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Original Article

EFFECT OF A NEW XENOGRAFT MATERIAL IN MANDIBULAR POST-EXTRACTION SITES: A CASE SERIES

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ABSTRACT

The alveolus bone is a tooth-dependent tissue. The extraction of the dental element determines the resorption of the alveolar crest, which trophism is linked to the presence of the periodontal ligament. Several materials have been used to improve alveolar bone healing and maintain alveolar ridge. The aim of the study is to evaluate the effects of a new matrix of bovine bone processed at low temperature in association with a membrane of the bovine pericardium in post-extraction sites using histological analysis comparing treated and untreated alveoli. Five patients with non-recoverable teeth were enrolled in the present study for teeth extraction. In treated sites, the alveolus was packed with Decellularized and Antigen-free Bovine Bone (RE-BONE® Ubgen, Padova, Italy) and subsequently covered with a bovine-derived pericardium membrane (SHELTER® FAST Ubgen, Padova, Italy). Four alveoli of two patients were left to heal spontaneously as control sites. The tissue sampling was performed during the implant site preparation four months after extraction. Specimens were decalcified, and sections were stained with hematoxylin and eosin. Bone histomorphometry of regeneration tissues from treated sites showed an average increase of 2.9% in bone tissue. However, no statistically significant differences can be detected since standard deviations are very high. Generally, the alveolar preservation technique is a valuable method to guarantee alveolar volume stability. The material studied here showed a slight increase in bone production after 4 months from a tooth extraction in treated sites, which is an expression of a good healing process. However, since the limited number of cases analyzed, additional studies are needed to verify the bone gain in alveolar bone healing.

KEYWORDS: *bone, graft, alveolus, mandible, lower jaw*

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INTRODUCTION

The alveolus bone is a tooth-dependent tissue. The extraction of the dental element determines the resorption of the alveolar crest, which trophism is linked to the presence of the periodontal ligament. Tooth extraction leads to a reduction of alveolar ridge (1-4).

Bone resorption is most evident in sites where the thickness of the cortical bone is thin (5) or where the root anatomy of the teeth is more prominent in the vestibular sense (6, 7). Furthermore, human and animal studies showed that most of the tissue lost in the initial phase occurred in the coronal part, while the apical part was less affected. In untreated sites, continuous remodelling occurs over time, and a significant variability in ridge resorption between subject/site exists (8, 9). The studies of Pietrokovski & Massler (2) and Schropp et al. (3) performed on plaster models in which one tooth was extracted on one side while the contralateral tooth maintained a more than double resorption in the vestibular compared to the lingual part. Authors reported a bone loss of 30% at 3 months and 50% after 12 months (with a mean > 6mm), while the mesial and distal parts underwent reduced resorbing.

Animal models were used to study graft materials to counteract ridge remodelling following extraction. In these studies, biomaterial was inserted in post-extractive sockets (10).

These studies failed to demonstrate that the biomaterials entirely prevent the resorption of the buccal wall and the remodelling of the ridge in a general sense but showed that their use, under certain conditions, significantly reduced alveolar crest resorption (8-11). Alveolar Socket Preservation (ASP) is defined as "any procedure undertaken at the time or following the extraction, aimed at minimizing the external resorption of the ridge and maximizing the formation of bone within the alveolus" (12).

Since a new biomaterial has been recently introduced in the marker (13-18), we decided to evaluate the healing process of the post-extractive socket after 4 months by inserting a new matrix of bovine bone processed at low temperature and covered with a membrane in the bovine pericardium.

MATERIAL AND METHODS

Patients were enrolled based on the following criteria: non-recoverable teeth due to destructive caries, traumatic events (i.e., vertical root fracture), endodontic treatments (i.e., teeth no longer retractable). Five test sites and four control sites were evaluated for the study. Three female patients and two males, mean age of 61 years, were enrolled for test sites. The five test alveoli were one premolar and four molars. Two male patients, mean age of 52.5 years, were enrolled as controls. One premolar and three molars were investigated.

