Endoscopic Injection Treatment of Vesicoureteral Reflux in Children: Meeting with the Factors Involved in the Success Rate

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ABSTRACT
The challenges and controversies in vesicoureteral reflux intervention guidelines resulted in a more individualized treatment planning. Endoscopic injection therapy is now widely used and is considered preferable, but still remains less successful than ureteral reimplantation. The endoscopic vesicoureteral reflux approach should be risk-adapted to current knowledge, so more experience and longer-term follow-up are needed. The precise of preoperative, intraoperative, and postoperative factors that affecting endoscopic injection therapy success rates and outcome have not yet been clearly determined.
The aim of this study was to investigate these associated factors. Although the reflux grade is the most well-known factor that can affect the success of the procedure, there is no agreement on which factors are the most influential for the efficacy of endoscopic reflux treatment. So, we carried out a broad review of published papers on this topic, and we presented all the potential predictive variables of endoscopic reflux resolution in children.

KEYWORDS
vesicoureteral reflux; children; endoscopic; injection; outcome; risk factors

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INTRODUCTION

Vesicoureteral reflux (VUR), as the most common and controversial urological abnormality in children, with a prevalence of 1% to 2%, is the most frequent predisposing risk factor for acute pyelonephritis, nephropathy with renal scarring, and decreased renal function (1). Renal damage may be congenital or acquired after infection, and the aim of the VUR-facilitating factor management is to prevent recurrent or breakthrough urinary tract infections, new renal scarring formation, and relative renal function deterioration (2). We can no longer view VUR as a homogeneous entity affecting all individuals equally. In contrary, we should take into consideration individual parameters and specific factors for a case by case and risk to benefit based deciding VUR management (1, 3).

Endoscopic injection treatment (EIT) became initially a popular alternative to open surgical ureteral reimplantation and long-term antibiotic prophylaxis in pediatric VUR management, as a minimally invasive and well tolerated method with a relatively short learning curve and low complication rate (4). Endoscopic treatment of a bulking agent was pioneered by Matouschek in 1981 (5) and was further developed and popularized by O'Donnell and Puri, who reported their experimental and clinical endoscopic Teflon injection results in 1984 (6). Since then, a lot of different substances have been used until the introduction of Deflux (7). EIT is nowadays recommended in selected centers as the first line therapy when intervention is needed (8, 9). It is considered preferable to ureteral reimplantation, which may be reserved for exclusive use in children not responding to EIT (9). In addition, parents of children with VUR are very likely to express a preference for EIT among all alternative options proposed (10). While open ureteral reimplantation has a maximum reported success rate of 98%, the most recently reported maximum radiographic EIT short-term cure rate is about 94% (11). Although EIT provides approximately a medium 80% cure rate, concerns about its long-term efficacy and delayed complications have resulted in a controversy over its real usefulness in recent years (4). Delayed post-injection ureteral obstruction is rare but may occur years postoperatively (11). EIT needs further evaluation of long-term outcomes (1).

Researchers have reported a variable success rate 50–94% of EIT, indicating differences in study design and methodology (12). There is significant disagreement on what a successful EIT constitutes, between the absence and the downgrading (presence of grade I-II or ≥2 grades improvement) of VUR, in combination or not with a recurrent febrile urinary tract infection (UTI) after injection (11, 12). One more or multiple reinjection procedures are frequently necessary in about 10–30% of cases with failed EIT (13, 14). Persistent VUR, is defined as that which is present three months after EIT, detected by follow-up cystography (15). In addition, reflux can recur in about 5–25% of children after a successful EIT (16, 17). Recurrent VUR, is defined as a proven VUR by repeated voiding cystourethrography (VCUG) in children with febrile UTI, any time after the first negative post-EIT cystography follow-up (15).

EIT success is evaluated in a short period of time, as in most studies it is determined after three months from the injection. If follow-up periods were longer, the recurrence rate might be higher (18, 19). There are reports of recurrence VUR rates as high as 26% after one year and 54% after two years from EIT (18). Fresh development of contralateral reflux after EIT for unilateral VUR is reported in the literature as well (9, 20). Persistent or recurrent VUR puts the children at risk for further UTIs and possible kidney damage. The precise pre/peri/post-operative factors affecting EIT success rates and outcome have not yet been clearly determined (21, 22). There is no agreement on which factors are the most influential for the endoscopic resolution of VUR, even with the use of artificial neural networks (23). We report the studies with positive correlation of factors with EIT cure rate, although there are many with opposite results.

EIT TECHNIQUE

EIT involves submucosal injection of a bulking agent to provide tissue augmentation and to improve the ureteral orifice valve mechanism (9, 24) (Figure 1). In the traditional STING (Subureteral Transurethral Injection) procedure, the needle is introduced under the bladder mucosa 2–3 mm below the refluxing orifice (9, 24). In the intraluminal HIT (hydrodistension implantation technique) technique, which has increased the success rate significantly (from 80% to 90%) (9, 16, 25–27), the needle is introduced into the mucosa inside the ureteral tunnel (9, 24). The advantages of HIT over STING include better visualization of the distal ureteral lumen with the aid of hydrodistension, more accurate placement of the injector needle at the desired position, better coaptation of ureteral orifice (27). In addition, the HIT technique has statistically significant success rate against STING technique for high grade reflux cases (28). Unlikely, other multivariate analyses have failed to demonstrate a significant difference in outcomes between the two techniques (22, 29, 30). The double HIT technique, currently achieving the highest success rates, involves one proximal and a second distal intraluminal ureteral injection site (9, 15). A combination of HIT and STING techniques can be performed in cases of HIT failure to coapt the ureter (31).

BULKING AGENTS

Over the years, many injectable agents have been investigated but only few of them are widely used. Thousands of children have been treated with different agents. Only four – Teflon, Deflux, Collagen, Silicone/Macroplastique – have been used in humans in large enough numbers to enable assessment of effectiveness (16, 32). The risk of new renal scarring is greatest among infants and young children aged under 5 years and therefore, the bolus created, using an injectable substance should persist for at least a period of 3 to 5 years (9). Deflux is now the most widely used and the most extensively studied bulking agent, which completely substituted the Teflon (16). Silicone/

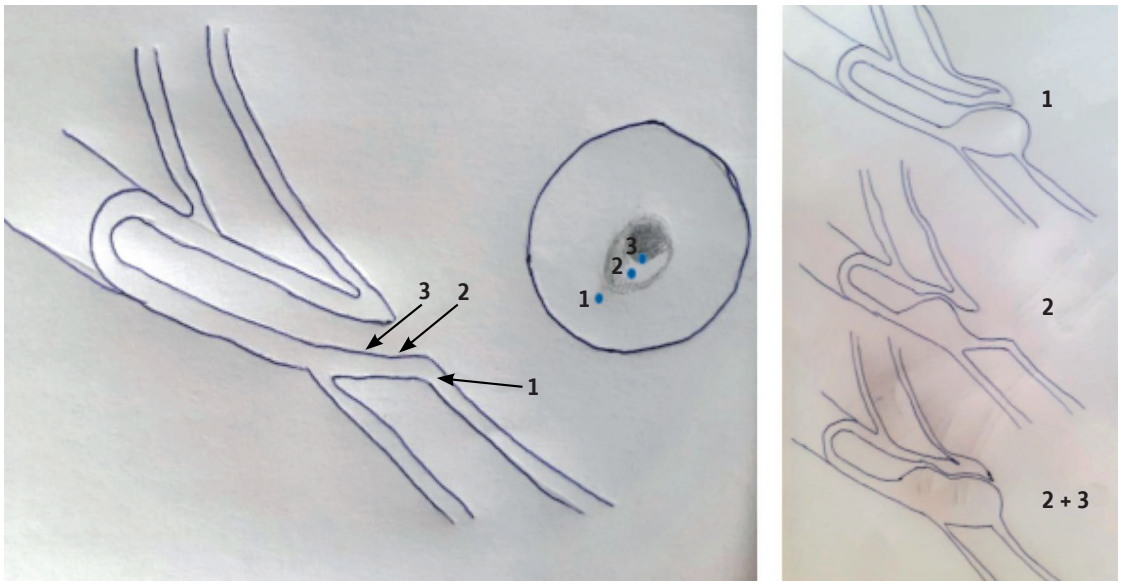

Fig. 1 Algorithm of endoscopic technique injection sites (1 = STING, 2 = HIT, 2 + 3 = Double HIT).

Macroplastique has been used with effectiveness that approaches that of Deflux (16). Biocompatibility of the substance, lack of potential for migration from the injection site, and absence of fibrosis or aggressive granulomatous reaction spreading to adjacent tissue are the ideal properties for the dominant or preferable injectable agent (4, 9, 12). The use of new polymer agents, like polydimethylsiloxane and polytetrafluoroethylene, is related with less recurrence rate in comparison with Deflux (33).

Studies have reported an increased success rate with the injected Vantris polymer agent and approved it as a more effective and promising material (with better stability and long-term durability) regardless of other confounding variables and the EIT technique (4, 34–39). In addition, Vantris presents more efficacy against Deflux in high grade VUR cure (40). However, ureteral obstruction and severe fibrosis on injection site are more commonly seen with Vantris use, and long-term data should be followed (4, 39–42). In a recent study, Vantris is presented as a completely effective agent for treating all high grade (IV–V) of primary (100% success rate after the second injection) and complex (100% success rate after the third injection) VUR in children, with the significant disadvantage of late (occurred within 3 years of EIT) severe ureteral obstruction in 8% of patients, that required surgical reimplantation (43).

PERIOPERATIVE FACTORS

There is a significant correlation between the endoscopically showed shapes of the ureteral orifice and their hydro-distension grade before the injection with the VUR grades and the EIT outcome (26, 44, 45). The cure rate in children with golf-hole type orifices is significantly lower than that of other types (46). The creation of a mound that elevates and coapts the orifice is the most important factor determining the success of EIT (4, 29). This volcano mound after the injection morphology of ureteral orifice is associated with a statistically significant increase in reflux resolution (19, 22, 45). The achievement of a mound mass height measuring 9.8 mm in maximal vertical diameter, at intraoperative ultrasound simultaneously with endoscopic procedure, maintains a significant correlation with EIT success (47). On the other hand, moderate injected volume >1 ml is a significant predictor of treatment failure (22). Intraoperative cystography following injection may help to determine immediate success and identify cases of new contralateral reflux but there is insufficient correlation with the standard 3-month postoperative VCUG (48). Thus, intraoperative cystography fails to show clinical utility to predict the EIT outcome (49).

LEARNING CURVE OF EIT

Independently of the technique used, there has been a learning curve associated with endoscopic VUR correction (50). EIT cure rates related to the surgeon are increasing and the requirement for reinjections decreasing as experience with the technique is improved (21, 46, 51). A combination of adequate experience and great skill in EIT is required to obtain results that could be favorably comparable to ureteral reimplantation, because there is a strong belief that nearly all endoscopic failures are related to unrecognized or unappreciated technical errors (9). In one study, success rates increased from 60% for the first 20 of 134 patients, to 80% for the last 20 cases (52). With increasing experience, not only high grade primary VUR, but also secondary reflux (duplex system), are considered eligible for EIT (51, 53). A multivariate analysis demonstrates that physician experience is an independent predictor of endoscopic VUR correction rates (54). Physician's experience and adjustments in clinical-surgical practice are associated with a reduced ureteral reimplantation rate (51, 55).

VUR GRADE AND MULTIPLE INJECTIONS

VUR grades II–III are considered as middle grade reflux and grades IV–V as high. In systematic reviews the reported success rates were 80–90% for grade I, 79–84% for grade II, 72% for grade III, 59–63% for grade IV, and 51–62% for grade V (56, 57). Lower success rates are presented in higher grades of VUR (19, 21, 46, 57, 58), and application of second and third injections increases the success rates (21, 46, 57). EIT is successful in about 50% of children with high grade VUR, and multiple injection procedures are frequently necessary to achieve a success rate about 85% (16). In a study, reflux was cured after the first STING injection in 100% of the ureters with grade II reflux, 65% of the ureters with grade III reflux and 50% of the ureters with grade IV reflux (28). The overall success rate increased with the second STING injection to 73% and 67% for grades III and IV reflux (28). Reflux was cured after the first HIT injection in 100% of the ureters with grade II reflux, 74% of the ureters with grade III reflux and 55% of the ureters with grade IV reflux (28). The overall success rate increased with the second HIT injection to 85% and 76% for grades III and IV reflux (28). Repeated EIT procedures are found to be successful in grade III and IV VUR, but children presenting with grade V should undergo ureteral reimplantation if the first trial of EIT results in failure as repeated injections have been proved unsuccessful in this grade (59). Recently, increasing evidence has been emerging to support the use of EIT in children with grade V VUR (24, 43, 60).

OTHER PREOPERATIVE FACTORS

Age is found to be a significant predictive factor of EIT success (40). Puri et al. presented their experience with endoscopic treatment of VUR in infants less than 12 months of age, asked about the necessity to overcome general anesthesia in such young patients with increased rate of VUR resolution over time (61). We can perform EIT in infants (62, 63), but we should rather not, because the effectiveness is lower than in children treated at an older age, there is not much experience, and the incidence of high-grade VUR is significantly higher as DMSA changes too (62). Age ≥6 years is a positive predictor (22), and age <1 year is a negative predictor of EIT success (33, 64). Younger age, especially age of 0–12-month-old, is a significant predictor of postoperative febrile UTI recurrence (65).

Radiologic success of EIT is statistically less common in males compared to females (66). There is a significant positive correlation between grade, bilaterality, recurrent preinjection UTIs, history of voiding dysfunction, defects on DMSA scan, and persistent or recurrent VUR after EIT (67). A study revealed that younger age, grade IV–V VUR and renal scarring are significant variables for the failure of endoscopic treatment after the first injection (68).

VCUG timing for VUR is found to be an independent factor for VUR resolution after EIT, and a filling reflux has significant lower success rate than a voiding reflux, especially in children with high grade VUR (69). Ureters that refluxed during the voiding phase have an approximately threefold independent odds of successful EIT, compared to those that refluxed during bladder filling (70). Distal ureteral diameter ratio at preoperative VCUG provides an objective measurement of VUR and appears as a predictive tool for clinical outcome and success after EIT (71, 72). It is significantly higher in children with high grade VUR and/or DMSA renal uptake ≤40% (72). Its predictive value for EIT success is more significant than VUR grade (72).

Renal scars on preoperative scintigraphy are significantly associated with postoperative febrile UTI and possible EIT failure (15, 21). Renal units with preoperative DMSA changes (hypoplasia, scars, uptake ≤40%) are at higher recurrence and lower cure risk, as a possible result of maldevelopment (15, 19, 46, 72, 73).

A factor that influences VUR resolution is voiding dysfunction, which refers to the presence of bladder filling and/or emptying lower urinary tract symptoms (urge, incontinence, weak stream, hesitancy, frequency, accompanying bowel problems) (1). Bilaterality is also a significant prognostic factor for the success rate of EIT (65–71). The success rate of EIT is significantly reduced in the presence of abnormal voiding habits, and additional injections are needed (21, 33, 74, 75). Duplicated systems and complex cases of VUR have lower cure rates (4, 31, 45), but are not associated with EIT failure (29, 31, 55). More recent studies have reported better success rates after a single injection (43, 55, 60, 76, 77).

It seems clear that a girl with high grade VUR and DMSA changes is at relatively high risk for recurrence than a boy with low grade VUR and no DMSA changes (15). Children aged less than two years or with ≥3 preoperative febrile UTI or with documented voiding dysfunction or with grade IV–V VUR, are 13 times more likely to have EIT failure (78). Children with ≥2 predictive factors, including febrile UTI, voiding dysfunction, and/or defects on DMSA, may not be optimal candidates for EIT (67).

POSTOPERATIVE FACTORS

Postoperative febrile UTI is significantly associated with EIT failure (21). Recurrences of febrile UTI may occur after 3 years of follow up and within the first 5 years after EIT (79, 80). Children with >3 episodes of recurrent preinjection UTI are 8.5 times more likely than those with only one episode to have an infection after EIT (67, 81). Female sex, older children and voiding dysfunction are the most important risk factors in the development of febrile UTI during long-term follow-up after successful EIT correction of VUR (81, 82). Furthermore, reinjections in children with postoperative febrile UTI and grade III-IV VUR seem to be unsuccessful (21). Mound detection at the first postoperative ultrasound is most critical factor than the intraoperative mound shape, and a strong and more reliable predictor of a successful EIT outcome (19, 45, 66). Calculated ellipsoid volume (CEV) of injected agent mounds is defined as $4/3\pi \times \text{height}/2 \times \text{length}/2 \times \text{width}/2$, based on 3-months postoperative ultrasound dimensions (22, 45). CEV>25% of injected agent volume is a positive predictor of EIT success (22, 31). The achievement of a maximal mound height in transverse vesical section measuring

at least 10 mm at three months postoperative ultrasound suggests a major predictive parameter for VUR resolution after EIT (64).

CONCLUSIONS

With the wide use of EIT and long-term follow-up, more treatment failures are being encountered, despite the accumulated endoscopic experience and the apparent by time success rates improvement. There is no agreement on which factors are the most influential for EIT successfulness. The risk factors affecting the outcome of EIT management for VUR studied herein are summarized in Table 1. The high grade VUR and treatment at infancy, as well as the presence of positive DMSA or urinary dysfunction, are factors associated with reflux persistence or recurrence after EIT. The preoperative filling than voiding VUR and the large ureteral diameter at VCUG are strong independent predictive factors for EIT failure. Duplex ureters are no longer considered as a contraindication for EIT. Even with widespread experience, the success rate in high grade VUR with a single injection of any of the available agents still fails to equal that of following ureteral reimplantation. Vantris is a new promising bulking agent for additional improvement of the double HIT method's results. Postoperative febrile UTI and/or poor ultrasound visualization of the ureteral orifice mound suggest negative predictive parameters for VUR cure after EIT. The endoscopic VUR approach should be risk-adapted to current knowledge. More experience and longer-term follow-up are needed. More prompt and aggressive approach with ureteral reimplantation is mandatory for children who have grade V VUR and low success rate at the beginning.

