arrowhead) adjacent to thymus parenchyma (arrow) [haematoxylin and eosin stain (H-E), 25 $\times$ ].



**Fig. 9** Cyst filled with tissue detritus and cholesterol crystals, lined internally by stratified epithelium (H-E, 100 $\times$ ).

nation should also focus on differential diagnosis between a cystic ectopic thymic remnant and a branchial cyst; in the latter case, a branchial cyst does not enlarge with Valsalva maneuver (27). Valsalva maneuver does not induce enlargement of the mass, when it does not extend to the mediastinum, as in our case. Mediastinal extension is seen in almost half of the cases of cervical thymic cysts (22).

Plain chest radiograph will reveal a normal thymic shadow in the mediastinum in children aged less than 2 years; thus, presence of mediastinal shadow beyond this age is suggestive of the mediastinal extension of a cervical thymic cyst (28). Performance of Doppler ultrasound enables the clinician to identify certain cyst characteristics, such as the presence of septae, the hypo- or anechoic content and its relationship with the adjacent anatomic structures (29). Occasionally, internal septations or mural calcifications can also be seen; however, cyst's content may be proteinaceous or hemorrhagic, thus creating a diagnostic dilemma (17). In such cases, magnetic resonance imaging (MRI) appears more accurate in distinguishing a thymic cyst from a solid thymic lesion (29). Therefore, MRI might be helpful, when evaluating a child with a solid cervical mass, in order to compare the derived imaging characteristics to those corresponding to an orthotopic mediastinal thymus (16).

In less than 15% of cervical thymic remnants diagnosis is made preoperatively (30, 31). In their retrospective analysis, Statham et al. managed 16 children with cervical thymic remnants, 3 solid and 13 cystic (27). Preoperative imaging techniques were not diagnostic in those patients having a solid thymic cyst, while they established the correct diagnosis in one fourth of those patients featuring a cystic thymic mass (27).

Thus, physical examination, preoperative imaging and intraoperative findings play a crucial role in the diagnosis of a cervical thymic cyst; however, diagnosis is usually established after histopathological examination (7, 22, 24).

A thymic cyst usually contains ectodermal derivatives, with its epithelium ranging from squamous to cuboidal or columnar. It also contains endodermal derivatives, for example thymic and parathyroid tissue (7). Presence of thymic tissue, lymphoid tissue, and Hassall's corpuscles within the cystic mass constitute pathognomonic findings (7, 25, 29).

Respiratory distress post resection of a thymic remnant is a known complication of cervical thymic masses. Presence of tracheal compression by the mass preoperatively is an important guide for postoperative respiratory care (27). In our case we reported a mild stridor that restored within a few minutes, without any signs of acute respiratory distress. Patients with respiratory distress symptoms during the postoperative period may be managed with emergent endotracheal intubation; however, no long-term sequelae is reported in most cases (25).

No immunological deficiency after removal of a thymic cyst is expected; this can be simply explained by the fact that it does not contain functional thymic tissue (19, 27). Although being extremely rare during childhood, complications in the presence of a thymic cyst that should be taken into account are thymic carcinoma and myasthenia gravis development (9, 19).

## CONCLUSIONS

1. Cervical thymic remnants represent a group of neck masses that pediatricians and pediatric surgeons should always consider in the differential diagnosis of both cystic and solid neck mass.
2. Most cervical thymic cysts are found in the lower third of the anterior neck, extending deep into the sternocleidomastoid muscle, with close anatomic relationship with the composites of the carotid sheath.
3. After ruling out the possibility of a subject immunologic deficit, elective surgical excision of the mass is recommended. No severe complications are described in the vast majority of cases.

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# Myelin Oligodendrocyte Glycoprotein Antibody Associated Transverse Myelitis

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## ABSTRACT

Antibodies against myelin oligodendrocyte glycoprotein cause inflammatory lesions of central myelin – in optic nerves, of the brainstem, and spinal cord. There are characteristic changes of CNS white matter, protein-cytological association in cerebrospinal fluid, MOG IgG antibodies, a very important differential diagnosis and a relatively mild course.