Exclusion criteria were as follows: patients smoking more than 10 cigarettes/day, pregnant women, patients with chronic diseases (diabetes, orofacial neoplasms, etc.), bisphosphonate therapy, or with an acute infection in progress, untreated periodontitis, autoimmune diseases, allergies to one or more materials, drugs used during treatment, alcohol and/or drugs intake.

Surgical procedure

During the extraction, an attempt was made to lift the flaps in the least invasive way possible to preserve the alveolus from further resorption due to surgical exposure. The alveolus was packed with Decellularized and Antigen-free Bovine Bone (RE-BONE® Ubgen, Padova, Italy) and subsequently covered with a bovine-derived pericardium membrane (SHELTER® FAST Ubgen, Padova, Italy). The same surgical procedure was used for the control sites, but no biomaterial was grafted, and the alveoli were left to heal spontaneously.

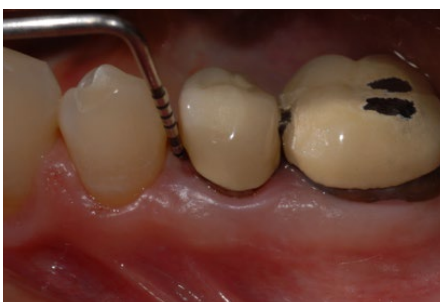

Fig. 1. *Pre-surgical image*

Compression sutures were performed in monofilament in e-PTFE (Gore-Tex®), removed after 10 days, anti-inflammatory therapy with Nimesulide was prescribed as well as soft and cold diet for at least 2/3 days, ice packs for few hours were delivered as well as rinses with Chlorhexidine 2/3 times a day for 15/20 days. Monthly checks were carried out until the fourth month, when bone sampling was scheduled.