Tab. 1 Risk factors affecting the outcome of VUR endoscopic injection therapy.

VUR grade
Re-injections
Endoscopic technique
Bulking agent
Surgical endoscopic experience
Age (≥ 6 or ≤ 1 years old)
Gender
DMSA scan defects (hypoplasia, renal scar, uptake ≤40%)
Recurrent preinjection UTIs
Post-injection UTIs
Voiding dysfunction history
Bilateral VUR
Double ureteral system
VCUG timing of VUR (filling vs voiding)
Preoperative-VCUG distal ureteral diameter ratio
Endoscopically showed shapes of the ureteral orifice
Hydro-distension grade of the ureteral orifice
Endoscopically creation of a volcano mound
Moderate injected volume >1 ml
Intraoperative ultrasound mound mass height
Postoperative ultrasound mound volume (3 months)
Postoperative ultrasound mound mass height (3 months)

Abbreviations: vesicoureteral reflux (VUR), urinary tract infection (UTI), hydrodistension implantation technique (HIT), voiding cystourethrography (VCUG).

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Is There a Correlation of TSI Levels and Incidental Papillary Thyroid Carcinoma in Graves Disease? A Review of the Latest Evidence

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ABSTRACT

Purpose: Our aim is to clarify if there is an association between the TSI levels and the development of thyroid carcinoma in patients with Grave's disease.

Methods: A systematic search concerning original studies from 2010 to 2020 was carried out through the databases PubMed, EMBASE and Cochrane, according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines. The terms used are 'Graves' disease' and /or 'Incidental Papillary thyroid cancer' and 'TSI' levels. Retrospective studies upon the subject were concluded in the analysis.

Results: Only three retrospective studies were found involving 916 patients with Graves' disease and Euthyroid goiter. No significant correlation has been found between TSI and the occurrence of thyroid carcinoma in patients with Graves' disease.

Conclusion: Very little research has been conducted upon the subject. More assays are required in order to identify a possible prognostic role of TSI levels in Papillary thyroid carcinoma in patients with Graves disease.

KEYWORDS

TSI; papillary; thyroid; cancer; Graves disease

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Tab. 1 The data of the current study.

Study		Patients (n)	Method	Results
1	Ergin et al., (2014) (4)	248 EG + 245 GD	Total thyroidectomy	PTMC was found in 28% in EG group, as compared to 26% in GD group. PTC Patients with GD were significantly younger (44 vs 59) and less likely to have compressive symptoms than with EG before surgery ($p < 0.001$). In GD group, patients with PTMC were also significantly older ($p = 0.009$) than those without, were more likely to have symptomatic goiter ($p < 0.001$), and to have a nodular disease ($p < 0.001$). TSI ab titer did not predict PTMC in GD group. Among patients with GD and incidental PTMC, 58% of patients had at least one nodule.
2	Menon et al., (2018) (6)	308	Primary surgery	Significant incidences of disease progression in patients with PTC associated with GD ($p = 0.034$; OR 2.747, CI 1.078–7.004). Disease progression as new distant metastases mostly in skeletal locations was high in this group compared to euthyroid group ($p = 0.027$; OR 4.121, CI 1.008–15.600). There was higher incidence of cumulative metastatic diseases in PTC associated with GD.
3	Boutzios et al., (2019) (5)	115	Total thyroidectomy	The mean TSI antibodies levels were 4.14 IU/L compared with patients who had not developed cancer, whose mean TSI antibodies levels were 9.26 IU/L ($p = 0.31$). Patients with GD and TC had lower mean levels of TSI antibodies, though statistically not significant, in comparison with patients without TC.

EG: Euthyroid Goiter; PTMC: Papillary thyroid microcarcinoma; GD: Grave's Disease; PTC: Papillary thyroid cancer; TC: Thyroid Cancer; TSI: Thyroid Stimulating Immunoglobulin

INTRODUCTION

Graves' disease (GD) is an immune system disorder resulting in the overproduction of thyroid hormones and thyrotoxicosis as a result of binding of circulating antibodies to certain thyrotropin receptors. Thyroid-stimulating immunoglobulins (TSI) are immunoglobulins G that bind to and activate the G-protein coupled thyrotropin receptors causing the growth of the thyroid gland and the increased synthesis of thyroid hormones. TSIs mimic the action of thyroid stimulating hormone (TSH) and their levels are high in persons with hyperthyroidism due to GD. In fact, GD is the most common cause of hyperthyroidism (50–80%) (1).

TSI levels are suspected to be responsible for the potentially increased incidence or aggressiveness of papillary carcinoma in that setting. The diagnosis of incidental papillary carcinoma in patients submitted to thyroidectomy for a benign disease is quite frequent. According to Pezolla et al., 75% of patients undergoing thyroidectomy for benign disease were diagnosed with thyroid cancer and 18 out of 30 were papillary carcinomas (60%) (2).

The aim of this review is to investigate possible correlation of TSI levels in patients with GD to incidental papillary thyroid cancer (PTC) and assess the ability to predict PTC.

METHODS

A systematic search was conducted using three electronic databases (EMBASE, PubMed and Cochrane library) for retrieval of potentially relevant articles published from 2010 through 2020. The search comprised the following terms "thyroid cancer", "incidental thyroid papillary carcinoma", "TSI levels" and "Grave's Disease". Articles were also searched from references of the original papers and review articles. Inclusion criteria were for the articles to be retrospective studies and follow a surgical approach. All duplicates were removed, and the remained records were screened for eligibility criteria. Studies suspected of bias were excluded. The research was conducted according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines as shown in Figure 1.

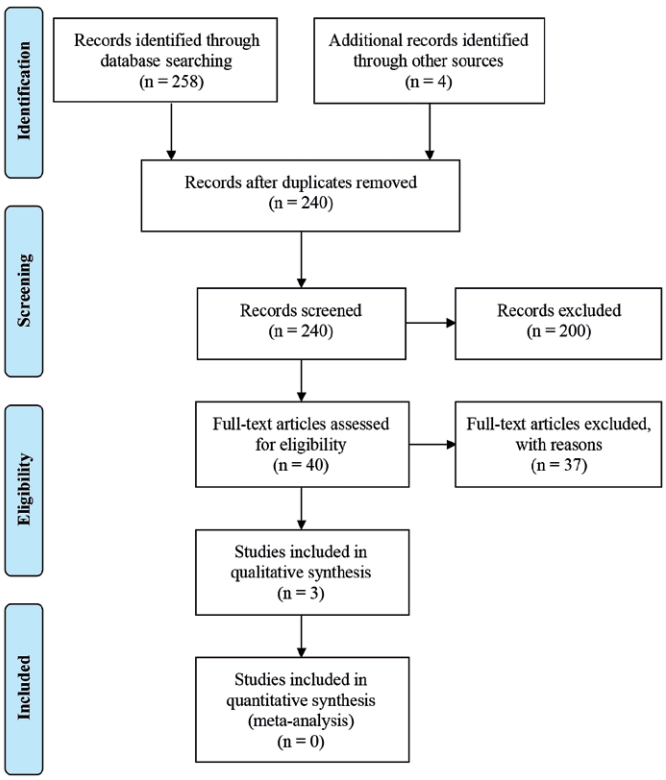

Fig. 1 PRISMA flow diagram for the current study.

RESULTS

The database search resulted in only 3 studies that met the inclusion criteria of relating the incidental PTC and TSI levels in patients with GD. A total of 916 patients with GD and euthyroid goiter were analysed in those studies. The data of the analysis can be seen in Table 1.

According to Ergin et al. (2014), the prevalence of incidental papillary carcinoma of the thyroid gland in GD is comparable to euthyroid patients with goiter (3). Each is increased compared to the general population. The age of papillary carcinoma presentation is lower in GD suggesting increased risk for patients with GD. Nodules >1 cm predict incidental papillary carcinoma. On the contrary disease duration and TSI titters do not. Boutzios et al. (2019), studied the association between the levels of TSI antibodies and thyroid cancer and found that among patients with cancer, the mean TSI antibodies levels were 4.14 IU/L compared with patients who had not developed cancer, whose mean TSI antibodies levels were 9.26 IU/L ($p = 0.31$). Those levels were not statistically significant (4).

Menon et al. (2018), conclude that papillary carcinomas in GD are more aggressive even when tumor characteristics are favorable. Aggressive progression with osseous metastases is also more frequent in PTC when GD is associated (13.6% in GD whereas the overall reported incidence ranges from 1.7–7%). TSI levels are elevated in GD raising the suspicion of potential relation with both the higher incidence and/or the aggressiveness of PTC in this setting. According to Menon et al., aggressive treatment and close follow-up are important in patients with GD compared to euthyroid counterparts diagnosed with PTC (5).

DISCUSSION

Thyroid carcinoma is the commonest endocrine cancer (6) and PTC – a well-differentiated thyroid cancer – usually appears as nodule or irregular mass, solid or cystic, in a normal parenchyma (7, 8). 11% of PTC patients present with metastases outside the neck and the mediastinum (9–12). Although thyroid carcinoma was originally thought to be rare in GD there are certain studies that have suggested an increased risk of thyroid malignancy in GD (13–15). Hypotheses about the carcinogenesis mechanisms centre around binding of thyroid stimulating antibodies and activating pathways of growth, invasion and angiogenesis (16). The American Thyroid Association states a frequency of < 2% of thyroid cancer in GD (17). Thyroid cancer may be associated with a nodule, detectable in 30–70% of the population on ultrasound (18). A fine needle aspiration (FNA) biopsy is always recommended in all detectable nodules, especially in high-risk patients (17, 18).

Thyroid cancer may occur concomitantly in GD. Its frequency varies from 0.15 to 15%. GD seems to be associated with larger, multifocal, and potentially more aggressive thyroid cancer compared to single hot nodules or multinodular toxic goiter. Patients with GD and nodules are at higher risk for thyroid cancer compared to patients with diffuse goiter (15).

Papillary thyroid microcarcinoma (PTMC) is a distinct entity; a thyroid carcinoma of ≤ 10 mm diameter usually an incidental finding during FNA biopsy or thyroidectomy for benign disease of the gland (19). Its prevalence in GD is estimated at 4.1% (20–22). Though rare, a small portion of microcarcinomas give distant metastases. The well-known prognostic biomarkers for thyroid cancer (BRAF and/or TERT) do not have an established role in PTMC patients (23). TSH has no prognostic value either in PTMC progression (24). No data exist concerning TSI levels in GD and their relationship with PTMC. Thus, further research is needed since both autoimmunity and inflammation are considered as independent risk factors for thyroid cancer (25).

Incidental thyroid carcinoma is considered the one occurring in patients with no suspicious features in any exam that may suggest the presence of cancer and no previous FNA biopsy (18). Both the prevalence and the clinical significance of incidental PTC in patients with GD remain uncertain.

The majority of thyroid cancers are PTMC. The prevalence and clinical significance of incidental PTC, which is well-differentiated thyroid cancer, in GD are uncertain but comparable to euthyroid patients with goiter and increased compared to the general population (3).

Papillary carcinomas of the thyroid express TSH receptors. The binding of the TSH to its receptors promotes the progression of cancer through growth of tumor cells (1). Similarly, TSI, present in GD, act through TSH receptor stimulating tumor cell growth. This may explain the higher incidence and/or the aggressiveness of PTC in patients with GD but no direct relationship between TSI levels and PTC has been established yet. It is also unclear whether concomitant GD affects the prognosis of papillary thyroid malignancy.

CONCLUSIONS

The conclusions of this review cannot be considered solid since the number of studies upon the subject is very limited. Further research is needed to understand the connections between cancer and thyroid autoimmunity, to correlate TSI levels with prediction or prognosis of papillary carcinoma and design a tailored therapy for these patients since both autoimmunity and inflammation are defined as independent risk factors for thyroid cancer.

CONFLICTS OF INTEREST

All the authors declare that there is no conflict of interest.

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Goeckerman Regimen Reduces Alarmin Levels and PASI Score in Paediatric Patients with Psoriasis

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ABSTRACT

Background. Psoriasis is a chronic systemic inflammatory disease with (extra-)cutaneous manifestations. Inflammation is associated with cellular stress and tissue damage which lead to the release of alarmins (signals of danger). Goeckerman regimen (GR) is a highly efficacious treatment consisting of the application of pharmaceutical crude tar and UVB light exposure. The reduction of inflammatory processes in the skin is accompanied by changes in the levels of inflammatory markers - alarmins (HMBG-1, S100A7, S1000A8, S100A9, S100A12, IL-17, IL-22, and IL-33).

Methods. The alarmin levels in sera of 19 paediatric patients with psoriasis were determined before and after GR using commercial ELISA kits. The Psoriasis area severity index (PASI) was used to determine the disease severity.

Results. GR reduced both PASI and the levels of all measured alarmins. The levels of S100A7, S100A9, IL-22, IL-33, and HMGB-1 were significantly decreased. Positive correlations between IL-22 and PASI, between S100A9 and IL-17, S100A9 and IL-22, and a negative correlation between S100A8 and IL-33 were found.

Conclusions. Goeckerman regimen is a very effective, safe and low-cost therapy. We confirmed, it modulates the immune system reactivity, ameliorates the severity of the disease and reduces the levels of alarmins reflecting the presence and intensity of inflammation.

KEYWORDS

alarmins; children; psoriasis; HMGB1; S100

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INTRODUCTION

Psoriasis vulgaris is a common chronic immune system-mediated inflammatory skin disorder that affects approximately 3% of population. It is relatively rare in childhood. Only a third of cases occur before the age of 18. Its pathogenesis consists of sustained inflammation that leads to uncontrolled keratinocyte proliferation, their dysfunctional differentiation, and reduced time of maturation. Macroscopically, it constitutes of psoriatic plaques, overlying inflammatory areas that are infiltrated by immune cells, mostly by dendritic cells, macrophages, T cells, and neutrophils (1).

Psoriasis is driven by immunopathological responses to stimuli causing "sterile" inflammatory processes triggered by endogenous danger signals and cytokines released from damaged or dying cells or during cell stress. These signals are called alarmins (DAMPs, PAMPs) (2).

Alarmin family includes a wide variety of endogenous molecules that are, in higher amounts, passively or actively released from the stressed, damaged, or dying cells. Intracellularly, alarmins have homeostatic functions. Conversely, the extracellular alarmins are recognized as a signal of danger and trigger a local or systemic inflammatory response; therefore, they might serve as potential markers of inflammation (3). Inflammatory conditions are associated with significantly increased levels of alarmins, one of them is psoriasis vulgaris (4). The levels of alarmins, including various chemokines, cytokines, are increased through all skin layers and in the circulation of patients with psoriasis. They act as potent immunostimulants sensed by chemotactic receptors and pattern recognition receptors (PRRs) through which inflammatory signalling pathways are stimulated. This stimulation causes the initiation of recruitment and activation of APCs, especially dendritic cells (DCs), which can stimulate innate and adaptive immune responses (5). The DCs are usually found accumulated in chronically inflamed tissues (just like presented in psoriasis). They activate naive T cells, enhance T cell proliferation, and promote their differentiation toward Th1 and Th17 phenotype (6).

To bring a deeper insight into the inflammatory processes associated with psoriasis and their changes influenced by the Goeckerman regimen we analysed the levels of 8 alarmins: HMGB1, IL-22, IL-33, IL-17, S100A7, S100A8, S100A9, and S100A12.

HMGB1 (amphoterin) is a ubiquitous, evolutionarily highly conserved protein helping to organize the DNA and co-regulating its transcription as its cofactor, while situated in the nucleus (7). HMGB1 binds and bends DNA to facilitate binding with other proteins (8). Posttranslational modifications of HMGB1 determines whether it will be present in the nucleus or not. Acetylation activates nuclear exclusion, translocation and accumulation of HMGB 1 in the cytoplasm (9).Outside the cell, in the extracellular matrix, HMGB1 plays the role as an alarmin and acts as a common mediator of inflammation (10).

IL-22 is a key effector molecule which is produced by activated T cells (Th22, Th17 and Th1 cells) as well as subsets of innate lymphoid cells. Although IL-22 can act synergistically with IL-17 or tumour necrosis factor, some important functions of IL-22 are unique to this cytokine. Increased production of IL-22 can result in keratinocyte hyperplasia, which causes a switch into an epidermal regenerative growth pathway (with increased synthesis of S100 proteins) leading to the faster growth of keratinocytes, which in conclusion accelerate the loss of surface keratinocytes and elimination of pathogens (4, 11, 12).

IL-33, a member of the IL-1 cytokine family, is constitutively expressed by epithelial and endothelial cell barrier, where it presents as an endogenous danger signal – alarmin (13, 14). IL-33 is found in higher serum levels in the circulation of patients with various inflammatory diseases such as allergic, autoimmune, and infectious diseases, with certain influence/participation in their pathogenesis (15–17). Innate and an adaptive immune response are involved via the interaction of IL-33 with its receptor ST2. When activated, ST2 triggers pleiotropic immune functions in multiple ST2-expressing immune cells.

IL-17, a family of proinflammatory cytokines, is synthetized mainly by Th17 cells, γδ T cells and innate lymphoid cells type 2 / 3 (ILC2 / 3). Production of IL-17 is driven by IL-23 stimulation which is secreted by activated monocytes, macrophages, and dendritic cells. IL-17 contributes to the development and progression of a variety of inflammatory conditions, including autoimmune and allergic inflammation. Furthermore, IL-17 mediates protective innate immunity to extracellular pathogens. IL-17 deficient mice are more susceptible to systemic bacterial and fungal infections (18–20).

S100A7 (psoriasin) is a member of the S100 family of alarmins and shares the typical calcium-binding domains that define this family of proteins (21). A close correlation between high expression and release of different S100 proteins with disease activity has been shown in many inflammatory diseases. S100A7 is overexpressed in keratinocytes found in psoriatic lesions, and there is growing evidence that S100A7 may be involved in the pathogenesis of psoriasis (22).