## KEYWORDS

myelin oligodendrocyte glycoprotein; inflammation; spinal cord; antibodies

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## INTRODUCTION

Myelin oligodendrocyte glycoprotein (MOG) is a component of myelin sheaths that is solely expressed in the central nervous system and thought to be an important target of inflammatory demyelinating disorders. The clinical features of MOG antibody-associated demyelination different onset phenotypes are important for prognosis of the disease (1). MOG-IgG-seropositive patients can be divided into four groups: MOG-ON (optic neuritis), MOG-TM (transverse myelitis), MOG-pure brain symptoms and MOG-ON+TM (2).

Because we have diagnosed a young man with transverse myelitis and a high titer of MOG-IgG antibody we have decided to publish this relatively new clinical disorder.

## CASE REPORT

A 33-year old up till now healthy clerk was referred to neurology from urology. A month earlier, the retention of



**Fig. 1** Sagittal T2 weighted MRI image of the thoracic spine – intramedullary hyperintense lesion: clearly seen at the Th3–5 level, marginally viewed at the distal part of lumbar spinal cord.

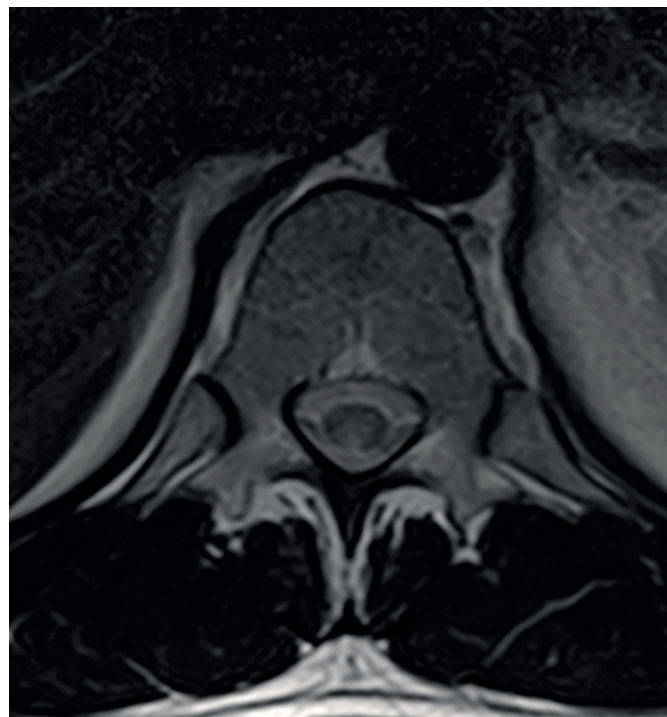
urine began to occur. After catheterization, 1.5 liter urine was let out. He also complained of a progressive burning of the feet, followed by ascendent hypesthesia of the leg and trunk up to above xiphoid (Th6). During the neurological examination done at admission, spinal cord symptomatology with hypesthesia up to Th6 level, mild spastic paraparesis and a spastic sphincter were established. The patient was able to walk, but slowly, without any support.

On the MR of the brain and spinal cord with gadolinium, a couple of focal spinal cord changes in cervical and thoracic level were found. One focal myelopathy was three spinal cord segments long (Fig. 1, 2, 3, 4). There were no clear focal changes in the white matter of the brainstem or brain. In the cerebrospinal fluid we found hyperproteinorhachia (2.5 g/liter; 0,2–0,4), decreased lactate (0.90 mmol/l; 1,20–2,10) and glucose (2.85 mmol/l; 2,50–4,50) and normal chlorides (120 mmol/l; 120–130). Pleocytosis was found with 349 cells and a prevalence of mononuclears-336 and polymorphonuclears -13.

At this point we consulted a specialist in infectious diseases who expressed suspicion of infectious disease and recommended intravenous therapy with ceftriaxone and herpesin. Once administered, the therapies recommended by the ID specialist did not result in an improved neurological condition of the patient.

We sent the cerebrospinal fluid to the reference laboratory (Topelex), but there was only a very small intrathecal production of immunoglobulins and no infectious agents were found (borreliosis, HSV 1 and 2, VZV, CMV, tick meningoencephalitis, neuroleues, chlamydia, mycoplasma, enteroviri, listeria monocytogenes, L. interrogans; including HIV in blood). MRZ reaction was not performed.

Because of prominent and long intraspinal focuses, we suspected an inflammatory origin, we thought it could



**Fig. 2** Axial T2 weighted MRI image of the thoracic spine – intramedullary hyperintense lesion at the dorsal part of spinal cord.