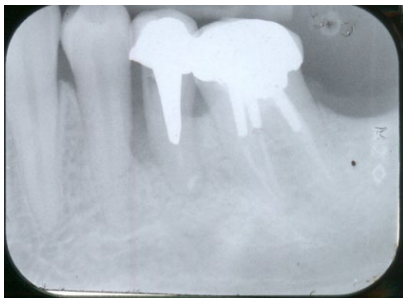

Fig. 2. *Pre-surgical radiograph*

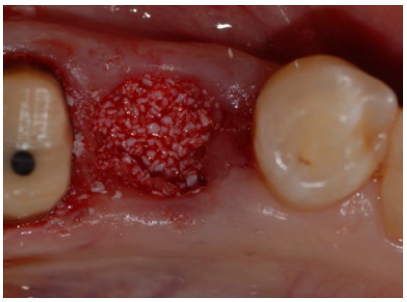

Fig. 3. *Socket preservation I*

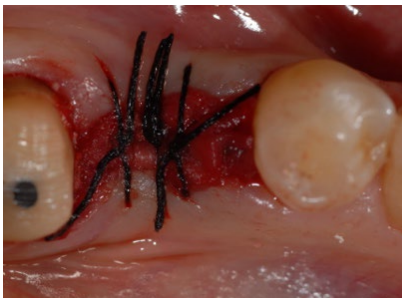

Fig. 4. *Socket preservation II*

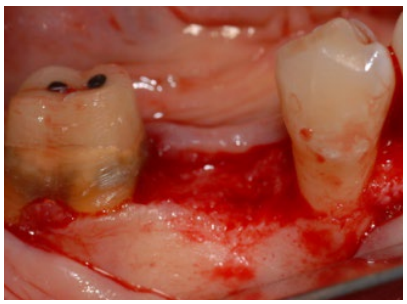

Fig. 5. *Re-entry after 4 months for implant insertion*

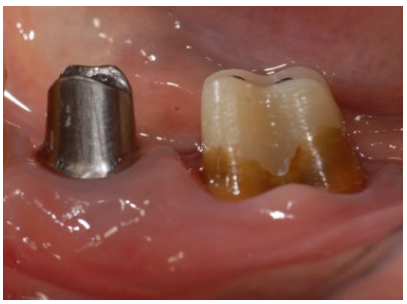

Fig. 6. *Abutment*

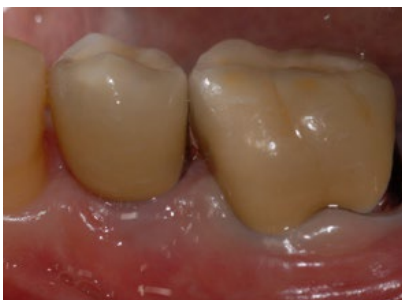

Fig. 7. *Prosthetic restoration*

The tissue sampling was performed during the preparation of the implant site, using a 2 mm core drill for a depth between 2 to 3 mm. The bone samples were placed in sterile and labelled blisters and immersed in formalin 10% (Merck, Darmstadt, Germany) and subsequently sent to the laboratory for histological evaluations (Fig. 1-8).

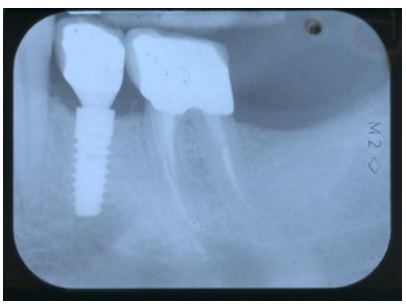

Fig. 8. *Radiographic check*

Histological analysis

The bone samples were decalcified with Osteosoft® and subsequently embedded in paraffin. A microtome (RM2025 Leica Instruments, Nussloch, Germany) was used to obtain a 5 µm thick section. These paraffin sections, collected on a microscope slide, were deparaffinated, rehydrated, and stained with haematoxylin and eosin. After staining, the sections were dehydrated in alcohol, cleared in xylene, and then preserved using a suitable mounting medium for morphological observations. All reagents were obtained from Merck (Darmstadt, Germany).

For the subsequent analysis, slides were scanned using an APERIO ScanScope slide scanner (Leica Biosystems, Buccinasco-MIlano, Italy), obtaining an image file with .svs (ScanScope Virtual Slide) format for every sample. Finally, the .svs files were viewed and analyzed using a free software program called ImageScope.

RESULTS

The tissue samples (obtained by a core drill during implant site preparation) were decalcified, and sections were stained with hematoxylin and eosin. The analysis by ImageScope software of the scanned histology slides quantified the length of the sample, the total area, the percentage of bone and fibrous tissue, and, when present, the area with residues of bovine-derived pericardium membranes.

Fig. 9 shows control and test samples with the respective magnifications of connective and bone tissues. On the left are the areas limited by red and green lines, which correspond to the total sample' area and connective tissue area. The

bone area is derived from total and connective tissue areas. The length of the sample, the total area, and the percentage of bone and connective tissues in the scanned histology slides were quantified by ImageScope software.

Table I summarized histomorphometric results showing an average increase of 2.9% in the bone area in the treated samples compared to the controls. However, no statistically significant differences can be detected since standard deviations are very high.

DISCUSSION

Healing in a post-extraction socket occurs through a series of events, including clot formation and maturation, matrix