S100A8 and S100A9 are Ca^{2+} binding proteins belonging to the S100 family, their complexes are the most abundant DAMPs in many autoimmune diseases such as psoriasis (23). These proteins are constitutively expressed by neutrophils and monocytes, are released actively during inflammation, and play a critical role in modulating the inflammatory response by stimulating leukocyte recruitment and inducing cytokine secretion. S100A8/9 could be used as a biomarker for diagnosis and follow-up as well as an indicator of therapeutic response to inflammation-associated diseases (24).

S100A12, also a member of the S100 family of proteins, is mainly secreted by activated neutrophils. It is overexpressed at local sites of inflammation, and a high concentration of S100A12 can be found, during an active inflammatory episode in the serum (25, 26). Protein S100A12 appears to be a valuable serum biomarker showing the closest association to psoriasis activity (27).

The Goeckerman regimen (GR) is used in the treatment of psoriasis vulgaris. It is an extremely efficient therapy consisting of topical application of pharmaceutical crude coal tar and exposure to ultraviolet light (28, 29).

Although the GR has a genotoxic effect, which was confirmed in both adults and children with psoriasis (higher

rate of chromosomal aberration, DNA adducts, the elevation of Hsps, and oxidative stress), the benefits of therapy often outweigh the potential risks. GR is proven to have anti-proliferative anti-inflammatory, anti-angiogenic potential (30, 31).

MATERIALS AND METHODS

STUDY GROUP

A group of 19 paediatric patients (7 males, 12 females) aged 5–18 (median; age 13.2 years) diagnosed with psoriasis was selected. All participants of the study underwent the Goeckerman regimen.

Psoriasis severity was measured using the PASI scoring system (Psoriasis Area and Severity index; erythema, induration, desquamation, percentage of affected area). PASI score was calculated before and after treatment for each patient. The informed consent from each participant (parents) was obtained before the beginning of the study.

The efficacy of GR (changes in PASI) and the levels of alarmins were assessed before and after the treatment.

GOECKERMAN THERAPY

The Goeckerman therapy (GR) started daily in the morning with whole body exposure to ultraviolet light (UVR). The exposure was extended individually during GR from one to maximally twenty minutes according to photo-type of patients and according to their skin reaction. The UV emitter Chirana 397 (Chirana Group a.s., Czech Republic) was used. The patients were exposed simultaneously to UVR-A (242 μW/cm^2) and UVR-B (131 μW/cm^2) from one source. The density of UVR was controlled daily with a spectroradiometer Sola-Scope 2000 (Solatell Ltd., United Kingdom). Pharmaceutical grade crude coal tar ointment (containing 5% Pix lithantracis) was applied on the patients' skin approximately one hour after exposure to UVR. It was not whashed away until the next morning. The ointment was applied only to lesions which presented 11–61% of body surface. Duration of whole GR was individualized according the severity of disease (7–31 days, average 16 days) and its clinical benefit was recorded by comparing PASI.

BLOOD SAMPLES

The peripheral blood samples from paediatric patients with psoriasis were collected from the cubital vein. The Vacutainer sampling tubes (Becton Dickinson) were used. Whole blood samples were incubated for 30 minutes at room temperature; then, the samples were centrifuged for 10 minutes at 1300 g (2500 rpm) and serum was isolated and stored under –70°C until analysis. All the samples were collected throughout the time period of year (2017).

SERUM ANALYSIS

Levels of HMGB1

The concentrations of HMGB1 in serum were evaluated using commercial sandwich ELISA kit – Human HMGB1 ELISA kit (IBL International GmbH, Hamburg, Germany) according to the manufacturer's instructions. The limit of detection of HMGB1 was 0.20 ng/ml.

Levels of IL-17, IL-33

The concentrations of IL-17 and IL-33 were measured in serum using commercial ELISA Quantikine ELISA Human IL-17 and ELISA Quantikine ELISA Human IL-33 Immunoassay (R&D System, Inc., Minneapolis, MN) according to the manufacturer's instructions. The limit of detection was 20 pg/ml and 0.357 pg/ml, respectively.

Levels of IL-22

The serum levels of IL-22 were detected by ELISA technique using commercial kit Quantikine ELISA Human IL-22 Immunoassay manufactured by R&D Systems, USA. The assay was run according to the instruction for use provided by the manufacturer. Samples were used undiluted. The limit of detection was 2.7 pg/ml.

Levels of S100A7 and S100A12

Both parameters were determined by commercial ELISA kits: Enzyme-linked Immunosorbent Assay Kit For S100 Calcium Binding Protein A7 (S100A7) and Enzyme-linked Immunosorbent Assay Kit For S100 Calcium Binding Protein A12 (S100A12) according to the manufacturer's instructions (Cloud-Clone Corp., Houston, TX, USA), respectively. ELISA kits sensitivities were 0.050 ng/ml and 0.031 ng/ml.

Levels of S100A8 and S100A9

The concentrations of S100A8 and S100A9 in serum were determined by sandwich enzyme-linked immunosorbent assay technique (ELISA) with Enzyme-linked Immunosorbent Assay Kit For S100 Calcium Binding Protein A8 (S100A8) and Enzyme-linked Immunosorbent Assay Kit For S100 Calcium Binding Protein A9 (S100A9). Both kits were manufactured by Cloud-Clone Corp., Houston, TX, USA. The limit of detection was 0.56 ng/mL for S100A8 and 0.58 ng/mL for S100A9. The assays were run according to the instructions for use provided by the manufacturer. Samples were diluted 100× (1 + 99).

Absorbance values were read at 450 nm/620 nm by the Multiskan RC ELISA reader (Thermo Fisher Scientific, USA).

STATISTICAL ANALYSIS

The data were statistically processed with the Statistica software version 13.5.0.17 (TIBCO Software Inc., Palo Alto, CA 94304 USA). Based on the D'Agostino-Pearson test for the data distribution, either the parametric or nonparametric test was used to ensure the proper test sensitivity. Associations between parameters were evaluated by Pearson's correlation test and Spearman's rank correlation test. Changes of parameters were assessed using T-test for dependent sample or the Wilcoxon matched-pair test. The

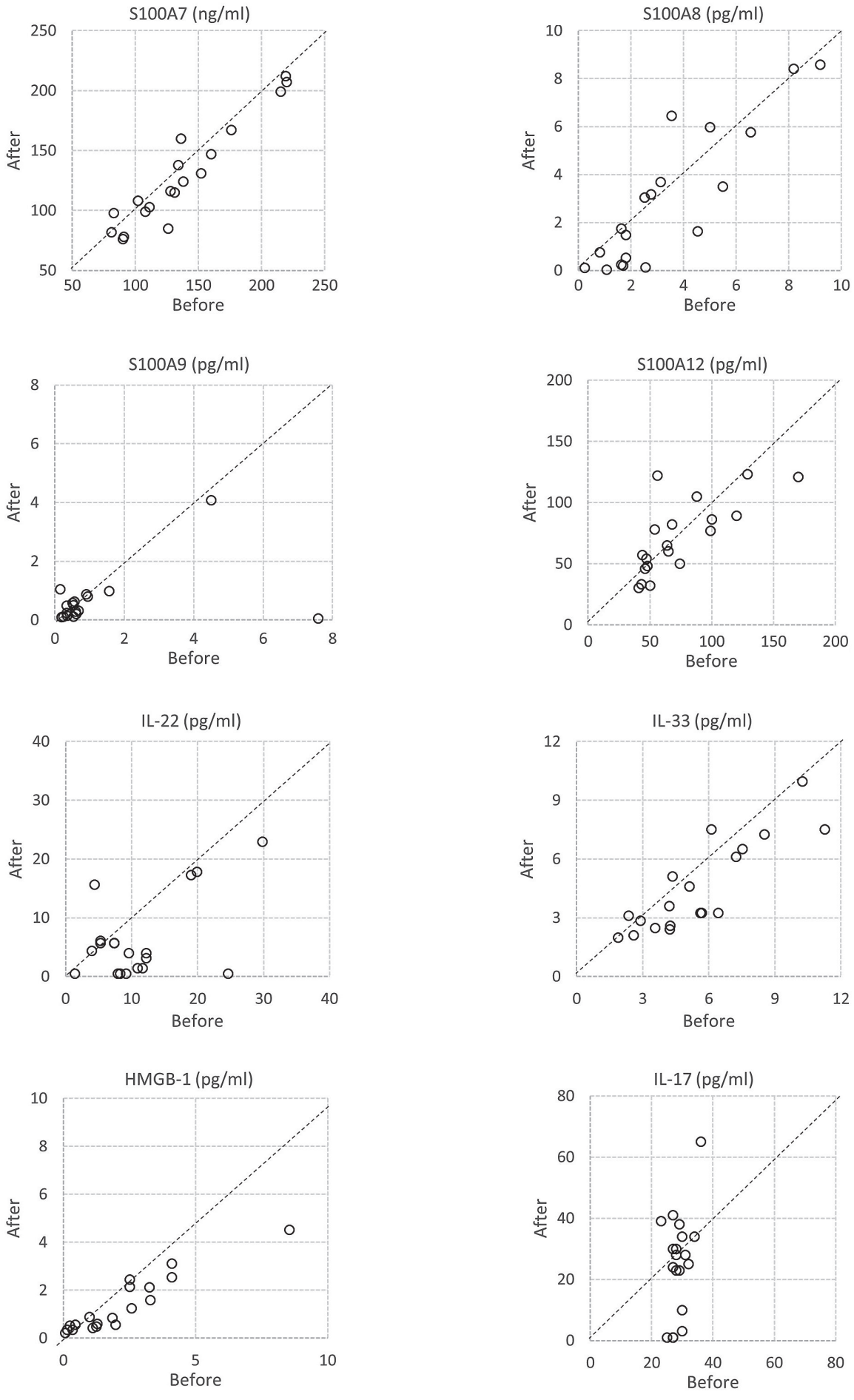

Fig. 1 The effects of GR on the levels of S100A7, S100A8, S100A9, S100A12, IL-22, IL-33, HMGB-1, and IL-17.

Tab. 1 Serum levels of selected alarmins before and after GR.

Values before and after GR	Valid N	Mean	Median	Min	Max	Lower	Upper	Test	p-value
PASI before/after	19	17.600	18.400	7.200	27.000	12.800	22.400	W	0.0001
	19	8.768	9.400	3.800	20.700	5.600	11.000		
S100A7 before/after (ng/ml)	19	136.895	131.000	81.000	220.000	102.000	160.000	T	0.0200
	19	128.684	116.000	76.000	212.000	98.000	160.000		
S100A8 before/after (ng/ml)	19	3.380	2.560	0.240	9.200	1.640	5.000	W	NS
	19	2.916	1.750	0.030	8.580	0.240	5.770		
S100A9 before/after (ng/ml)	18	1.178	0.550	0.160	7.590	0.340	0.910	W	0.0200
	18	0.642	0.405	0.050	4.080	0.150	0.800		
S100A12 before/after (ng/ml)	19	74.000	64.000	41.000	170,000	47.000	99.000	W	NS
	19	71.474	65.000	30.000	123.000	48.000	89.000		
IL-17 before/after (pg/ml)	19	28.842	28.000	23.000	36.000	27.000	30.000	T	NS
	19	25.158	28.000	1.000	65.000	10.000	34.000		
IL-22 before/after (pg/ml)	19	11.079	9.200	1.400	29.800	5.300	12.200	W	0.0040
	19	6.190	4.000	0.500	22.900	0.500	6.100		
IL-33 before/after (pg/ml)	19	5.476	5.110	1.890	11.260	3.560	7.240	W	0.0090
	19	4.497	3.250	1.980	9.950	2.590	6.520		
HMGB-1 before/after (ng/ml)	19	2.142	1.850	0.080	8.560	0.450	3.250	W	0.0030
	19	1.341	0.850	0.210	4.520	0.480	2.140		

Legend: GR, Goeckerman regimen; N, number of samples; Lower, lower quartile (Q1); Upper, upper quartile (Q3); PASI, Psoriasis Area and Severity Index; NS, statistically insignificant; Test, statistical test used; W, Wilcoxon matched-pair test; T, T-test

differences were considered statistically significant when the probability level (p) was below the alpha level of 0.05.

APPROVAL OF THE ETHICS COMMITTEE

The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the University Hospital in Hradec Králové, the Czech Republic (project identification code: PROGRES Q40-09, Q40-10, and Q40-11; reference number: 201705 183P; date of approval: May 2, 2017). Informed written consent was obtained from all persons.

RESULTS

The median PASI score in the group of children before the Goeckerman therapy application was 18.4 (N = 19; interquartile range 12.8–22.4) after GR the median PASI score was 9.4 (N = 19; interquartile range 5.6–11.0).

The serum levels of observed alarmins overall declined after GR. The reduction of S100A7, S100A9, IL-22, IL-33, and HMGB-1 achieved statistical significance. All the significant changes in alarmin levels are visualized in figure 1 and detailed in table 1.

A significant relationship was found in the group of children before GR between S100A8 and IL-33 (Spearman's rho = −0.469, $p < 0.043$), S100A12 and calendar age (Spearman's rho = 0.603, $p < 0.006$), IL-22 and PASI (Spearman's rho = 0.569, $p < 0.01$). After the GR, there was a significant relationship between S100A9 and IL-17 (Spearman's rho = 0.842, $p < 0.00001$), S100A9 and IL-22 (Spearman's rho = 0.528, $p < 0.02$), IL-22 and IL-17 (Spearman's rho = 0.680, $p < 0.001$).

DISCUSSION

Alarmins, signals of danger, are potent immunostimulators, markers of inflammation. The changes in their levels might reflect the severity of inflammation, especially in patients with psoriasis (3).

Our study aimed to investigate the relationships among changes of the alarmins concentrations and psoriasis severity depending on GR. Only a few studies are focused on the efficacy of GR in paediatric or adult patients with psoriasis. The studies mostly described the impact of GR on PASI score or detected a lower number of inflammatory markers compared to our study.

It is known that the child's immune system (numbers of immune cells, reactivity) differs from adults, but the basic pattern of the immune reactions is similar for all age groups (31).

In our study, 19 paediatric patients with psoriasis were included and the efficacy of GR (PASI score) and levels of alarmins S100A7, S100A8, S100A9, S000A12, IL-17, IL-22, IL-33, and HMGB-1 were determined. GR improved the PASI score in all patients and significantly reduced

the levels of S100A7, S100A9, IL-22, IL-33, and HMGB-1. This fact implies that GR therapy has an anti-inflammatory effect, despite causing oxidative stress, cell DNA damage, and apoptosis which support a pro-inflammatory state.

The GR reduced the mean of PASI score from median 18.4 before GR to 9.4 after GR ($p < 0.0001$). Our results correspond with those described in previously published studies in which the efficacy of the Goeckerman regimen was confirmed. DesGroseilliers et al. treated 200 patients with an ambulatory GR, chosen regimen cleared psoriasis in 86% of patients (32). Kortuem et al. reviewed the data of 65 paediatric patients with psoriasis (1983–2003): GR therapy ameliorated psoriasis in all patients. The improvement was higher than 80% in 55 patients (33). Petrozzi et al. and Fitzmaurice et al. described that GR is effective even in patients with psoriasis refractory to the biologic therapy (34, 35). Archid et al. performed the histological examination of lesional skin samples obtained from psoriatic patients after GR and revealed that the treatment was associated with a significant decrease of PASI, capillary and papillary diameters (36). The improvement of PASI score after GR was accompanied by the changes in measured molecules. We found that S100A7, S000A9, HMGB1, IL-22, and IL-33 are well-responsive molecules to the GR.

IL-22 has been proven to be a significant factor involved in the pathogenesis of psoriasis. It stimulates proliferation and differentiation of keratinocytes, promotes their viability (anti-apoptotic effect), and induces their stemness in cooperation with IL-17 (37, 38). In our study, the level of IL-22 after GR not only decreased (median; before GR 9.20, after 4.00 pg/ml; $p < 0.004$) but also positively correlated with PASI before GR ($p < 0.01$), and with IL-17 after GR ($p < 0.001$).

This agrees with the results of a variety of studies showing that anti-psoriatic/anti-inflammatory therapy lowered the level of IL-22. Fatiadou et al. confirmed that the levels of IL-6, IL-17A, IL-22, and IL-23 are higher in patients with psoriasis compared to controls. Moreover, the levels of IL-17A, IL-22, and IL-23 were significantly enhanced in patients with active disease compared with those with stable diseases (39). Olejniczak-Staruch et al. and Gkalpakiotis et al. documented that long-term biologic therapy with anti-TNFα drugs improved the PASI score, reduced systemic and local inflammation, and decreased the markers of inflammation such as CRP, IL-2, IL-22, etc (40, 41). Correlation between IL-22 and severity of disease and between IL-22 and IL-17 was proven in studies by Sobhan et al. and Elala et al. Sobhan discovered that the level of IL-22 was significantly higher in patients with psoriasis compared to healthy controls and correlated with PASI (42, 43). The study by Elala et al. focused on the levels of IL-17, IL-22, and FoxP3 in patients with vitiligo. The results showed that the levels of IL-17, IL-22 positively correlated with the severity of the disease and each other, and negatively with FoxP3; thus the decrease of both interleukins was associated with immunosuppressive response (43).

Although IL-17 is described in a wide range of studies with adult subjects as a crucial player in the pathogenesis and progression of psoriasis and reduction of its level is associated with the clinical improvement, in our study, the IL-17 decrease was not statistically significant (median/mean; before GR 28.000/28.842, after GR 28.000/25.158 pg/ml).

Nevertheless, our results are consistent with those reported by Kim et al. They analysed markers of inflammation in the lesional skin of both adult and paediatric patients with psoriasis. The level of IL-17 was significantly lower in children compared to the adults; thus, the level of IL-17 was not the crucial marker associated with psoriasis in paediatric patients. In children, the more important role is played by TNF-α. We measured the level of IL-17 in serum, not in the skin as Kim et al., but previous studies proved that the higher level of IL-17 in the skin of psoriatic patients is associated with a higher level of IL-17 in plasma/serum, thus we might assume that the level of IL-17 in the serum of children with psoriasis is lower compared to adults as well (44, 45). Therefore, it might imply that Th-17 did not play such an important role in the pathogenesis of psoriasis in children. Kim et al. accentuate the role of TNFα in the pathogenesis of psoriasis in children. According to the study by Borska et al., the levels of TNFα are also influenced by GR in paediatric patients (46).