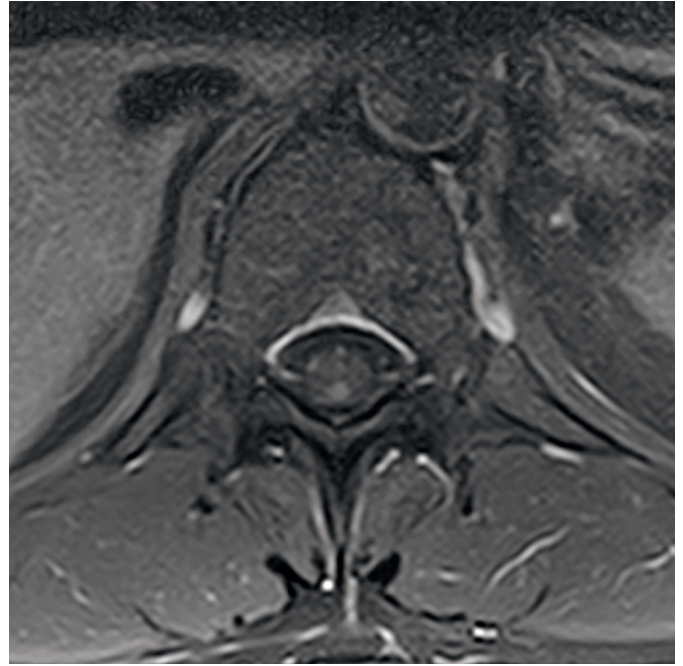


**Fig. 3** Axial T1 weighted MRI image of the thoracic spine after gadolinium administration – inhomogeneous nodular opacification of the lesions same as picture No. 1.

be an atypical finding of multiple sclerosis – but no oligoclonal bands were found. As a second option we thought of neuromyelitis optica spectrum disorder – but no changes of the optic nerves (MRI, VEP, OCT) or aquaporin-4 antibodies were found. Because of suspected sarcoidosis we treated the patient with methylprednisolone 5 g intravenously. After 2 g of methylprednisolone the urologic complaints, hypesthesia and also spastic paraparesis began to improve while sarcoidosis was excluded.

We have excluded sarcoidosis and, according to MRI spinal cord changes with negativity of aquaporin-4-antibodies, we indicated anti-MOG antibodies (myelin oligodendrocyte glycoprotein) and it was positive in serum. The positivity anti-MOG antibodies was established in a specialized reference laboratory Topelex. The cell-based assay is used and with help of fluorescence is evaluated either on native cell culture and also on transfected cells. There is only qualitative evaluation – antibody titer higher than 1 : 10 is expressed as positive.

At the admission of this patient we supposed that it could be an infections diseased of bacterial or viral origin.



**Fig. 4** Axial T1 weighted MRI image of the lumbar spine after gadolinium administration – inhomogeneous nodular opacification of the lesions.

Our suspicion increased with the proteino-cytological association in cerebrospinal fluid and also a prominent positivity of a chemokine CXCL13, that is usually found in borreliosis. But the patient did not improve after combination (cetriaaxone and herpesine) therapy and tests for the most common neuroinfections were not positive. We concluded, that the patient has transverse myelitis with anti-MOG antibodies, a new clinical disorder. At discharge he had improved, with mild spastic paraparesis, hypesthesia only of the low extremities and minimal dysuria. At the time, he was being treated with a small dose of medrol (8 mg/d). He will be monitored and treated in the multiple sclerosis centre.

## DISCUSSION

Over the past few years, new generation cell-based assays have demonstrated a robust association of autoantibodies to human MOG with optic neuritis, myelitis and brainstem encephalitis (3). Disorders based on anti-MOG demyelination are immunopathogenetically distinct from both classic multiple sclerosis and aquaporin-4-IgG positive neuromyelitis optica spectrum disorder (NMOSD) (4). Based on evidence from immunopathological studies suggesting a direct pathogenic impact of MOG-IgG, neuropathological studies demonstrating discrete histopathological features, serological studies reporting a lack of aquaporin-4-IgG in almost all MOG-IgG-positive patients, and cohort studies suggesting differences in clinical and paraclinical presentation, treatment response and prognosis, MOG-IgG is now considered to denote a disease entity in its own right (1).