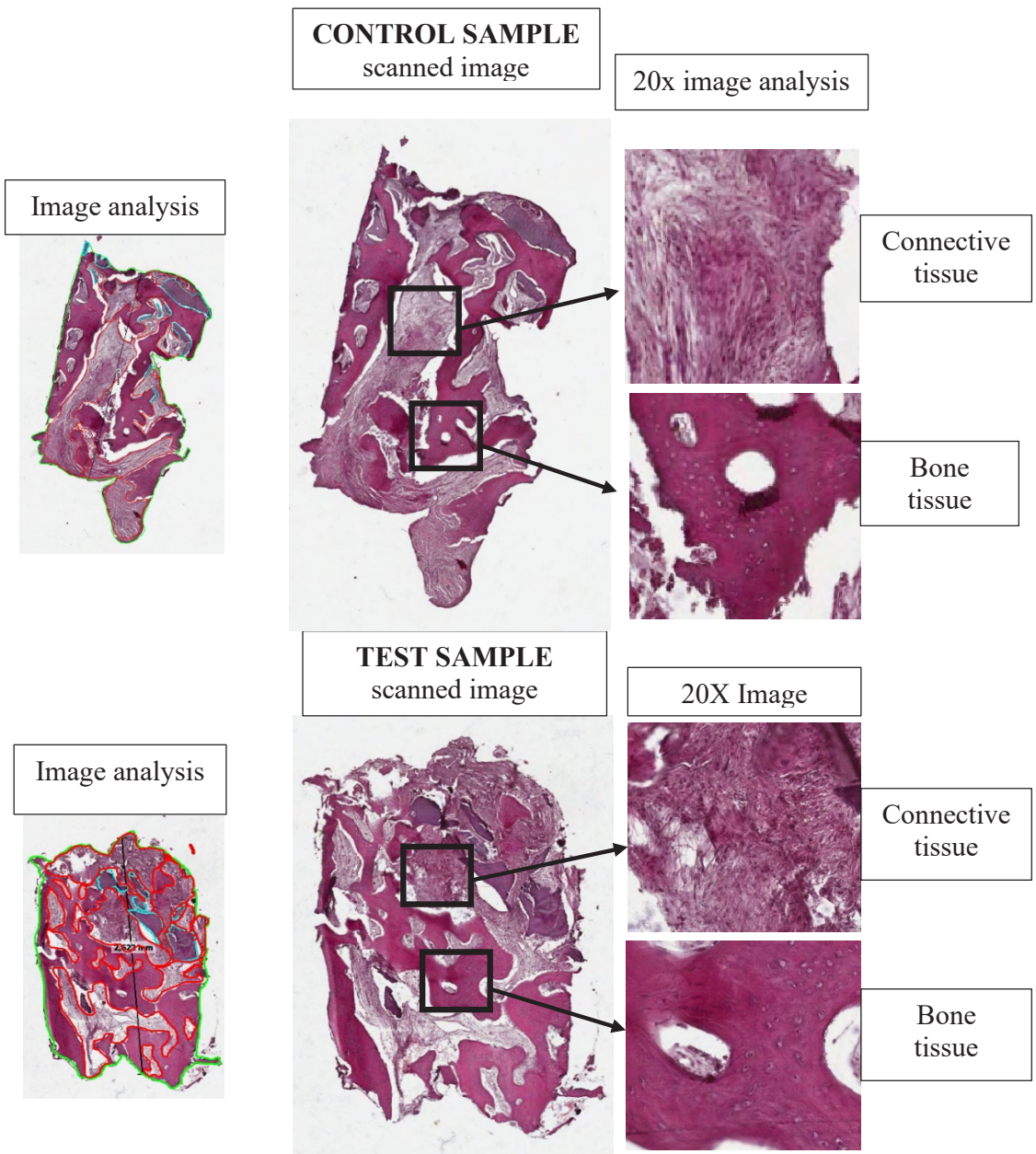

Fig. 9. *Images of a control sample and a test sample and the respective magnifications*

Table I. *Average values obtained from the analysis.*

	Average values					
	Age	Length of samples	Analyzed area	% bone	% connective	% shelter® residues
Control samples	52.5	2.2 (±1.1)	3.1 (± 1,1)	59.6 (± 5.8)	35 (± 3.4)	5.4 (± 2.8)
Test samples	60.8	2.3 (±0.3)	2.7 (± 0.8)	62.5 (± 18.1)	31.8 (± 31,8)	5.7 (± 9.9)

deposition and mineralization. Usually, the residual ridge decreases by 15% at six months, both vertically and horizontally (19). This dimensional change can lead to aesthetic and functional disadvantages for the subsequent placement of the implant since an adequate residual ridge width is one of the main prerogatives for the long-term success of prosthetically guided implant therapy (19).