We also confirmed that a close connection between IL-17 and IL-22 exists. The level of IL-22 positively correlated with IL-17 ($p < 0.001$). It is documented that the distinct T cell subsets, neutrophils, and ILC subsets that coexpress these cytokines (47–49). Their inhibition resulted in the reduction of both cytokine levels.

Besides IL-22, GR effectively reduced the levels of all analysed S100 proteins. Members of S100 proteins have the potential to amplify the immune system response, activate immune cells, and stimulate the production of proinflammatory cytokines. Importantly, the expression of each S100 protein is not linked with that of others. Their expression depends on distinct stimuli (50).

Borsky et al. documented that S100 proteins are elevated in patients with psoriasis (51), the same results published Wilsmann-Theis et al. and D'Amico et al. Wilsmann-Theis documented that proteins belonging to the S100 group of alarmins are valuable markers reflecting the activity of psoriasis. Subjects with psoriasis had elevated levels of all subtypes of S100 proteins in the skin lesions compared to healthy controls, subjects with atopic dermatitis and lichen ruber. The therapy with anti-TNFα decreased the level of S100A7, 8, 9, and 12 proteins (27). D'Amico et al. revealed that the expression of S100A7 in psoriatic skin is elevated and the reduction of its level is caused by the biologic therapy consisting of anti-TNFα or anti-IL-12/23 drugs (52).

Our results are consistent with both papers. We documented that levels of S100A7 and S100A9 were significantly decreased by GR (median; before GR 131.0, after 160.0 ng/ml; $p < 0.02$, and 0.550, 0.450 ng/ml; $p < 0.02$) which was associated with the clearance of psoriatic lesions.

Although the reduction of S100A7 and S100A9 was significant, the impact of GR therapy on the levels of S100A8 and S100A12 was, surprisingly, insignificant (median; before GR 2.560, after 1.175 ng/ml; 64.0 and 65.0 ng/ml) (27, 52).

Mentioned studies, we compared our results with, emphasized only the proinflammatory properties of S100

proteins, but the recently published study by Defrêne et al. demonstrated that S100A8 and S100A9 has not only the proinflammatory effect but also anti-inflammatory effect. In the mice imiquimod-induced psoriasis model, the abrogated activity of extracellular S100A8 and S100A9 increased PASI and elevated the production of IL-17. Additionally, S100A8 regulated differentiation and inhibited proliferation of keratinocytes; thus, prevented the development of skin hyperplasia (53). An almost unchanged level of S100A8 in our study might have had an ameliorating effect on the severity of psoriasis and might slightly reduce the level of IL-17.

Interestingly, according to our results, the S100A12 expression is related to the patients' age (correlated with age); the younger patients exhibit lower molecule production than older ones. This correlation was found both before and after GR. The reason for this relation is unclear. We suggest it might be due to the immune system maturation and different reactivity. The main source of S100A12 is neutrophils. The counts of neutrophils vary depending on a person's age. The higher age of a child, the higher number of neutrophils; therefore, the production of S100A12 naturally increases during the maturation of the child and its immune system (54). Our results correspond to the results of Walscheid et al. They investigated the serum of children with juvenile idiopathic arthritis-associated uveitis and showed that the expression of S100A12 positively correlated with age (55).

We also discovered a positive correlation between SA1009, but not S1008A, and IL-17 and between S100A9 and IL-22. The expression of S100A9 depends on the stimulation of target cells by IL-17 and IL-22. Limited accessibility of both cytokines might lead to the dose-dependent reduction of S1009A secretion. The study of Behnsen et al. evaluated the impact of IL-22 on the release of S100A9 and confirmed that the deficit of IL-22 in mice resulted in the lower production of S1009A, but S1008A was also reduced (56).

Surprisingly, a negative correlation between IL-33 and S100A8 was found ($p < 0.043$). IL-33 is expressed constitutively by keratinocytes and its expression might be induced by a wide range of cells (fibroblasts, endothelial cells, dendritic cells, monocytes, etc.). Its expression is enhanced in an inflammatory microenvironment and reflects the inflammatory response intensity. In mice studies, the intradermal application of IL-33 induced a psoriasis-like skin disease; on the other hand, the deficit of IL-33 ameliorated the inflammation in psoriatic skin lesions (57). Therefore, IL-33 is involved in the induction and progression of psoriasis. Mitsui et al., as well as Borsky et al., showed that the level of IL-33 is significantly increased in patients with psoriasis compared to the healthy controls; furthermore, the level of IL-33 is reduced by anti-inflammatory biologic therapy (anti-TNFα) (52, 58). In agreement with mentioned studies, we discovered that GR was able to reduce the level of IL-33 (mean; before GR 5.4758, after 4.4974 pg/ml; $p < 0.01$).

To complete the set of important alarmins, we detected HMGB-1 which has been implicated as a pro-inflammatory alarmin in the pathogenesis of various inflammatory conditions, including psoriasis (59). We confirmed that GR significantly lessens the level of HMGB-1 (mean; before GR 1.85, after 0.85 ng/ml; $p < 0.003$). As reported by Watanabe et al. the levels of HMGB-1 are increased not only in serum of psoriatic patients but also in the lesional skin when comparing to the healthy controls and patients with atopic dermatitis (58). Moreover, Bergmann et al. and Kamel et al. revealed that the amount of HMGB-1 in serum depends on the severity/progression of disease but we did not confirm the correlation between HMGB-1 and PASI (61, 62).

Based on results, we conclude that the Goeckerman regimen successfully diminishes alarmin levels among a wide range of age, from 5 to 18 years, and ameliorates the symptoms of psoriasis (reduces PASI score). This might imply that GR has not only local but also more profound systemic effect.

CONCLUSION

The results of our study show that the GR is very effective in the reduction of the severity of psoriasis, significantly reduced PASI score and clinical symptoms of psoriasis, and thus might improve the quality of life of patients. Moreover, GR significantly decreased the levels of almost all measured alarmins which play an important role in the pathogenesis of psoriasis and inflammation, and the correlation between the decrease of IL-22 and PASI was documented; therefore, the results of this study support the idea that GR therapy does not have only local, but also systemic effect and balances the immune system activity. These findings highlight the potential usefulness of GR in paediatric patients with psoriasis.

STUDY LIMITATIONS

Several limitations of our study need to be acknowledged. A rather small number of participants ($n = 19$) was enrolled in the study; however, the numbers were high enough for the power of the study to be high enough. We achieved statistically significant differences among the values before and after therapy in most studied parameters.

We did not include healthy controls; thus, we can only assume that the baseline values of alarmins in patients were higher than in healthy children, but previous studies focusing on the levels of cytokines and alarmins in the samples of patients with psoriasis, even children, provide sufficient evidence that the levels of alarmins in patients are higher than in controls.

The factor of age might play important role in the immune system response to the GR. Among our patients, there is a wide age range (5–18 years). Especially among children, the reactivity of the immune system might slightly differ depending on their age. A more homogenous group of patients might have provided more accurate results; on the other hand, we did not document statistically significant age-dependent changes in the levels of alarmins. Only S100A12, as we mentioned in the results and discussion, was influenced by age.

Furthermore, there were 5 smokers among patients. Smoking might impair the results of GR treatment and the inflammation; however, the number of smokers was too

small to ever achieve a statistical significance when comparing to non-smokers.

Although the study has its limitations, the results are robust enough to provide an insight into the pathology of alarmins in children with psoriasis.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

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Determination of Cadmium and Chromium in Fruit Spirits Intended for Own Consumption Using Graphite Furnace Atomic Absorption Spectrometry

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ABSTRACT

Introduction: Analysis of the occurrence of cadmium and chromium in selected samples of fruit spirits intended for own consumption.
Material and methods: In our pilot study, we analysed 89 samples of fruit spirits intended for own consumption. The samples were mineralized with use of microwave decomposition system MULTIWAVE 60 50 Hz and analysed by atomic absorption spectrometry with a graphite furnace (AAS GBC XPLORAA 5000 with GF 5000).
Results: Most of the analysed samples originated from plums (39), apples (38) and pears (5). The average ethanol concentration was 53.7%. Cadmium and chromium were detected in all samples. The highest concentration of chromium and cadmium was found in the apple spirit (31.9 ± 6.6 µg/l and 40.1 ± 8.3 µg/l).
Conclusions: The ethanol concentration in the samples was higher than in distribution spirits. Concentrations of chromium in all samples did not exceed the limit given by the Slovak legislation or the limit of the AMPHORA. The permissible cadmium concentration (10 µg/l according to the AMPHORA) was exceeded in 9 samples. This indicates the potential importance of cadmium compared to chromium. Due to the lack of information in this field, the study presents an important starting point for further research.

KEYWORDS

fruit spirits; cadmium; chromium; concentrations; analysis

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INTRODUCTION

The main risk factor in alcoholic beverages is ethanol, which has been classified as Group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) in 2012. In terms of ethanol concentration, fruit spirits intended for own consumption present an increased risk because they usually have higher ethanol concentrations compared to alcoholic beverages in the distribution network (< 40% vol.) (1). In addition to the higher concentration of ethanol in fruit spirits intended for own consumption, presence of xenobiotics such as cadmium or chromium have been considered as another possible risk factors (2). There is an insufficient information about concentration of cadmium and chromium in alcoholic beverages intended for own consumption. The reason is that these alcoholic beverages fall only partially under regulatory control (determination of the volume of ethanol, methanol and higher alcohols) and are not subject to full regulatory control compared to alcoholic beverages in the distribution network.

The European project *"The European study Alcohol Measures for Public Health Research Alliance (AMPHORA)"* was dealing with the issue and pointed out relatively high mortality rate on alcohol attributable diseases in some countries (Hungary or Slovenia) only partially corresponding with amount of alcohol consumed (3). For this reason, several studies evaluated concentrations of certain possible contaminants. Among them, heavy metals can play a significant role at increased mortality rate on alcohol attributable diseases. IARC (International Agency for Research on Cancer) considers cadmium as a human carcinogen being associated with several types of cancer, e. g. liver, kidneys etc. Moreover, it is well known as a risk factor for cardiovascular diseases. Hexavalent chromium is associated with allergic reactions, skin irritation and lung and digestive tract cancer (4). At the same time, ethanol is also a significant risk factor for the above-mentioned diseases (2). There is possibility that the above diseases may occur due to the interaction of heavy metals with higher concentrations of ethanol, but current knowledge in this area is unknown.

The aim of this pilot study is to identify and measure the content of cadmium and chromium in various types of fruit spirits intended for own consumption originating from Slovakia. Another goal of the study was to evaluate the measured levels against the limits set by the AMPHORA project as well as valid Slovak legislation. The results significantly contribute to understand the extent of the issue and can present an important starting point for further systematic research in the field.

MATERIALS AND METHODS

SAMPLES

We analysed 89 samples of legal fruit spirits intended for own consumption. Samples were distilled in local growing distilleries in Martin (Northern Slovakia). The samples of fruit spirits were taken during the winter period 2018/2019. Most of the analysed samples were from plums (39), apples (38) pears (5) and apricots (2). We had only one sample from black elderberry, rose hip, cherry, grapes and raspberry.

The bottles were used to collect samples, which were soaked in 10% nitric acid for 24 hours with HNO_3 and afterwards washed twice with ultrapure water Type 1 (up H_2O) with minimum resistivity of 18.2 MΩ cm. The ethanol content was determined by alcoholometric tables. Samples were diluted (up H_2O) to 10% ethanol and mineralized by microwave decomposition system manufacturer (Multiwave 60 50 Hz) (Table 1). High-performance reaction vessels with pressure-activated-venting for routine and quality control applications made of PTFE-TFM with a volume of 40 ml were used for mineralization. Immediately before the mineralization, we prepared the 15 ml samples consisting of 10 ml trace metal grade (TMG) HNO_3 and 5 ml of 10% distillate. After mineralization, the mixture was made up to 15 ml with ultrapure H_2O. As stated by

Tab. 1 Digestion program of alcoholic beverages for MULTIWAVE 60 50 Hz.

Step	Ramp time (mm:ss)	Temp. (°C)	Hold time (mm:ss)	Fan
1	20:00	130	0:01	1
2	5:00	180	5:00	1
3		70		3

Tab. 2 Graphite furnace temperature program for the study of cadmium in spirits.

	Final Temp. (°C)	Ramp Time (s)	Hold Time (s)	Gas Type
1				
2	40	2.0	1.0	Inert
3	120	5.0	10.0	Inert
4	130	5.1	10.0	Inert
5	300	5.0	10.0	Inert
6	300	0.0	2.0	None
7	1800	1.0	1.5	None
8	2300	1.0	2.0	Inert

Tab. 3 Graphite furnace temperature program for the study of chromium in spirits.

	Final Temp. (°C)	Ramp Time (s)	Hold Time (s)	Gas Type
1				
2	40	5.0	10.0	Inert
3	90	10.0	10.0	Inert
4	120	10.0	10.0	Inert
5	1100	5.0	10.0	Inert
6	1100	0.0	2.0	None
7	2500	1.0	2.4	None
8	2900	1.0	2.0	Inert

the manufacturer, even at the 10% concentration limit, alcohols may strongly react with HNO_3 even in the cold. It is necessary let the mixture pre-react under the fume hood without closing and performing the digestion or use a safer method of sample preparation. Samples were analysed using a graphite furnace atomic absorption spectrometer (AAS GBC XplorAA 5000 with GF 5000) (Tables 2 and 3).

INSTRUMENTATION

Sample preparation consisted of previous mineralization (Multiwave 60 50 Hz). The samples were analysed by graphite furnace atomic absorption spectrometry using GBC XplorAA 5000 instrument equipped with GF 5000 graphite furnace. We used graphite cuvettes with a pyrolytic surface without a platform. We used the deuterium lamp background correction method. For specific cadmium analysis we used hollow cathode lamp with wavelength of 228.8 nm and lamp current 3 mA. For chromium we also used hollow cathode lamp, but with wavelength equal to 357.9 nm and lamp current 6 mA. The temperature program used to determine the cadmium by GF AAS is shown in Table 2. Chromium is in Table 3. The temperature mode has been set by the instrument manufacturer and adapted to measure the cadmium and chromium content in the presence of HNO_3. Argon was used as the inert gas at 300 ml/min.

CHEMICALS AND REAGENTS

In the analysis we used ultrapure water Type 1 (up H_2O) with minimum resistivity of 18.2 MΩ cm. Other used chemicals were nitric acid (trace metal grade – TMG HNO_3) and standard (Sigma-Aldrich: Cd; Sigma-Aldrich: Cr) for AAS with concentration of cadmium 1000 ± 4 µg/l and concentration of chromium 1000 ± 4 µg/l. The dosing volume of the sample without modifier was 25 µl and with modifier 20 µl. The modifier was used only for cadmium analysis. As the modifier, we used ammonium phosphate ($NH_4H_2PO_4$) in a volume of 5 µl according to the device manufacturer's recommendations. The standard for cadmium was diluted to 2.6 µg/l and for chromium was diluted to 16.0 µg/l (max. recommended concentration by AAS manufacturers). From each of these standard solutions, two additional calibration solutions with a quarter and a half concentration - three-point calibration - were programmed in the measuring device for each calibration. Blank was prepared from up H_2O. The concentration is expressed in µg/l. Limit of detection (LOD) and limit of quantification (LOQ) were different for each sample considering various dilution level (to achieve the same ethanol concentration before mineralisation).

RESULTS

Chromium was detected in all samples. The highest concentration of chromium was in the sample of the apple spirit K81 (31.9 ± 6.6 µg/l), K87 (31.6 ± 6.6 µg/l) and the pear spirit K31 (30.7 ± 6.4 µg/l). The average concentration of chromium in our samples was 19.1 ± 4.5 µg/l. Similarly, as chromium, cadmium was also detected in all samples. The highest concentration of cadmium was in the sample of the apple spirit K1 (40.1 ± 8.3 µg/l), the plum spirit K25 (30.8 ± 6.4 µg/l) and plum spirit K70 (23.8 ± 4.9 µg/l). The average concentration of cadmium in our samples was 6.0 ± 1.2 µg/l. The average concentration of ethanol was 53.7% (Table 4).

DISCUSSION

In the study, we compared our results with the standards set by Slovak legislation (5) as well as with the limits set by the AMPHORA project (3). According to the average ethanol concentration in all analysed samples (53.7%), we set the limit concentration of chromium at 462 µg/l, which was calculated from the limit value for chromium in other foods (0.5 mg kg^{-1}). This level was not exceeded. The AMPHORA project set the maximum concentration of chromium in alcoholic beverages at 500 µg/l, our results were not exceeded in any case. Following the application of the drinking water standard (50 µg/l), this concentration was not exceeded even in this case. All previous

Tab. 4 Results for Cr and Cd concentration in spirits.