In patients with transverse myelitis there is a number of various diseases that should be taken into account

(Table 1). Second to clinical findings, MRI imaging is the most important examination (5). Also cerebrospinal fluid examination, tests for infectious diseases, and an assessment of auto-antibodies are unavoidable in order to achieve a correct diagnosis (6). In our 33-year old, active man, the first suspicion was of an infectious disease. According to a consulted Infectious Disease Specialist, neuroborreliosis. Yet, this was not confirmed. Multiple sclerosis was the second diagnosis – but oligoclonal bands were not found. Extensive transverse myelitis may be part of *neuromyelitis optica*. An MRI of the optic nerves, visual evoked potentials, and optic coherence tomography resulted in normal findings. Antibodies against aquaporin – 4 were not found. According to the spinal cord MRI, we also suspected spinal sarcoidosis, which was later not confirmed. Finally, antibodies against MOG in cerebrospinal fluid were found and we could achieve the correct diagnosis – MOG-IgG transverse myelitis.

**Tab. 1** Causes of transverse myelitis.

Demyelinating disorders	Multiple sclerosis Neuromyelitis optica spectrum disorders Acute disseminated encephalomyelitis (ADEM) Adrenoleukodystrophy
Non-infectious inflammatory disorders	Post-infectious transverse myelitis Primary vasculitis of CNS Lupus erythematoses Paraneoplastic myelopathies (breast, lung, lymphoma) Sjögren syndrome
Vascular disorders	Spinal cord infarctions Spinal dural arteriovenous malformations
Infectious disorders	Viral (herpes, CMV, West Nile virus, HIV) Bacterial (staphylococcus, streptococcus, borrelia, mycobacterium) Parasitary (schistosomiasis)

Patients with anti-MOG transverse myelitis are young – 24 years at onset (2), have higher titer of anti

MOG IgG than patients with anti-MOG optic neuritis, and have increased cerebrospinal fluid leukocytes. Though patients with anti-MOG-TM tend to relapse more frequently, the outcome is favorable – with only 2% becoming wheelchair-dependent. Intravenous methylprednisolone during acute attacks have proven to be the most successful therapy. Only a small number of patients were treated with intravenous immunoglobulin or plasma exchange, which resulted in only a weak effect. Only children were treated with rituximab or azathioprine in the first line. For chronic maintenance therapy corticosteroids with azathioprine or mycophenolate mofetil were used. In our 33-year old patient we succeeded with intravenous methylprednisolone and then with decreasing doses of medrol.

## CONCLUSION

Anti-MOG transverse myelitis is a newly established disease. The differential diagnosis is especially against neuromyelitis optica and multiple sclerosis. Though the disease tends to relapse, the outcome is favorable in most patients.

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# Interactions between Anti-Vegf Therapy and Antitumor Immunity as a Potential Therapeutic Strategy in Colorectal Cancer

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## ABSTRACT

There is a growing corpus of evidence indicating that anti-VEGF therapy may normalize the abnormal tumor vasculature with the potential to re-program the tumor immune microenvironment to a more immunosupportive profile. Tumor vessel normalization increases tumor perfusion, and, consequently, oxygen and nutrient supply, and thus can be assumed to improve the general response to anticancer immunotherapy. The increased antitumor immunity responses seen following anti-VEGF therapy may also be associated with the inhibition of the immunosuppressive action deployed by VEGF on effector T cells. Bearing in mind the recent advances of combination immunotherapy, combinations of anti-VEGF therapy with immune checkpoint inhibitors now appear to represent an attractive strategy. Key to the successful implementation of a combination strategy for treating cancer is understanding the interaction of these two therapeutic interventions, particularly in regards to appropriate reprogramming of the tumor immune microenvironment to improve antitumor immunity.