Biomaterials and/or biological agents such as autologous bone, bioactive glasses, hydroxyapatite, human-derived bone (allografts), and especially animal-derived bone (xenografts) were used and analyzed to counteract the alveolar ridge resorption and make the site available for the insertion of an implant (20, 21). It has been shown that these biomaterials could be embedded in a newly formed bone, kept as inactive fillers, or reabsorbed by the host tissue during its natural remodelling course (22).

Although the ability of biomaterials to decrease resorption and preserve adequate edentulous ridge volumes has been extensively documented in the literature, the quality of grafted tissue has not yet been widely understood.

A De Risi et al. (21) meta-analysis showed that no histological differences exist between the different procedures compared to spontaneous healing. The highest percentage values of regenerated bone at 3 months came from procedures using allografts (54.4%), while the lowest at 5 months were those using xenografts (23.6%). Regarding the presence of connective tissue in the grafted sites, the highest value at 7 months was referred to allografts (67%), and the lowest to the alveoli treated with alloplast (27%). As for the residual biomaterial, the lowest percentages were attributable to sites with allografts (12.4 - 21.11%), while those with xenografts and alloplast showed better results at 7 months (37.14 - 37.23%) (21).

In our report, although a slight increase in bone formation was detected in treated alveoli (2.9%), no statistically significant differences were obtained due to the great standard deviation value. This fact is probably related to the small sample size.

In the literature, no differences were highlighted regarding the superiority of an alveolar preservation technique over others (i.e., GBR, site filling, site sealing) regarding the three-dimensional preservation of the site, bone formation, amount of keratinized tissue and complications (20-23).

In a systematic review, Chan et al. (24) analyzed the proportion between bone and connective tissue in grafted and untreated alveoli. They found that in ungrafted sites, the percentage of vital bone and connective tissue was 38.5% ± 13.4% and 58.3% ± 10.6%, respectively.

Chan et al. (24), reported that four studies investigating the effect of xenografts gave the most contrasting results: the presence of vital bone ranged from - 22% (decreased) to + 9.8% (increased), instead alloplastic grafts increased the amount of vital bone from 6.2% to 23.5%. Furthermore, many residual biomaterials were noted when hydroxyapatite and xenografts were used, ranging from 15% to 36% of the healed alveolus.

Using grafting materials for ASP might change the proportion of vital bone compared to sockets allowed to heal without grafting. In 2020 Koo et al. (25) compared two xenografts, one of bovine and one of porcine derivation. Histology was comparable in the percentages of newly formed bone, residual connective tissue and residual graft particles at 4 months.

CONCLUSION

In conclusion, the English literature shows that alveolar preservation techniques provide acceptable tissue volumes for implant therapy, but bone quality does not show significant differences between the various biomaterials with respect to spontaneous healing. In our report, although a slight increase in bone formation was detected in treated alveoli (2.9%), no statistically significant differences were obtained due to the great standard deviation value. This fact is probably related to the small sample size, so additional studies are needed.