Distilled fruit	Number of samples	Ethanol average (%)	Concentration of Cr (µg/l)			Concentration of Cd (µg/l)		
			Median	A. mean ± MU*	Range	Median	A. mean ± MU*	Range
plums	39	53.7	18.6	18.5	3.0–26.1	6.6	6.7 ± 1.4	<LOQ–30.8
apples	38	54.9	19.3	19.5 ± 4.1	12.9–31.9	4.8	5.8 ± 1.2	<LOQ–40.1
pears	5	51.4	19.5	21.8 ± 4.5	19.2–30.7	3.9	4.2 ± 0.9	2.1–6.7
apricots	2	46.0	19.3	19.4 ± 4.1	15.9–18.8	–	<LOQ	–
elder	1	43.2	14.7	14.8 ± 3.1	13.2–16.4	1.9	2.0 ± 0.4	1.9–2.3
grapes	1	52.6	19.6	19.6 ± 4.1	17.6–21.7	5.7	5.8 ± 1.2	5.2–6.5
cherries	1	49.8	22.3	22.3 ± 4.6	19.9–24.7	5.9	6.2 ± 1.3	5.5–7.2
raspberries	1	52.6	14.0	14.5 ± 3.0	13.0–16.6	1.5	2.1 ± 0.4	2.3–2.5
rose hip	1	52.4	18.5	19.0 ± 4.0	17.0–21.5	–	<LOQ	–

* arithmetic mean, ± measurement uncertainty (expanded uncertainty by 2), <LOQ lower than the limit of quantification

studies evaluating chromium concentrations in alcoholic beverages (wine, beer, spirits) consistently found out chromium present in very low concentrations in alcoholic beverages (2, 6). In a study by Lendinez et al. several types of alcoholic beverages were analysed, such as wine, beer, apple cider, brandy, rum whiskey, gin, vodka and aniseed liqueurs, with the highest concentration of chromium being 25.0 μg/l (6). In our results, the highest concentration of chromium was 31.9 ± 6.6 μg/l present in the sample of apple spirit, the average concentration of chromium was 19.1 ± 4.5 μg/l. Chromium concentrations in our samples did not exceed the limit value for drinking water. Considering our findings in the context of the findings of other studies and existing body of knowledge, chromium concentration in alcoholic beverages intended for own consumption is mostly not very high, generally not exceeding requirements for drinking water.

Similar as for chromium, according to the average ethanol concentration in all analysed samples (53.7%), we set the limit concentration of cadmium at 28 μg/l, which was calculated from the limit value for cadmium in alcoholic beverages (0.03 mg kg^{-1}) (5). This level was exceeded in two analysed samples, namely in the sample of apple spirit K1 (40.1 ± 8.3 μg/l) and in the sample of plum spirit K25 (30.8 ± 6.4 μg/l). The AMPHORA project determined the recommended limit in alcoholic beverages to be 10 μg/l (3). Concentrations lower than 10 μg/l were recorded in several cases taken, namely in 4 apple spirit samples: K65 (17.8 ± 3.7 μg/l), K52 (13.1 ± 2.7 μg/l), K50 (12.2 ± 2.5 μg/l) and K1 (40.1 ± 8.3 μg/l). In samples of plum spirits in 5 cases: K25 (30.8 ± 6.4 μg/l), K70 (23.8 ± 4.9 μg/l), K27 (18.3 ± 3.8 μg/l), K30 (15.4 ± 2.2 μg/l) and K49 (11.5 ± 2.4 μg/l).

Serbian study by Bonic et. al. evaluated cadmium in plum spirits. The cadmium concentrations in their study were below the limit of quantification (<LOQ). However, they defined, considering the used method, the limit of quantification more than 20 μg/l, which can be considered as a relatively high level (limit for cadmium concentration from AMPHORA project is set at 10 μg/l) (3, 7). Study Mena et al. evaluated cadmium in apple cider originating in Spain and the cadmium concentration varied between 0.2 μg/l and 0.7 μg/l (8). However, in our samples we found a considerably wider range with much more higher values extending from less than 0.6 μg/l up to 40.1 ± 8.3 μg/l. Two studies analysed samples of "orujo" distillate (a distillate made from grape marc) in Spain. Cadmium concentrations ranged from less than 0.01 to 1.9 μg/l (9) and from 1.0 to 1.9 μg/l (10). Again, compared to our grape samples (5.8 ± 1.2 μg/l), concentrations found in Spain were much lower. However, the main limitation of our result is small number of samples, as we only had one sample available.

From our results it is clear, that the concentrations of cadmium vary independently from ethanol concentration. As for as a role of used fruit, we can able to compare only plum and apple spirits due to a similar number of samples (39 samples of plum spirits and 38 of apple spirits). The mean cadmium concentration in plum spirits was 6.9 ± 1.4 μg/l and in apple spirits 6.1 ± 1.2 μg/l. The mean cadmium concentration from all samples was 6.0 ± 1.2 μg/l. The difference calculated from the average of cadmium in apple and plum spirits was 0.8 ± 0.2 μg/l, which is a higher value in plum spirits. This higher concentration of cadmium in plum spirits may be due to the presence of a fruit stone in the yeast (11). According to current knowledge, we know that the maximum concentration of cadmium in plum stones is 67 μg/kg, in peaches 6 μg/kg and in cherries 76 μg/kg (11). From the study by Y. Sultanbawa et al. it is known that the content of cadmium in the plum stones of *Terminalia ferdinandiana* was up to 100 μg/kg, from which we can assume the presence of cadmium in plum stones intended for yeast preparation in our samples (12). It is necessary to take into account that in our study we recorded only 8 cases of respondents who were pitting plums. A limitation in proving the effect of the fruit stone on the cadmium content in the final product is the lack of samples of plum spirits from fruit stone-free yeast and fruit stone-fermented yeast, so we cannot evaluate the effect responsibly. At the same time, in only two cases the respondents reported the growth of plums nearby public road, so we cannot evaluate the influence of this factor on the occurrence of cadmium concentrations either. The last significant factor of possible contamination of fruit spirits intended for own consumption is storage material intended for yeast. In neither case was a material other than plastic barrel used for the yeast, which means that the yeast was not exposed to the possibility of contamination from the surface treatment of the fermentation barrel.

CONCLUSIONS

Our study points out that the cadmium is a frequent contaminant of fruit spirits intended for own consumption, with concentrations above the recommended level of Slovak legislation and the limits of the AMPHORA project. Considering insufficient information on this issue, our results represent a significant insight as well as an important starting point for further research in this field.

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Mixed Infections (Mucormycosis, Actinomycosis and Candidiasis) Leading to Maxillary Osteomyelitis in a Diabetic Mellitus Patient in Post COVID Phase: First Case Report

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ABSTRACT

Background: The second wave of COVID-19 has emerged with the addition of vivid types of oral manifestations. Immunosuppression caused by COVID-19 results in an exacerbation of pre-existing infections. Recently, in the backdrop of COVID-19 expression, a notable rise in the incidence of secondary infections, both fungal and bacterial, have been reported either during the disease or as a post-COVID manifestation.

Case presentation: A 70-year-old male diabetic COVID-19 patient reported with a chief complaint of pain in the right side maxillary region for 3 months and the passage of content from the oral cavity into the nose. Intraoral examination revealed missing teeth i.r.t. 12 to 17, denuded mucosa with exposed necrotic bone and an oroantral opening. Sequestrectomy was done and the tissue was sent for histopathological examination which revealed necrotic bone interspersed with broad aseptate fungal hyphae branched at right angles along with actinomycotic colonies and Candidal hyphae in few areas. Based on histopathological findings, a final diagnosis of mixed infections leading to Maxillary Osteomyelitis was given. No recurrence was noticed after 3 months of follow up.

Conclusions: The occurrence of oral infections even after the remission period of COVID-19 signifies an alarming sign both for the patient and clinicians monitoring the oral health status during the follow-up period. To our knowledge, this is the first such case (three oral infections as a post covid manifestation in a single diabetic patient) reported in the literature till date.

KEYWORDS

actinomycosis; candidiasis; COVID; diabetes; mucormycosis; osteomyelitis

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ABBREVIATIONS

ACE: Angiotensin convertase enzyme, AIDS: Acquired immunodeficiency syndrome, CMV: Cytomegalovirus, CNS: Central nervous system, COVID: Coronavirus infectious disease, DM: Diabetes mellitus, H&E: Hematoxylin & eosin, HIV: Human immunodeficiency virus, HT: Hypertension, ICU: intensive care unit, IL: interleukin, PAS: periodic acid Schiff, PNS: paranasal sinus, TNF: Tumour necrosis factor.

INTRODUCTION

The coronavirus infectious disease-19 (COVID-19) caused by Severe acute respiratory syndrome- coronavirus-2 (SARS-CoV2) has emerged to be a lethal viral infection resulting in high morbidity. Although the remarkable presentation of this disease is respiratory symptoms, its clinical features reported are not considered to be highly specific. With the spread of COVID-19, the signs and symptoms of this disease have varied from patient to patient. This is a contagious disease that puts patients with underlying comorbidities such as diabetes mellitus (DM), hypertension (HT), obesity, old age, trauma, organ transplant, hematopoietic malignancies, or any cardiac or renal disorder, at higher risk (2).

Studies on SARS-CoV and SARS-CoV-2 have shown that both viruses belong to the same species and have similar biological and clinical characteristics (3). It has been observed in previous studies that microbial infections were very frequent in SARS patients as a leading cause of death in 25–73.7% cases (4). These results signify an alarming sign for the clinicians to pay attention to the probability of secondary infections accompanying COVID-19 disease. Recently, in the backdrop of COVID-19 expression, a notable rise in the incidence of both fungal and bacterial, infections have been reported either during the disease or in post-COVID phase (5–7). Association of uncontrolled DM, SARS-COV2 and secondary infections predominantly mucormycosis has been reported by various researchers in the literature (8). Here we represent a case of a DM patient with mixed infections (mucormycosis, actinomycosis and candidiasis) leading to maxillary osteomyelitis as a post COVID sequelae. To our knowledge, this is the first such case (three infections as a post covid manifestation in a single DM patient) reported in the literature till date.

CASE REPORT

A 70-year-old male patient reported with a chief complaint of pain in the right side maxillary region for 3 months and the passage of content from the oral cavity into the nose. The patient had been a known case of type II DM for last 20 years and was currently taking oral hypoglycaemics (Metformin, 1000 mg BD; before breakfast and dinner). He was non-smoker, non-alcoholic without any allergic history. The patient suffered from fever 4 months ago and was diagnosed with COVID-19 infection. During his treatment for COVID-19, he was administered steroids along with a cocktail of other drugs (Ivermectin, Remdesvir, and Tocilizumab). After hospital discharge, he presented with the complaint of pain and noticed denuded bone over the right maxillary alveolar ridge region. On general examination, he was moderately built, with normal gait and posture and well- oriented to the time, place and surroundings. His vital signs were normal. An intraoral examination revealed missing teeth i.r.t. 12 to 17, denuded mucosa with exposed necrotic yellow-coloured bone extending from the distal aspect of right maxillary central incisor to the right side of the posterior maxillary tuberosity region (Figure 1A), and an oroantral opening was noticed on the palatal side of the necrotic alveolar bone. On palpation, the affected area was rough in texture with mild tenderness. The paranasal sinus (PNS) view showed haziness in the right maxillary sinus (Figure 1B). Based on history, clinical and radiographic findings, a provisional diagnosis of Osteomyelitis of the maxilla secondary to Mucormycosis was given. Other differential diagnoses included deep fungal infections, malignant neoplasms, etc. Sequestrectomy was planned as further management. Before operating,

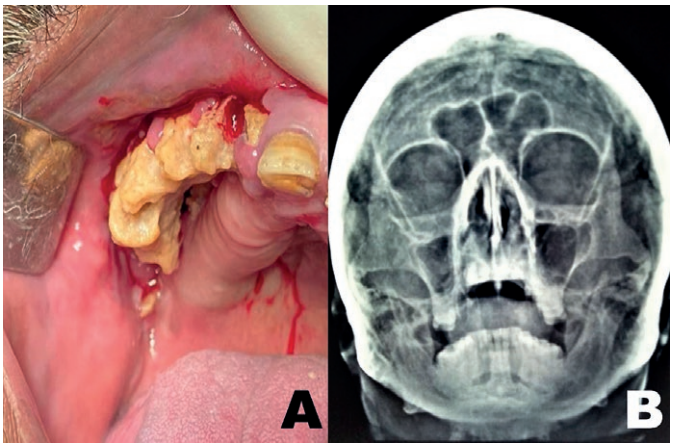

Fig. 1 A) Clinical photograph showing necrotic denuded bone; B) Paranasal sinus radiograph showing haziness of right maxillary sinus.

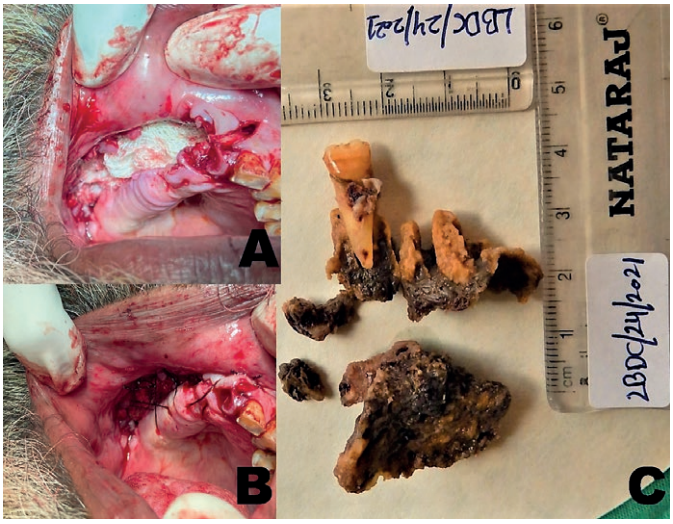

Fig. 2 A) Intraoperative photograph of resection of dead dentoalveolar right maxillary segment; B) Primary closure of incision site after resection; C) Photograph of gross excisional bony sequestrum showing blackish fungal colonies on the necrotic bone.

his blood sugar level was checked. His fasting blood sugar was 90 mg/dl on the day of surgery. His glycaemic index reported was 71. Routine blood tests were normal. Sequestrectomy (Figure 2A and 2B) was done for the dead right maxillary dentoalveolar segment along with curettage for the right maxillary antrum and nasal floor (Figure 2C), and the tissue was sent for histopathological examination. The Haematoxylin and eosin (H&E) stained sections revealed necrotic bone interspersed with fungal hyphae. These hyphae were broad aseptate with branching at right angles. Special staining with periodic acid Schiff (PAS) showed numerous magenta pink coloured non-septate fungal hyphae showing branching at 90° (Figure 3A and 3B). Further, necrotic bone showed sclerosis with actinomycotic colonies (Figure 4A and 4B), exhibiting club shaped filaments arranged in a radiating rosette pattern within the necrotic tissue and trabecular surface. On the surface of dead bone, candidal hyphae were also seen in a few areas (Figure 4C). Based on histopathological findings, a final diagnosis of mixed infections (Mucormycosis (BLACK FUNGUS), Actinomycosis (YELLOW FUNGUS), along with Candidiasis (WHITE FUNGUS) leading to Maxillary Osteomyelitis was given. The patient was asked to keep his sugar under control and was administered Posaconazole (400 mg BD × 3 months) along with oral Clindamycin (300 mg TID × 6 weeks). The patient was referred to the prosthetic department for an obturator, but the patient declined for it. The patient appears to be in remission and no recurrence was noticed after 3 months of follow-up.

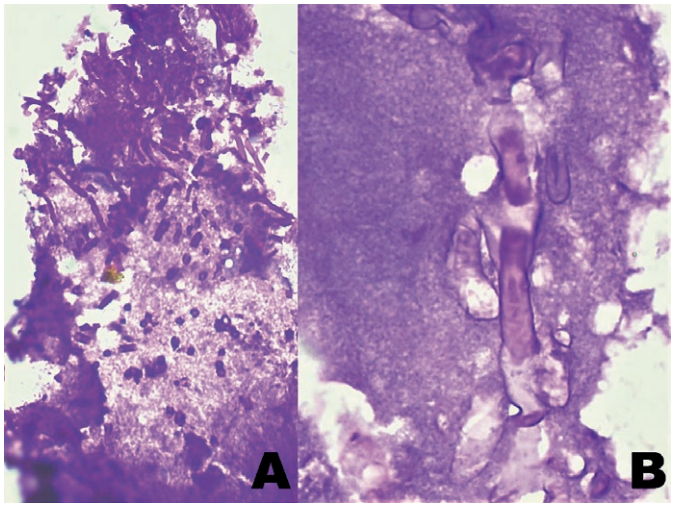

Fig. 3 A) Photomicrograph showing magenta pink colored Aseptate broad fungal hyphae (PAS stain, 100×); B) (PAS, 400×).

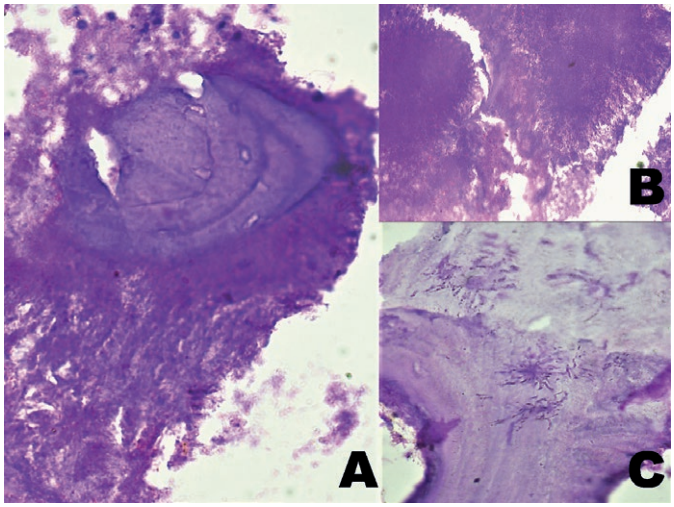

Fig. 4 A) Dead bone with Actinomycotic colonies on trabecular surface of bony spicules (PAS, 400×); B) Actinomycotic colonies exhibiting club shaped filaments arranged in a radiating rosette pattern in the necrotic tissue (H&E stain, 400×); C) Candidal Hyphae on the surface of necrotic bone (PAS, 100×).

DISCUSSION

It has been suggested that COVID-19 infection is associated with a marked reduction in CD4+ T cells, CD8+ T cells, B cells, NK cells along with lymphocytes, monocytes, and eosinophils and increase in neutrophil count, pro-inflammatory markers, such as interleukin (IL)-1, IL-6, and tumour necrosis factor alpha (TNF-a). Also, there is an overactivation of T cells caused due to an increased level of Th17 and over cytotoxicity of CD8+ T cells. Thus, overall leading to immunosuppression and increased susceptibility to infections (9). Meanwhile, the SARS-CoV2, can damage airway tissue and blood vessels, putting people at a higher risk of secondary infections (10). Also, SARS-CoV2 has been found to have an increased affinity for angiotensin convertase enzyme-2 (ACE-2) receptors (11). These receptors have also been reported in the oral mucosa making the oral mucosa as a portal for the virus entry. Existence of ACE-2 receptors in the epithelial cells of the salivary glands leads to dry mouth or xerostomia that might predispose the individual to develop various oral infections (12).