## KEYWORDS

vascular endothelial growth factor inhibition; antitumor immunity; colorectal cancer

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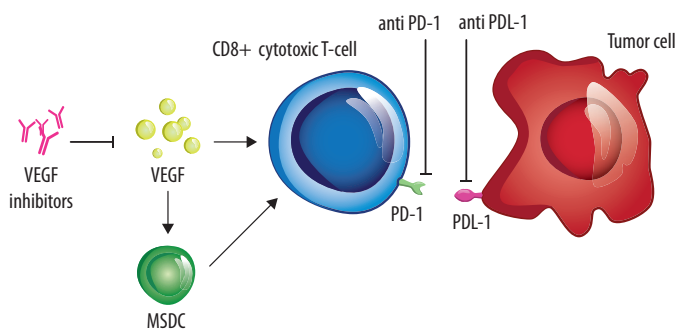
## INTRODUCTION

There is a growing corpus of evidence indicating that anti-VEGF therapy may normalize the tumor vasculature with the potential to switch the tumor immune microenvironment to a more immunosupportive profile (1).

The efficacy of anticancer immunotherapy using immune checkpoints blockade is compromised by hypoxia and poor T cell infiltration within the tumor resulting from poor perfusion in the disorganized tumor vessels. Abnormal tumor vessels also limit the adhesion and extravasation of leukocytes and impair leukocyte infiltration into the tumor tissue. Hypoxia increases the immunosuppressive nature of the stromal tumor microenvironment, by impairing T-cell effector functions including T-cell receptor signaling, proliferation, and cytokine production. Hyperoxia also increases the performance of cytotoxic T-cell, which may result in better clinical responses to the blockade of the immune checkpoints, e.g. programmed death receptor 1 (PD-1) (2). The hypoxic tumor is genetically unstable, giving rise to a new genotype with increased production of angiogenic factors. This transformation, known as the angiogenic switch, initiates the angiogenesis process.

The inhibitors of VEGF enhance the influx of immune cells into the tumor by restoring vessel integrity, increasing tumor perfusion and decreasing interstitial fluid pressure. The normalized tumor vasculature not only results in reduced tissue hypoxia and improved delivery of cytotoxic agents as well as oxygen (enhancing the effect of radiation therapy), but also augments anti-tumor immunity (3). In addition, deprivation of nutrients including glucose impedes T-cell proliferation and activation of CD8+ effector cells. Hence, tumor vessel normalization may be, consequently, expected to enhance the overall anticancer immunotherapy response (2).

The increased antitumor immune response seen with anti-VEGF therapy might be also related to the counteracting of the direct and indirect immunosuppressive activity of VEGF on effector T cells (Fig. 1). VEGF can inhibit the T cell function while increasing the recruitment of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs), and suppressing the differentiation and activation of dendritic cells (4). Hence, blocking VEGF sign-



**Fig. 1** Interactions between VEGF inhibitors and PD-1/PD-L1 inhibitors in colorectal cancer microenvironment. Abbreviations: PD-1 – programmed cell death protein 1; PD-L1 – programmed cell death protein ligand 1; MDSC – myeloid-derived suppressor cells; VEGF – vascular endothelial growth factor.

aling enhances effector T-cell function by increasing the activation and trafficking to the tumor due to both tumor vessel normalization and by inhibiting the VEGF-induced upregulation of inhibitory immune checkpoints (3, 5).

## VASCULAR ENDOTHELIAL GROWTH FACTOR

Vascular endothelial growth factor (VEGF) has two fundamental roles, first, in developing and maintaining blood vessels, and, second in regulating vascular endothelial cell permeability (6). Immunohistochemical studies have shown that VEGF is not expressed in normal colorectal mucosa, but significantly expressed in adenocarcinomas. VEGF expression is an early event in the transformation sequence from adenoma to adenocarcinoma. Neovascularization, underpinned by increased expression of VEGF is required for both tumor nutrition and hematogenous spread (7). In colorectal adenocarcinoma high VEGF expression has been demonstrated to correlate with poor prognosis and higher incidence of liver metastases.

VEGF inhibition is used successfully in the treatment of metastatic colorectal adenocarcinoma with bevacizumab, aflibercept and regorafenib and of advanced gastric adenocarcinoma with ramucirumab. In addition, radiosensitivity may be increased by the anti-angiogenic effect of bevacizumab. Bevacizumab has been incorporated into phase I-II studies of preoperative chemoradiotherapy for rectal cancer but the toxicity pattern and surgical complications observed in some studies prevented its routine use. The absence of a predictive biomarker for the therapeutic response to VEGF inhibitors means the selection of patients with a higher chance of response is currently impossible (8).