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Article

IN-VITRO EVALUATION OF DIFFERENT POLISHING METHODS AFTER BRACKET DEBONDING

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ABSTRACT

This study evaluated the performance of two different polishing methods on the enamel surface roughness of teeth following adhesive removal with a tungsten carbide bur. To this end, we examined 45 premolar teeth, randomly divided into three groups (C, SLD, and BG) with 15 teeth per group. To experiment, we attached stainless steel brackets to all three groups and later removed the brackets with debonding pliers. Afterwards, the composite resin remnant on the enamel surface was removed with a composite finishing tungsten carbide bur in all three groups. Group C did not receive any polishing after adhesive removal. Group SLD and BG underwent a polishing process using Sof-Lex discs (SLD) and Brownie-Greenie (BG), respectively. Subsequently, the areal enamel surface roughness parameters were analysed using the Keyence VK-X100 laser scanning microscope (LSM) at 10x, 20x, and 50x magnification. The results demonstrate significantly less surface roughness following both polishing methods compared to the control group. Furthermore, group SLD showed significantly less surface roughness compared to BG ($p<0.01$).

KEYWORDS: *bonding, dental adhesives, orthodontic brackets, dental resins, surface roughness*

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INTRODUCTION

Currently, the use of fixed appliances for orthodontic tooth correction is the most common treatment method. This method requires bonding and subsequent debonding of the brackets (1–3). The composite remnants are then removed with dental burs (i.e., tungsten carbide burs, diamond burs, composite burs, or stainless-steel burs). The debonding process increases the enamel surface roughness of the teeth and the cracks and removes the outermost fluoride-rich enamel layer that may cause damage to the teeth. In addition, resulting surface roughness, caused by resin removal using dental burs, increases accumulative dental plaque biofilms and food residue attachment.

Consequently, this increases the cariogenicity and risk of white spot lesion formation. Polishing the tooth surface after debonding can reduce these damages (4-8). Therefore, various polishing methods have been suggested, including polishing with Sof-Lex discs (SLD), Sof-Lex spiral wheels polishing brushes, and Brownie-Greenie (7, 9, 10). However, no consensus exists on the most suitable polishing method. There is also a lack of comparative studies regarding surface roughness following the use of SLD and BG.

Furthermore, studies have used different methods of measurement, such as scanning electron microscope (SEM), profilometer, and micro-computed tomography, which are mostly contact type or destructive type methods, making before and after comparisons impossible (11–17). Therefore, this research aims to compare two polishing methods of SLD and BG using a laser scanning microscope (LSCM).

MATERIALS AND METHODS

The present in vitro study was conducted with 45 extracted intact premolar teeth. The exclusion criteria included visible crack lines, caries, restorations, dental anomalies, and a history of previous orthodontic treatments.

The teeth were cleansed from visible blood, food, and tissue debris. They were then cleansed utilising a polishing brush (Nylon brush, Komet Dental, Germany), polishing paste (Super Polish, Kerr Dental, Switzerland), and dried with a mild oil-free air stream. They were then stored in a physiological saline solution at room temperature.

Subsequently, the enamel surfaces of the teeth were etched with a 35% phosphoric acid solution (Ultra-Etch etchant, Ultradent Products, Inc., USA). Afterwards, all tooth surfaces were carefully cleaned and rinsed with water spray for 60 seconds. Following the enamel conditioning procedure, iBond (iBond Self Etch, Heraeus Kulzer, Germany) was applied to the tooth surfaces.

The prepared surfaces were cured by light using a Mini LED (Acteon Groups, Satelec, France) with light for 20 seconds. The light-curing nanohybrid composite Grandio SO (VocoGmbH, Germany) was applied to the back of metal edgewise premolar brackets (Silver Motion, World Class Orthodontics, Ortho Organisers GmbH, Germany), and the prepared brackets were then placed on the teeth. The excess composite was removed with an explorer, and the Mini LED was used to cure the composite for 40 seconds (10 seconds each for the mesial, distal, occlusal, and gingival surfaces).

Afterwards, the brackets were debonded with an angulated bracket removing plier (Hu-Friedy, Chicago, USA). Next, the adhesive remnants were removed with a 30-blade ultra-fine tungsten carbide finishing bur with a low-speed contra-angle handpiece (Gentlepower Lux 20 LP 1:1, Kavo Dental, Germany), operating at up to 20,000 rpm, using water coolant for 35 seconds per tooth. Subsequently, teeth were randomly divided into three subgroups (15 teeth per group): group SLD, group BG, and group C as the control group.