Mucormycosis/Zygomycosis is a rare but lethal fungal infection caused by fungi (Mucor, Rhizopus, Lichtheimia, Cunninghamella) belonging to mucormycetes family. The mould is ubiquitous, growing in soil, plants, manure, and decayed matter. The fungus is non-pathogenic in immunocompetent individuals. However, in immunocompromised individuals like DM, organ transplantation, Human-immunodeficiency virus / Acquired immunodeficiency syndrome (HIV/ AIDS), corticosteroid therapy, malignancy, burns, trauma etc, it becomes pathogenic resulting in an invasive infection. Main route of infection is through spore inhalation that results in germination of spores in the nasal cavity and spreads to PNS further invading the palate, orbits and brain, often leading to death. The fungus exhibits a remarkable affinity for arteries forming thrombi within the blood vessels resulting in reduction of blood supply leading to tissue necrosis. In DM patients with ketoacidosis, the binding of iron to transferrin is inhibited and results in elevated iron levels, which promotes the growth of mucormycosis (13). Six common subtypes of mucormycosis are; rhino cerebral, pulmonary, gastrointestinal, involving Central nervous system (CNS) and disseminated. Oral mucormycosis mainly involves PNS, nose and palate. Symptoms include headache, fever, lethargy, painful eyes, nasal or sinus congestion, ophthalmoplegia, meningoencephalitis, proptosis, facial swelling, partial

loss of vision, coughing, shortening of breath, and altered mental status. In the oral cavity, the infection is manifested as necrotizing ulceration of palate, blackish slough formation and exposure of bone, tenderness over maxillary sinus area, tooth loss etc. Cases with oroantral opening have also been reported same as in our case (14). Diagnosis of mucormycosis includes imaging aids and histopathology. Histologically, mucormycosis is characterized by pathognomonic broad, non-septate hyphae with the branching at right angles. Both medication and surgical management strategies are employed in mucormycosis. Amphotericin B (liposomal) is the most commonly used drug. Combined therapy of amphotericin B and Posaconazole have shown synergistic effects against fungal hyphae formation.

Actinomycosis is a rare anaerobic bacterial infection caused by gram positive, non-motile, non-acid fast filamentous bacterial rod '*actinomyces Israeli'*. Clinically it is of three subtypes; cervicofacial, thoracic, and abdominal. Cervicofacial type is the most common. The infection is characterized by contagious spread, suppurative and granulomatous inflammation leading to multiple abscess formation and sinus tracts that may discharge sulphur granules. With the progression of disease, infection may invade jaws, gums, and internal organs like lung, heart, kidney, liver, appendix etc. When affecting the jaw, the disease is termed as 'lumpy jaw' (15). It manifests as fever, chills, painless/painful soft-tissue swelling in peri mandibular region and sinus formation leading to woody consistency of jaw resembling some malignancy. Infection may spread to adjacent bone and muscles too. In the jaw bone, it can result in osteomyelitis. Main contributory factors for the development of cervicofacial actinomycosis are; trauma to oral cavity, dental treatment undergone, poor oral hygiene, dental caries, periodontal disease, local tissue damage caused by neoplasm or radiation therapy. The infection is more susceptible to the immunocompromised patients same as of mucormycosis. Histopathologically, actinomycosis shows chronic granulomatous inflammation with central abscess formation demonstrating sulphur granules colonies of organisms that appear as round or oval basophilic masses with eosinophilic terminal "clubs" giving rise to ray fungus on H& E staining. But these granules are not always recovered in the cultured sections. And these are not specific to this infection and can be produced by other infections such as botryomycosis, chronomycosis too. Special stains such as Gram stain, Gomori methenamine silver (GMS), and Giemsa are used to demonstrate these granules (16). The management of the lesion includes drainage of pus, sinus tract excision, long term use of antibiotics such as Penicillin, Tetracycline and Erythromycin.

Candidiasis is the most commonly encountered opportunistic fungal infection in the oral cavity caused by yeast like fungi '*Candida albicans'*. The microbe is a common inhabitant of oral mucosa, which becomes pathogenic under certain immunocompromised conditions such as DM, malignancy, ingestion of chemotherapeutic drugs or steroids, HIV infection and Cytomegalovirus (CMV). Salivary gland infections, dentures, increased carbohydrate diet, smoking, old age, obesity, hyposalivation are other risk factors for oral candidiasis. Decreased secretion of antimicrobial proteins are observed in individuals with hyposalivation which are related to decreased antifungal properties and may predispose the occurrence of oral candidiasis (17). *Candida auris* is found to be another emerging fungus that can has exerted outbreaks of severe infections in healthcare facilities and units during COVID-19. Cases of candidiasis in COVID-19 have been reported in large number among the patients admitted to intensive care units (ICU). Researchers suspect that these outbreaks may be related to lack of routine infection control practices due to the health crisis such as insufficiency of gloves and gowns, possibility of faulty cleaning and disinfection (18). The diagnostic methods for oral candidiasis include exfoliative cytology, microbial culture, potassium peroxide staining, salivary assays, and oral mucosal biopsy. H&E stained sections may reveal the presence of candida hyphae. Special staining procedure such as PAS is helpful in definitive diagnosis. Candidiasis is a superficial fungal infection. But in the present case, the candidal hyphae were seen invading the deeper bony tissue, signifying an important finding. The management of candidiasis includes antifungal therapy; Nystatin used topically, Amphotericin B, Clotrimazole, Ketoconazole and Fluconazole used Systemically.

In the present case, clinical and radiographic features provided a provisional diagnosis of Osteomyelitis secondary to Mucormycosis. But when confirmed histologically, a final diagnosis of mixed infections (Mucormycosis, Actinomycosis and Candidiasis) leading to osteomyelitis was concluded. Osteomyelitis very rarely occurs in the maxilla due to its rich vascular supply, but some fungal infections such as mucormycosis can cause maxillary osteomyelitis. Contiguous spread of infection from surrounding soft tissue and bones due to hematogenous seeding or direct inoculation of microbes into bone results in the disease origin (19).

Management of such infections is done by providing antifungal and antibacterial agents. And in DM patients, the main factor of concern is the regulation of blood sugar to prevent the emergence of other secondary infections. Postoperatively, the present patient was instructed to monitor his sugar level and was administered Posaconazole along with oral Clindamycin. And no recurrence was noticed after months of follow up.

COVID-19 DM patients with secondary infections have been reported in the literature, but we could not find any such case documented till date in which three infections manifested in a single DM patient in the post-COVID phase. This makes the present case both interesting and rare.

Cases of secondary infections (especially fungal and bacterial) in COVID-19 patients are being notified to a great extent in India in comparison to other regions of the world. There may be various factors associated with this predominance, and the same can be contemplated for the present case also. However, evidence-based studies are still required.

1. Prolonged use of steroids leading to immunosuppression. It is hypothesized that steroids tend to reduce the inflammatory storm in this infection and also minimise the end organ damage. But, these drugs can cause immunosuppression and rise in blood sugar levels,

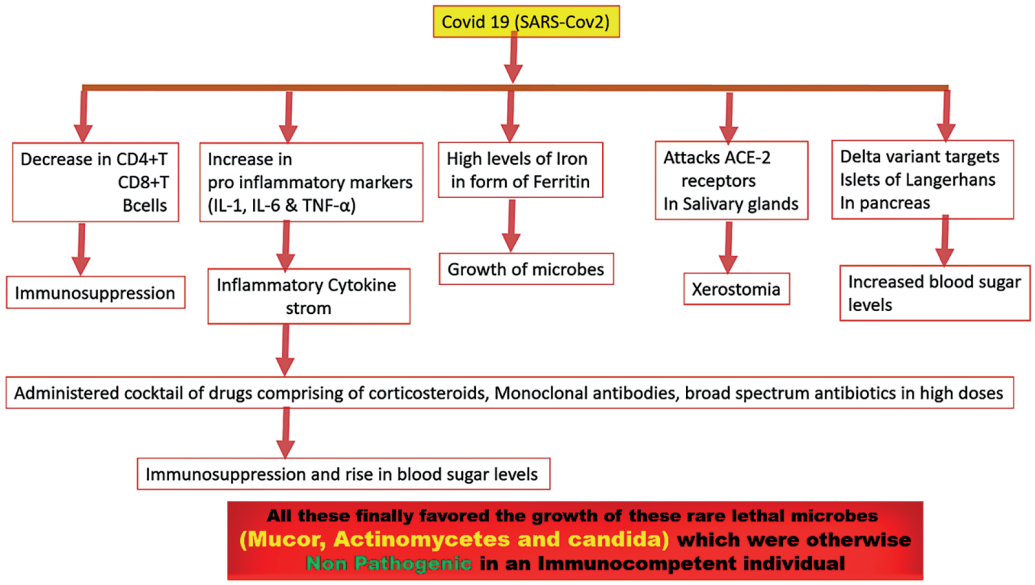

Fig. 5 Possible factors and mechanisms of developing secondary infections in diabetic patients as a post COVID sequelae

further resulting in secondary infections as a post COVID sequalae (13).

2. SARS-CoV2 has the potential to damage blood vessels and airway tissues, leading to more infections.
3. During the peak of the second wave of COVID-19, there was an acute shortage of medical grade oxygen, hence hospitals had to shift to industrial oxygen. Although no major difference has been noticed in both types, there is a strong possibility of the use of contaminated accessories during inhalation of oxygen by patients.
4. Humidifiers like distilled water or tap water may also be suggested as one of the predisposing factors for associated infections.
5. A high level of iron in the form of ferritin is found in COVID-19 and DM patients, which is also favourable for the growth of secondary infections like mucormycosis.
6. Therapeutic administration of Zinc in India was prescribed on a large scale, which also favoured the growth of such microbial agents.

It is well understood that DM patients are more prone to infections. High blood sugar level act as milieu for the microbial growth dysregulating the glycaemic homeostasis (20). According to a recent data, India stands second amongst the top 10 countries in the world, with 77 million people with diabetes and another 36.5 million with prediabetes which is a high-risk condition for diabetes and cardiovascular disease (8). Diabetic patients are at more risk of developing COVID-19 disease, conversely Covid-19 infection can worsen diabetes control and some treatments used for COVID (e.g. steroids) can exacerbate hyperglycaemia.

In the present case, the patient had a history of DM, COVID-19 infection, hospitalisation along with widespread use of steroids, monoclonal antibodies, broad spectrum antibiotics as a part of the armamentarium against COVID-19. All of these factors might have created the perfect storm in which secondary infections took root and thrived. From the above discussion, it can be hypothesised that the development of secondary infections in this patient in remission stage could be the result of multiple factors involved.

CONCLUSION

The second wave of COVID-19 has emerged with the addition of vivid types of oral manifestations. Immunosuppression caused by COVID-19 results in an exacerbation of pre-existing infections. While corticosteroids are considered to be life-saving in this pandemic, they have proved to be a double-edged sword and indiscriminate use has led to some deleterious results. Diabetes mellitus patients are already at a risk of developing vivid secondary oral infections. The occurrence of oral infections even after the remission period of COVID-19 signifies an alarming sign both for the patient and clinicians monitoring the oral health status during the follow-up period. Early diagnosis and treatment planning is mandatory to prevent further complications. The present case report of a DM patient with mixed infections (mucormycosis, actinomycosis, and candidiasis) in the post-COVID phase adds further important evidence of the increasing number of such cases during this lethal pandemic.

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Cervical Cystic Lymphangioma in an Adult Patient. A Case Report of a Rare Entity

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ABSTRACT

Cystic lymphangioma (CL) is a rare benign tumour that arises from the lymphatic vessels. The most common site of presentation is the posterior triangle of the neck. 90% of the lesions are diagnosed before the age of two years old and only a small number is reported in adults. In this paper, we describe the diagnostic and treatment approach of a cervical CL in an adult male.

KEYWORDS

cystic lymphangioma; neck; treatment; adult patient

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INTRODUCTION

Lymphangioma of the neck is an uncommon benign tumour arising from the cervical lymphatic vessels. Almost 90% of these lesions are diagnosed before the age of two years old and only a few cases of lymphangiomas are reported in adult patients (1). There are three morphological types described: capillary, cavernous and cystic (1). Despite the rarity of this pathology, lymphangioma should be included in the differential diagnosis of cervical masses (2). Diagnosis is based on previous medical history followed by ultrasonography (US) of the neck, magnetic resonance imaging (MRI), contrast-enhanced computed tomography (CECT), while fine needle aspiration cytology (FNAC) may also be used to assist in the diagnosis (1, 3). However, a definitive diagnosis is provided by histological examination (2). Complete surgical resection should be considered as the treatment of choice (3).

In this paper, we present the diagnostic and treatment approach of a cervical cystic lymphangioma (CL) in an adult male.

CASE PRESENTATION

An 85-year-old male was referred to the outpatient clinic of the Department of Oral and Maxillofacial Surgery with a history of a left-sided neck mass. The mass has been present for the last 15 years, but its size had increased during the last three months. The main complaints were a rapid increase in size and mild hoarseness of voice.

A nontender, soft and mobile mass was palpated in the left side of the neck. The overlying skin was intact. US showed a fluid-filled mass 9.0 × 3.5 cm in size in contact with the left thyroid lobe. FNAC was performed and 48 cc of bloody fluid were aspirated. Cytologic examination revealed a great number of small lymphocytes as well as histiocytes and no malignant cells. CL was suggested as a possible diagnosis. MRI of the neck showed a well-circumscribed lobulated mass, 9.0 × 5.4 × 5.0 cm in size with fluid-like intensity, located under the sternocleidomastoid muscle. The mass was in contact with the common carotid artery and caused mild deviation of the larynx (Figure 1).

The patient underwent complete surgical excision of the lesion with dissection and preservation of the vital anatomic structures (Figure 2). A soft dark grey cystic mass 9.0 × 5.4 × 5.0 cm in size was removed in entirety from the left lateral neck (Figure 3). Histological examination confirmed the diagnosis of CL (Figure 4). The post-operative period was uneventful, and the patient was discharged 5 days after the surgery. He remains asymptomatic 1 year later with no signs of recurrence.

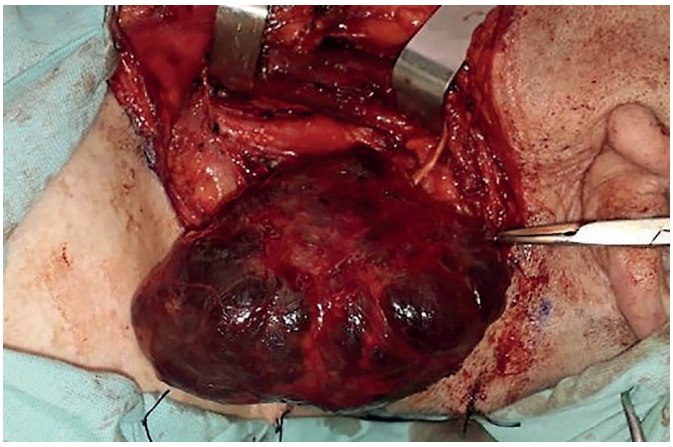

Fig. 2 Intra-operatively the mass was dark grey in color, and appeared fluid filled and lobulated.

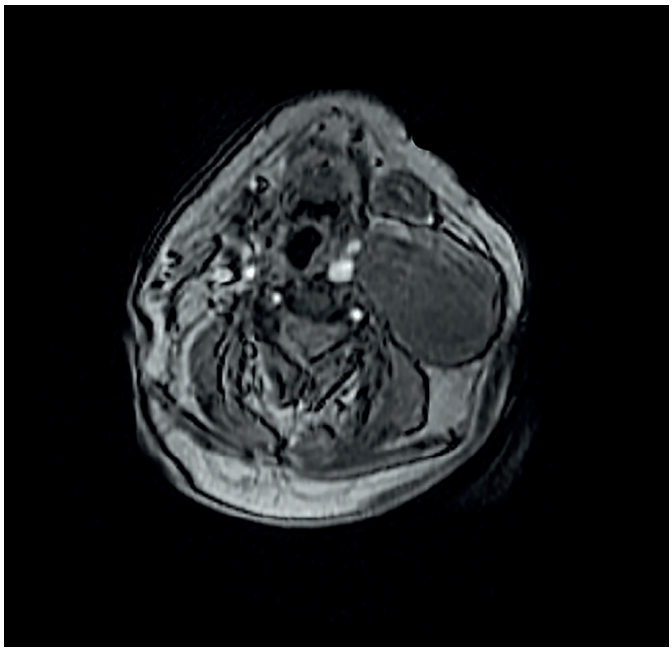

Fig. 1 MRI scan (axial view) of the neck, showing a well-defined mass located in the left side deep to the sternocleidomastoid muscle near to the carotid artery.

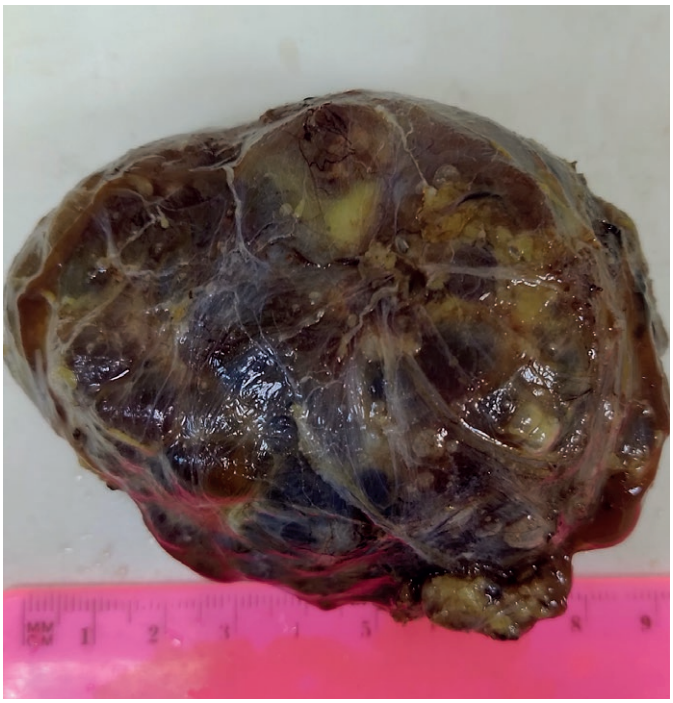

Fig. 3 Macroscopic view of the specimen.