## IMMUNOSCORE

Tumor infiltrating lymphocytes (TIL) are frequently found in colorectal tumors, indicating that these tumors are capable of triggering an immune response (9). The final effectors of antitumor adaptive immune response are predominantly cytotoxic T lymphocytes recognizing nonself antigens, leading ultimately to tumor cell killing. Several studies have reported that high abundance of CD8+ cytotoxic TIL is associated with a positive clinical outcome across various different primary tumors, including non-small cell lung cancer, colorectal carcinoma, esophageal cancer, breast cancer as well as urothelial cancers and melanoma (10).

All types of immune cell may be encountered in the tumor. Analysis of the location, density and functional orientation of different immune cell populations is referred to as the immune contexture (11). Thorough intra-tumor analysis demonstrates, that these immune infiltrates are not distributed randomly. The combination of two markers (CD3+ TIL and CD8+ TIL) in two regions (center of the tumor and its invasive margin) has been validated for standard clinical practice in colorectal cancer. The Immunoscore is a prognostic tool, which seems superior to the tumor-node-metastasis (TNM) classification in colorectal cancer (12).

A study of three independent cohorts of 415, 119 and 69 patients with stage I–III. colorectal cancer found a significantly lower recurrence rate and longer overall survival in patients with a high density of CD3+, CD8+, CD45RO+ TIL and granzyme B. The type, density, and location of immune cells in colorectal cancer was superior to and independent of those of the UICC-TNM classification (13).

A study in 411 patients with stage I and II colorectal cancer showed a favorable prognostic value of high-density CD8 + and CD45RO + TIL (14).

### IMMUNE CHECKPOINT INHIBITORS IN THE TREATMENT OF METASTATIC COLORECTAL CANCER PATIENTS

Abundant infiltration by CD8+ TIL is characteristic for colorectal cancers with microsatellite instability that represent approximately 15% of sporadic colorectal cancer cases. Microsatellite instability is caused by deficiency of DNA mismatch repair (MMR) and associated with 10–50 times higher mutational load compared to colorectal tumors without MMR defects. There is evidence that cancers with high gene mutational load respond better to immune checkpoint inhibitor therapy (15). Tumour microsatellite instability testing is strongly associated with response to immune checkpoint inhibitors when treating metastatic colorectal cancer patients. Patients with high microsatellite instability respond to PD-1 inhibitors (e.g. pembrolizumab or nivolumab) and PD-L1 inhibitors (e.g. atezolizumab) (16) alone or in combination with CTLA4 inhibitors (e.g. nivolumab with ipilimumab) (17, 18). The number of clinical trials that assess the efficacy of the checkpoint inhibitors in the treatment of colorectal cancer with or without combination with radiotherapy is increasing (Table 1) (19).

**Tab. 1** Clinical trials currently underway (20 June 2019) evaluating the efficacy of the checkpoint inhibitors in the treatment of colorectal cancer with or without combination with radiotherapy, according to <http://clinicaltrials.gov> (19).

Checkpoint inhibitor	Target	Number of clinical studies
Pembrolizumab	PD-1	70
Pembrolizumab + RT	PD-1	5
Nivolumab	PD-1	67
Nivolumab + RT	PD-1	8
Durvalumab	PD-L1	28
Durvalumab + RT	PD-L1	7
Atezolizumab	PD-L1	26
Atezolizumab + RT	PD-L1	4
Avelumab	PD-L1	17
Avelumab + RT	PD-L1	4
Combinations with ipilimumab	CTLA4	24
Combinations with ipilimumab + RT	CTLA4	3

### COMBINATION OF ANTI-VEGF THERAPY WITH IMMUNE CHECKPOINT INHIBITORS

The rationale of combining the VEGF blockade with the blockade of immune checkpoints has been reviewed above. This combined blockade represents an emerging strategy and probably a new standard of clinical management of renal cell carcinoma (20). Given the activity of both anti-VEGF agents and immune checkpoint inhibitors in colorectal carcinoma, this combination therapy represents an attractive approach that is being investigated also in patients with metastatic colorectal carcinoma.

### CONCLUSION

Bearing in mind the recent successes for immunotherapies, combinations of anti-VEGF therapy with immune checkpoint inhibitors now appears an attractive strategy. Key to the successful implementation of a combination strategy for treating cancer is understanding the interaction between these two therapeutic interventions, particularly in regards to appropriate reprogramming of the tumor immune microenvironment to improve antitumor immunity (2, 21).

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