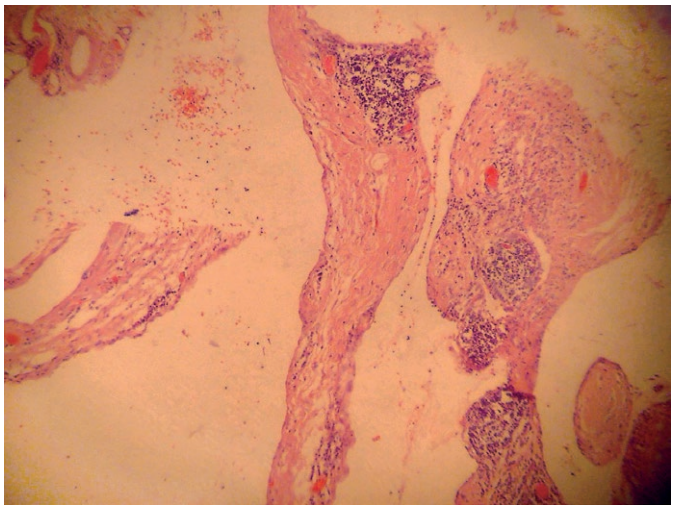

Fig. 4 Microscopic view of the cyst wall. Vascular spaces are large, irregular and dilated. The stroma consists of fibrous tissue and within it there are lymphoid aggregates.

DISCUSSION

CL is an extremely rare lymphatic vascular malformation in adults. The most common sites of presentation include the posterior triangle of the neck (75%), axilla (20%), mediastinum (5%), groin, retroperitoneal space and pelvis (4).

Cervical CL may present as a painless, soft and mobile mass of variable size in the neck region (5, 6). Most patients are asymptomatic, although it may rarely cause compression symptoms, such as dysphagia, hoarseness of voice and airway obstruction (3, 5, 7). Mild pain may also be noted (3).

In adult patients, the differential diagnosis includes branchial cleft cysts, dermoid cysts, and lymph node neoplasms (3).

Accurate preoperative evaluation plays an important role in the management of CL. US and MRI scan are the best-used imaging modalities (5). CECT can also be used (3). The role of FNAC is still controversial, since it may be complicated by infection, haemorrhage or recurrence (6). However, FNAC may rule out malignancy (1). Eventually, histological examination provides a definitive diagnosis (3).

Complete surgical removal with preservation of the vital anatomic structures and functionality is the mainstay of treatment (5). However, in some instances, this might be not possible, since the lesion may surround vital structures (1). In addition, a lack of a capsule can lead to infiltration of the neighbouring structures, making surgical excision harder (7). Incomplete removal of the lesion is associated with higher rates of recurrence (4). Alternative treatment approaches are radiofrequency ablation, CO_2 laser, electrocoagulation, cryotherapy and sclerotherapy (3, 8). These approaches can be used in combination with surgery in larger lesions (3).

The recurrence rate of CL is near 15% (5), whereas partial resection of the lesion can have a recurrence rate as high as 88% (3). Long-term follow-up time may vary depending on the extent of the resection.

CONCLUSION

Despite its rarity, CL should be included in the differential diagnosis of cervical masses. Diagnosis depends on medical history, US of the neck, MRI, CECT and FNAC. Total surgical excision of the mass is the treatment of choice. In our case, the patient was mainly asymptomatic except for mild hoarseness of voice. FNAC suggested the lymphovascular origin of the lesion and the MRI depicted a well-defined lobulated mass that mildly deviated the trachea. Total excision of the mass was accomplished. He remains asymptomatic 1 year later.

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Dual Fungal Infections (Aspergillosis and Mucormycosis) in a Diabetic Mellitus Patient Leading to Maxillary Sinusitis as a Post-COVID Manifestation: First Case Report

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ABSTRACT

Coronavirus infectious disease-19 caused by Severe acute respiratory distress syndrome-coronavirus-2 has emerged to be an emergency global health crisis for more than a year. And, as the disease has spread, a number of new clinical features have been observed in these patients. Immunosuppression caused by this disease results in an exacerbation of pre-existing infections. While corticosteroids are considered a life-saving therapeutic intervention for this pandemic, they have proved to be a double-edged sword and their indiscriminate use has produced some deleterious results. Recently, in the backdrop of this expression, a notable rise in invasive fungal infections has been identified even in the post-remission phase. Mucormycosis, Aspergillosis, and Candidiasis are the three most common opportunistic fungal infections among those observed. COVID-19 patients with diabetes mellitus are already at a higher risk of developing such secondary infections due to impaired immunity. Here we present a rare case report of a 50-year old male diabetic mellitus patient diagnosed with dual fungal infections (Aspergillosis along with Mucormycosis) leading to maxillary sinusitis as a post-COVID manifestation. To our knowledge, this is the first such case reported till date.

KEYWORDS

aspergillosis; COVID-19; diabetes; Maxillary Sinus; mucormycosis

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INTRODUCTION

For more than a year, Coronavirus infectious disease-19 (COVID-19) caused by Severe Acute Respiratory Distress Syndrome (SARS-CoV2) has emerged as an emergency global health crisis. And, as the disease has spread, a number of new clinical features have been observed in these patients (1). Recently, in the backdrop of this expression, a notable rise in invasive fungal infections has been identified either during the disease or in the post-remission phase (2–4). Mucormycosis, Aspergillosis and Candidiasis are the three topmost opportunistic fungal infections noticed among those. COVID-19 patients with diabetes mellitus (DM) are already at a higher risk of developing such secondary infections due to impaired immunity (5). Here we present a rare case report of 50-year old male DM patient with dual fungal infections (Aspergillosis with Mucormycosis) leading to maxillary sinusitis as post-COVID manifestation. To our knowledge, this is the first such case reported til date.

CASE REPORT

A 50-year-old male patient presented with a chief complaint of slow-growing swelling and continuous pain on the left side of his face since 1 month ago. He was a known case of long-standing DM (more than 10 years) and was on oral anti-hyperglycaemic drugs. He gave a history of fever and COVID-19 infection one and a half months back. He mentioned a drug history of Prednisolone, Remdesivir, and Tocilizumab administration during his treatment for COVID infection in the hospital. He reported a complaint of continuous pain and gradually increasing swelling on the left side of his face. On inspection, extra oral examination revealed a gross facial asymmetry due to swelling on the left side of the mid-face region; it was about 3.0 cm × 2.0 cm in size. On palpation, the swelling was firm and non-tender.

The computerised tomography (CT) scan of paranasal sinus (PNS) revealed a soft tissue density mass in the left maxillary sinus extending into osteomeatal complex resulting in complete blockage with bone erosion of the anterolateral wall, suggestive of fungal infection or malignancy (Figure 1A). A soft tissue density thickening was also noted along the walls of right maxillary sinus. X-ray chest revealed left sided perihilar opacity along with non-homogenous infiltrates in all zones of both lung fields, suggestive of transient pulmonary infiltrates (Figure 1B). With this suspicion, a cadwell-luc procedure was performed under local anaesthesia to incise the mass (Figure 1C) in the left maxillary sinus and sent for histopathological examination. The microscopic examination revealed a pseudostratified ciliated columnar epithelium with intact basement membrane all over and underlying connective tissue was fibro cellular with chronic inflammatory cell infiltrate. Special staining with periodic acid Schiff (PAS) stain showed acute and chronic inflammatory infiltrate along with branched septate hyphae at acute angles (Figure 2A, 2B) which are characteristics of aspergillosis. Apart from hyphal forms, many fruiting bodies and spores of fungus were also seen (Figure 3A). The

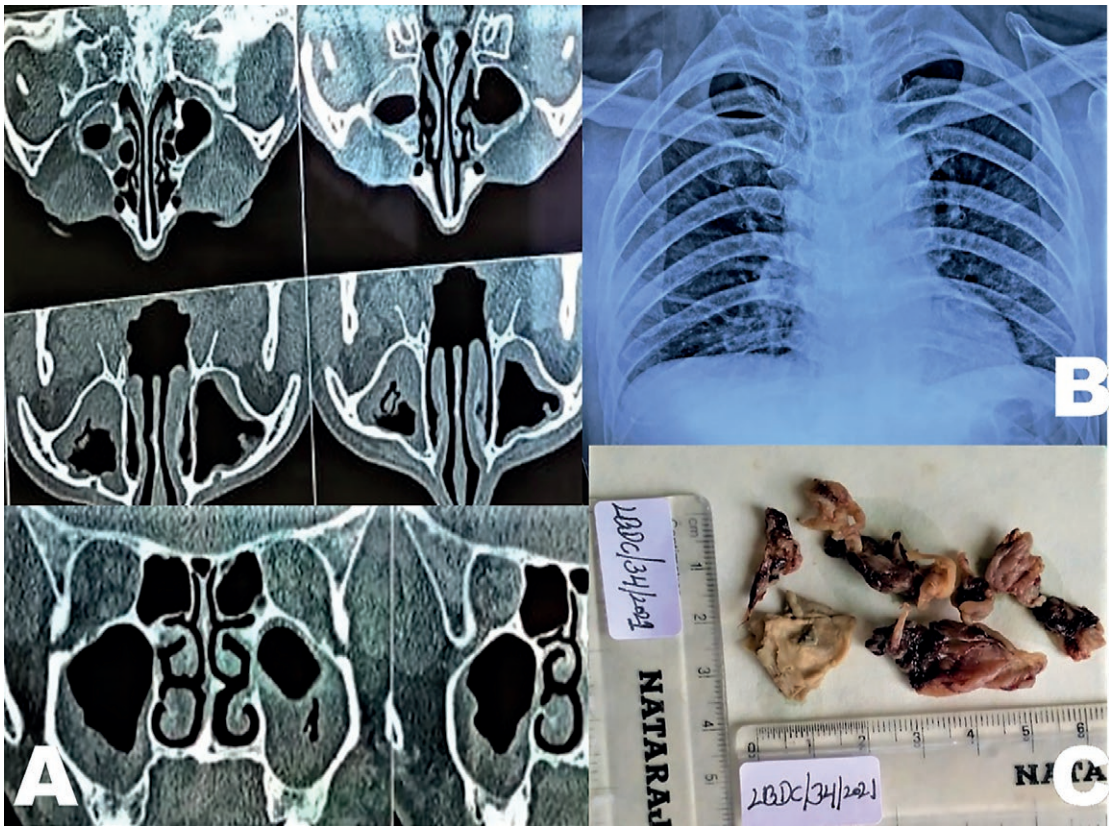

Fig. 1 A) CT scan of paranasal sinus showing soft tissue density mass in maxillary sinus; B) Chest radiograph showing perihilar opacity along with non-homogenous infiltrates in all zones of both lung fields; C) Photograph of gross incisional biopsy mass.

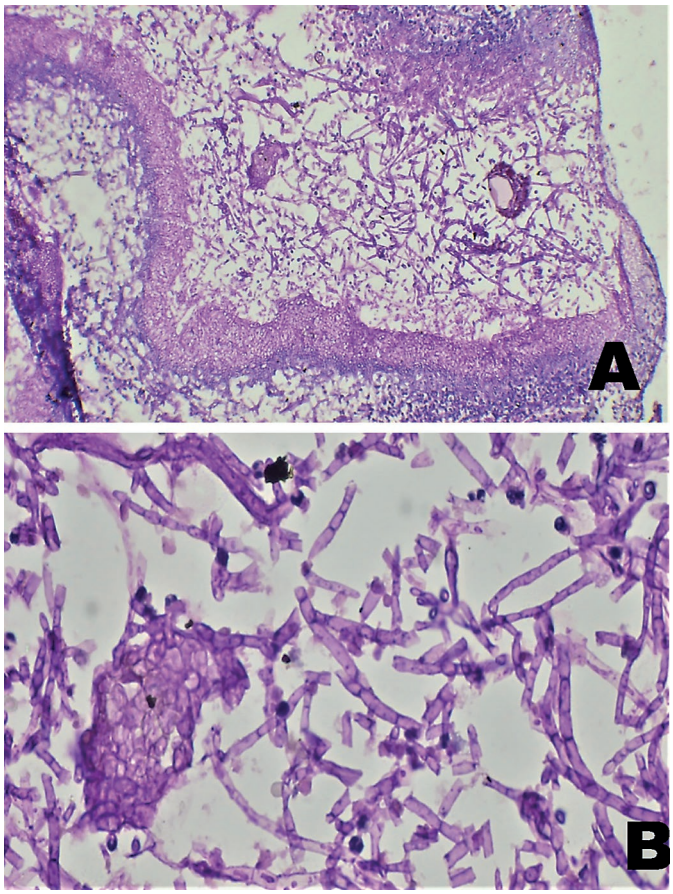

Fig. 2 A) Photomicrograph showing magenta pink coloured septate broad fungal hyphae of an Aspergillus species (PAS stain, 100×); B) (PAS, 400×).

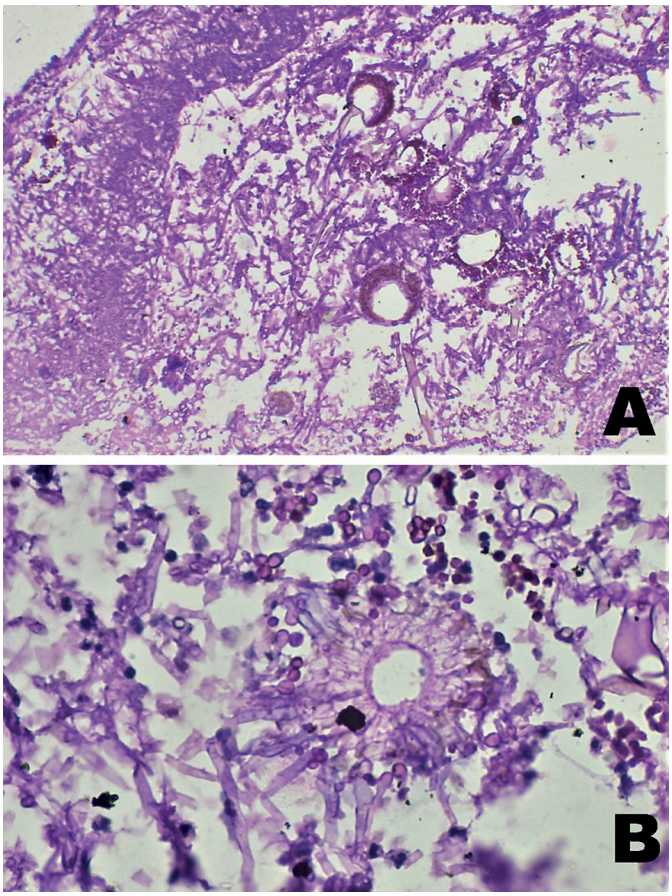

Fig. 3 A) Photomicrograph showing septate branching fungal hyphae along with numerous conidiophores and conidia (PAS stain, 100×); B) The conidial head is composed of a vesicle, which is crowned by one layer of phialides (PAS stain, 400×).

conidial head (fruiting bodies) was composed of a vesicle, which was crowned by one layer of phialides (sterigmata) (Figure 3B), ending in chains of conidias at the extremity. PAS staining also showed few broad aseptate hyphae with branching at 90° resembling mucor (Figure 4), intermingling with numerous narrow septate hyphae. After reviewing clinical, radiographic, and histopathological findings, diagnosis of Maxillary sinusitis secondary to dual fungal infection (predominantly Aspergillus along with Mucormycosis) was made. Sequestrectomy was done for the dead left maxillary dentoalveolar region along with curettage for left maxillary antrum and the tissue was sent for histopathological examination that further confirmed the histopathological diagnosis previously made on incisional biopsy tissue.

Postoperatively, the patient was asked to keep his sugar under control, and intravenous voriconazole was started the day before surgery and continued for 10 days (two doses of 6 mg/kg on day 1, followed by 4 mg/kg twice daily, followed by 200 mg orally twice daily). He was also prescribed Posaconazole (400mg BD × 4 months). After 4 months of follow-up, the patient appeared to be in remission and a CT scan showed no radiological evidence of disease, so any further treatment was interrupted. To this date, the patient is asymptomatic and shows no clinical or radiographic evidence of recurrent disease.

DISCUSSION

Researchers have reported marked immunosuppression resulting from COVID-19 that may give origin to vivid secondary infections either during the disease or even after

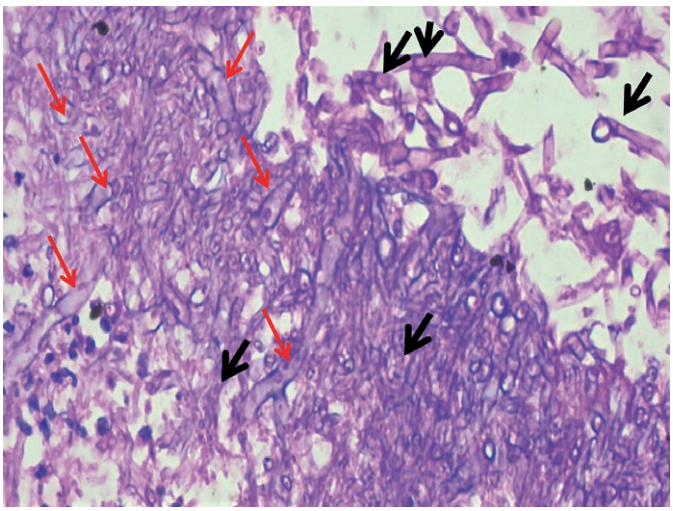

Fig. 4 Photomicrograph showing numerous broad aseptate (Red arrow) Mucor-like hyphae intertwined with narrow septate Aspergillus hyphae (black arrow) (PAS stain, 400×).

the recovery phase (6). Studies on SARS-CoV and SARS-CoV-2 have shown that both viruses belong to the same species and have similar biological and clinical characteristics (7). It has been observed in previous studies that fungal infections were very frequent in SARS patients as a leading cause of death in 25–73.7% of cases (8). These results indicate an alarming sign for clinicians to pay attention to the probability of fungal infections accompanying COVID-19 disease. Recently, a rapid rise in the number of fungal infections has been documented in COVID-19 patients. Mucormycosis, Aspergillosis, and Candidiasis are the most commonly encountered fungal infections in patients admitted to hospitals, in the intensive care units (ICU), and as post-COVID sequelae (2–4).

Mucormycosis is a rare but lethal fungal infection caused by fungi (Mucor, Rhizopus, Lichtheimia, Cunninghamella) belonging to the mucoromycetes family. The fungus is non-pathogenic to immunocompetent individuals. However, in immunocompromised patients, it results in invasive infection (9). The main route of infection is through spore inhalation that results in germination of spores in the nasal cavity and spreads to the PNS, further invading the palate, orbits, and brain, often leading to death. The fungus exhibits a remarkable affinity for arteries, forming thrombi within the blood vessels, resulting in a reduction of blood supply and leading to tissue necrosis (10). In DM patients, the inhibition of binding of iron to transferrin results in elevated iron levels, promoting the growth of mucor hyphae (11). Mucormycosis is classified into six subtypes: rhinocerebrovascular, pulmonary, gastrointestinal, central nervous system (CNS), and disseminated. Symptoms include headache, fever, lethargy, painful eyes, nasal or sinus congestion, ophthalmoplegia, meningoencephalitis, proptosis, facial swelling, partial loss of vision, coughing, shortening of breath, and altered mental status. In the oral cavity, the infection is manifested as necrotizing ulceration of the palate, blackish slough formation and exposure of bone, tenderness over the maxillary sinus area, tooth loss, etc. (12). Imaging aids and histopathology are used to diagnose mucormycosis. Histologically, mucormycosis is characterised by pathognomonic broad, non-septate hyphae with the branching at right angles. Both medication and surgical management strategies are employed in mucormycosis. Amphotericin B (liposomal) is the most commonly used drug. Combined therapy of amphotericin B and Posaconazole has shown synergistic effects against fungal hyphae formation (13).

Aspergillosis is the second most common oral opportunistic fungal infection caused by *Aspergillus fumigatus,* followed by *Aspergillus flavus, Aspergillus niger*, and *Aspergillus terreus* (14). The main routes of infection are through invasion of vascular tissues, leading to thrombosis and infarction. Clinically, this infection represents three subtypes: non-invasive, invasive, and destructive non-invasive. The invasive type is characterised by the invasion of the fungus into tissues, causing slow-progressing and destructive lesions or highly aggressive and lethal lesions. Non-invasive forms evolve as aspergilloma, fungal balls, mycetoma, and allergic sinusitis. Destructive non-invasive types are characterised by local tissue destruction without deep invasion. The destruction caused is due to the toxic and lytic products released by the fungal pathogen, such as phospholipases, proteases, aflatoxin, gliotoxin, and haemolysin and phthioic acid (14). The infection is more common in immunocompromised individuals, such as those with DM, haematological malignancies, bone marrow transplantation, etc. (15). The fungus is mainly an airborne pathogen, invading lungs and bronchopulmonary tissues. It also affects the PNS, larynx, eyes, ears, or oral cavity. Oral aspergillosis is caused by an invasion of the soft tissue lining of the maxillary sinus, which spreads to the oral mucosa and progresses to the underlying bone and palate, resulting in black or yellow necrotic tissue discoloration. Diagnosis of the fungus is made by microbiological culture and histopathological examination. Histologically, the fungus shows septate hyphae branched at an acute angle, 3 to 4 microns in size, invading adjacent small blood vessels. Occlusion of the vessels often results in necrosis (16). On Sabouraud's dextrose agar culture medium, the fungus shows fluffy granular blue-green colonies. The fungal organism exhibits centrifugal linear growth, developing into ball-shaped masses. The centre of the mass contains calcium phosphate, which appears as a foreign body on radiographic examination. Management of invasive aspergillosis of the sinus includes both surgical and medicinal therapy. Systemic administration of amphotericin B, voriconazole, itraconazole, and caspofungin is recommended. Local debridement with amphotericin B is employed as an adjunct treatment modality following surgical treatment (17).

In the present case, clinical and radiological features led to a provisional diagnosis of some fungal infection or any malignancy. To confirm the diagnosis, a histological examination was performed that showed features of fungal infection, predominantly aspergillosis, along with a few hyphae of mucor. A final diagnosis of maxillary sinusitis secondary to dual fungal infection (predominantly Aspergillus along with Mucormycosis) was made. Several cases of pulmonary and rhino-orbital aspergillosis have been reported in COVID-19 patients with DM. But maxillary sinus aspergillosis has been very rarely noticed. The presence of dual infection in a single DM patient as a post-COVID manifestation makes this case unique and intriguing. To our knowledge, this is the first such case reported till date. In 2021, Moorthy et al. in a retrospective study observed that out of 18 COVID-19 patients, only one case showed evidence of mixed fungal infections, i.e., aspergillosis and mucormycosis, but the patient was non-diabetic (4). In 2021, EI-Kohley et al. in a longitudinal prospective study, depicted the presence of such dual infection in 3 out of 36 COVID-19 patients, but all were non-DM (18).

Several factors have been contemplated for the increasing surge of COVID-linked fungal infections in India as compared to other regions of the world. And the same can be hypothesised for the present case also (19).

1. Prolonged use of steroids: Steroids are being implemented as the main line of drug therapy during this pandemic, and it is suggested that they tend to prevent end organ damage by reducing the inflammatory cytokine storm. But, the indiscriminate use of these

anabolic steroids has been shown to cause immunosuppression and a rise in blood sugar levels, providing a medium for the growth of more microbes causing infections.
2. SARS-CoV2 has the potential to damage blood vessels and airway tissues, leading to more infections.
3. It has been observed that during the peak of the second wave, there was an acute shortage of medical grade oxygen. Hence, most hospitals had to shift to industrial oxygen. There is a strong possibility of the use of a contaminated accessory during the inhalation of oxygen by patients.
4. The humidifiers used, i.e., distilled water versus sterile water or even tap water, were also alleged to be a source.
5. There is a high level of iron, in the form of ferritin, among COVID-19 patients, which is favourable for the growth of fungal pathogens.
6. In India, zinc was prescribed as a preventive or therapeutic agent, which is also conducive to fungal growth.
7. Prolonged use of contaminated masks and gloves without changing them leads to infections.

It is well understood that DM patients are more prone to infections. High blood sugar levels act as milieus for microbial growth, dysregulating the glycaemic homeostasis (20). According to recent data, India stands second amongst the top 10 countries in the world, with 77 million people with diabetes and another 36.5 million with prediabetes, which is a high-risk condition for diabetes and cardiovascular disease (4). Diabetic patients are at higher risk of developing COVID-19 disease. Conversely, COVID-19 infection can worsen diabetes control and some treatments used for COVID (e.g., steroids) can exacerbate hyperglycaemia.

In the present case, the patient had a history of DM, COVID-19 infection, and hospitalisation along with widespread use of steroids, monoclonal antibodies, and broad spectrum antibiotics as a part of the armamentarium against COVID-19. All of these factors might have created the perfect storm in which secondary infections took roots and thrived.

CONCLUSION

The rapid rise of fungal infections in the maxillofacial region reported in COVID-19 patients even after recovery signifies an important clinical finding. Multiple factors have been suggested for the co-existence of fungal infections in COVID-19 patients. The present case report of a DM patient with dual infections (Aspergillosis along with mucormycosis) as a post-COVID manifestation adds another important evidence of the increasing number of such cases during this lethal pandemic. Early diagnosis and treatment planning of such lesions are necessary to prevent further complications in both DM and COVID patients.

ABBREVIATIONS

CNS: Central nervous system; COV: Coronavirus; COVID: Coronavirus infectious disease; CT: Computerized tomography; DM: Diabetic mellitus; PAS: Periodic acid shiff; PNS: Paranasal sinus; SARS: Severe acute respiratory syndrome.

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Emergency Ilio-femoral Bypass during Kidney Transplantation due to External Iliac Artery Dissection: Case Report

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ABSTRACT

Intraoperative iliac artery dissection during kidney transplantation is a rare but serious complication that requires prompt intervention. We present a case of right external iliac artery dissection during deceased donor kidney transplantation. A 57-year-old male patient underwent standard pretransplant evaluation and had no signs of either significant aortoiliac occlusive disease or peripheral arterial occlusive disease. Diabetic nephropathy, arterial hypertension and smoking were the underlying causes of the patient's end-stage renal disease. Transplantation was performed in the standard fashion. The kidney was positioned in the right iliac fossa and the venous end to-side anastomosis was performed first. A significant dissection of the right external iliac artery was found on arteriotomy. Immediate ilio-femoral bypass with a vascular prosthesis was performed. During two years of follow-up the kidney function is stable and there are no signs of lower limb vascular insufficiency.

KEYWORDS

kidney transplantation; peripheral arterial disease; vascular grafting

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INTRODUCTION

Kidney transplantation is the best method for treating end-stage renal disease (1), but graft rejection, infections and vascular complications remain major causes of kidney allograft loss. The frequency of vascular complications is relatively low, and arterial complications are more severe than venous. An increased incidence of peripheral arterial occlusive disease (PAOD) is found in patients with chronic renal failure (2), and additional risk factors include smoking, arterial hypertension, and diabetes. PAOD significantly reduces the quality of life since it can lead to acute or chronic lower limb ischemia and patients with PAOD have an increased risk of death due to myocardial infarction or stroke (2). Symptomatic and significant PAOD in a kidney transplantation setting is rare: it occurs in 1.8% of patients eligible for transplantation (3), and is not necessarily a contraindication for kidney transplantation. There are reports of successful kidney transplantations after vascular reconstruction with favorable outcomes (4–6). Dissection of the external iliac artery (EIA) is a rare but serious complication that can occur during kidney transplantation. Combination of atherosclerotic plaque, poor vessel wall quality, clamping injury and suturing of the anastomosis may lead to dissection, which can cause vessel occlusion and graft loss. Arterial dissection during kidney transplantation is an emergency that requires a well-organized interdisciplinary approach to save both the lower extremity and the transplanted kidney.

CASE REPORT

A 57-year-old male patient underwent deceased donor kidney transplantation for end-stage renal disease that was a result of diabetic nephropathy from type 2 diabetes mellitus and arterial hypertension, as well as a 30 pack-year smoking history. Hemodialysis was initiated eighteen months before transplantation, first via arteriovenous fistula (AVF) and later, due to AVF thrombosis, via a temporary intravascular catheter. The patient underwent standard pre-transplant recipient evaluation. This included a kidney, ureter and bladder (KUB) x-ray study that described moderate left side external iliac artery calcifications and lower extremities color Doppler ultrasound which showed hemodynamically insignificant minimal calcifications of the common and external iliac arteries bilaterally. CT angiography in our institution is not routinely performed for every candidate but is indicated only for patients with moderate and severe iliac artery calcifications diagnosed by KUB X-ray. Since the evaluation didn't reveal any contraindications for transplantation, the patient was placed on the Eurotransplant waiting list. The deceased donor allograft was a left kidney with one renal artery, one vein, and a normal ureter.

End-to-side venous anastomosis to the right external iliac vein was performed first. This was followed by the clamping of the right EIA at the best available sites because the artery was moderately atherosclerotic. During the arteriotomy an intimal dissection occurred, but it was immediately recognized, and cold dressings were applied to the kidney. The vascular surgeon was called in to assist with the vascular reconstruction. Further exploration of the AIE revealed a dissection, 5 cm in length, distal to the arteriotomy site, so a right ilio-femoral bypass using an eight-millimeter diameter silver knitted vascular prosthesis (Integard Silver Knitted Straight Graft®, Intervascular, La Ciotata Cedex, France) was done. A renal artery to vascular prosthesis arterial end-to-side anastomosis was then performed. After completion of arterial anastomoses, good pulsations of the vascular prosthesis and renal artery were noted, and femoral and dorsal pedal artery pulsations were palpable. Upon reperfusion, the transplanted kidney regained normal color. The total cold ischemia time was 12 hours and 30 minutes, the bypass anastomosis time 40 minutes and the vascular prosthesis-renal artery anastomosis time 15 minutes. The patient received a standard post-transplant prophylactic low-molecular weight heparin (LMWH) regime during the early postoperative course alongside prophylactic doses of acetylsalicylic acid, which were continued after discharge. Hospitalization was prolonged due to delayed graft function, most likely caused by acute tubular ischemia which was confirmed by renal scintigraphy, followed by fungal pneumonia and recurrent urinary tract infection caused by P. aeruginosa. The patient was discharged after three weeks with good diuresis, a serum creatinine value of 180 ng/µmoL, and normal circulatory status of the right leg.

Eight months after transplantation the patient reported intermittent claudication of the right leg with a walking distance of 200 meters. A stenosis of the right superficial femoral artery was diagnosed on angiography, and successfully treated by percutaneous balloon angioplasty. The patient was discharged with a recommendation for long term LMWH therapy. During a two-year follow up period after kidney transplantation, the kidney function is normal with stable serum creatinine values. There are no signs of lower limb circulatory insufficiency.

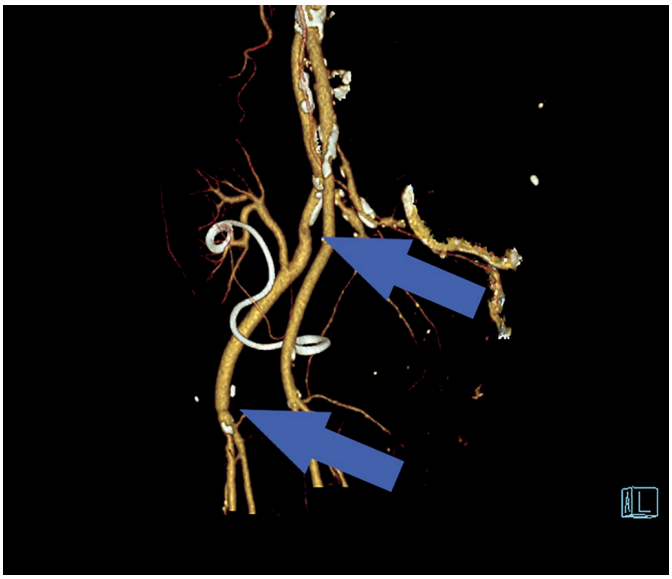

Fig. 1 CT arteriography showing ilio-femoral bypass (blue arrows) with normal allograft perfusion.

DISCUSSION

In general, kidney transplantation procedure involves arterial clamping which causes minor trauma to the vessel and in most cases does not lead to serious consequences. Rarely, combined with PAOD, it can result in iliac artery dissection, an emergency event that may lead to acute lower limb ischemia and, if the arterial anastomosis of the kidney has already been carried out, allograft hypoperfusion or acute thrombosis caused by arterial occlusion due to intimal flap. The most probable cause of the dissection in our case is the cascade facilitated by atherosclerosis: the clamping of the stiffened vessel initiates the separation of the intima that with arteriotomy progresses to splitting and dissection. The dissection of the EIA can occur during and after the procedure (7). In most reported cases it is diagnosed intraoperatively and is usually noticed because of the mottled and poorly perfused graft appearance after completion of the renal artery anastomosis and removal of vascular clamps, along with loss of distal arterial pulses (7). This situation requires resection of the arterial anastomosis, after which a vascular reconstruction can be performed. Uniquely in our case, the dissection developed at the beginning of the procedure, which allowed for immediate visualization of the extent of dissection and rapid intervention. Despite the relatively short dissection, we selected the bypass over endarterectomy because the allograft artery anastomosis needs to have a minimal risk of further vascular complications, such as thrombosis, which is greater with endarterectomy than bypass. After the bypass was in place, an end-to-side renal artery to prosthetic graft anastomosis was performed resulting with adequate perfusion for both the lower limb and the allograft. There are only 14 literature reports of intraoperative iliac artery dissection during kidney and/or pancreas transplantation procedure (7–14). The most used reconstruction technique is synthetic vascular graft (seven cases) (9, 11, 12), while other methods are implemented less frequently: donor iliac artery graft (four cases) (13), endarterectomy (one case) (7), endarterectomy followed by subsequent endovascular stenting (one case) (14) and recipient saphenous vein graft (one case) (10). The choice of vascular reconstruction depends on the extent of the dissection, time of diagnosis, the possibility of renal artery anastomosis, surgeon preference and experience, and technology/material availability. In our case, the dissection occurred, and was noted, intraoperatively, but it can also develop later in the postoperative period and present a diagnostic challenge. Once diagnosed, usually with CT angiography, immediate intervention/reconstruction is mandatory. In this setting an alternative intervention to surgery is minimally invasive endovascular stenting (15), if feasible without jeopardizing the renal artery anastomosis. As in most reported cases, the bypass in our case was performed with a synthetic vascular prosthesis, and this has proven to be a good choice because synthetic grafts in this location have satisfactory long-term patency. Reported short term transplant and limb revascularization outcomes are excellent with reports of 100% 1-year kidney survival rate without major vascular issues (7–14). The longest follow-up of a patients with simultaneous pancreas and kidney transplant is 45 months, reported by Moon et al. (13).

CONCLUSION

Acute EIA dissection, a rare, but serious intraoperative vascular complication, can be successfully managed with prosthetic vascular bypass grafting and renal artery to bypass-graft anastomosis with favorable short- and mid-term allograft and lower limb survival outcomes.

AUTHOR'S NOTE

The Institutional Ethical Committee approval for this case report was obtained and the patient provided signed informed consent for publication of the case report and accompanying images.

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