The teeth in group C did not receive any polishing treatment. The teeth in group SLD were first polished with Sof-Lex discs (Pop-On, 3M ESPE, USA) for 15 seconds and afterwards with a polishing brush (Komet Dental, Brasseler, Germany) for 7 seconds. Polishing in group BG was performed using Brownie (Shofu Dental, Kyoto, Japan) and Greenie (G Shofu Dental, Kyoto, Japan) for 10 seconds per tooth.

The teeth were then embedded into a silicone key (Silagum Putty, DMG, Germany), and their surface roughness was measured with a VK-X100 3D laser scanning microscope (Keyence, Japan). The samples were placed on the rotating stage, and the laser beam measurements were made in the same level longitudinal slices of the samples. We could then analyse the surface roughness using the VK application by using autofocus to achieve optimal sharpness of the images. The results of this study demonstrated strong inter-rater reliability based on the Intra-Class Correlation Coefficient (ICC) of> 0.9999 and a p-value <0.01.

Data analysis and visualisation were carried out using the statistical program BiAS (Epsilon Verlag, Germany). The areal roughness parameters [3D surface roughness (texture) parameters] were used to compare the quality of the two polishing methods and their differences with the untreated tooth surface. In addition, the arithmetical mean height (Sa), maximum peak height (Sp), maximum pit height (Sv), maximum height (Sz), and surface texture (Str) parameters were considered for all surface measurements for three groups with the conditions in order to analyse the surface roughness. During this research, all operators wore surgical masks to prevent the spread of the respiratory system virus (18) and maintain office hygiene (19, 20).

RESULTS

The Sa parameter is used to determine surface roughness and represents the differences between the height of each point and the arithmetical mean of the surface (21). Factors such as diet, differences in the tooth surface, enamel surface porosity, and iatrogenic damage to the enamel surface during tooth extraction cause changes to the surface roughness and Sa value. The Sa was measured on five teeth with a magnification of 10x, 20x, and 50x.

Although the first and second observations were made on the same tooth at 50x magnification, a difference was observed in the Sa values due to the different adjusted examination fields set under the bracket. Thus, with deviations of up to 0.273 µm, we used the average value for Sa. To determine the inter-rater reliability, we selected two data sets with five teeth in each data set; each tooth was measured three times. During refocusing of the first data set, among the five teeth measured, the maximum difference in the Sa value was 0.002 µm, while the maximum difference during repositioning was 0.004 µm. The ICC obtained in refocusing the first test data set was 0.999962 (p-value <0.01), while the ICC for repositioning was 0.999921. In the second data set, the maximum difference in Sa during refocusing was 0,001µm with an ICC = 0.999981, while the maximum difference for repositioning was 0.003 µm with an ICC = 0.999932. For each sample in the second data set, repositioning and refocusing had a p-value of <0.01. The maximum difference of both test subjects for refocusing was 0.002 µm, and for repositioning was 0.003 µm (ICC= 0.999955 and p-value<0.01).

The comparison of Sa values between the groups SLD and BG showed significant differences in the surface roughness with $p<0.01$, whilst between groups BG and C (control group) there was a major difference with $p<0.01$, the difference between groups SLD and C was $p<0.05$. One of the causes for the differences between the SLD and BG polishing methods may be the rough properties of the BG instruments. Even though polishing with SLD highly reduced the roughness, it did not eliminate the entire microscopic surface grooves. None of the SLD and BG polishing methods was able to remove all the remaining composite residues in some areas of the enamel surface, resulting in the increased roughness of the enamel surface (Fig. 1-3).

Thus, roughness plaque accumulation may occur in all methods due to the increased enamel surface. However, it is possible to manage plaque accumulation following the SLD polishing method with adequate oral hygiene. Nevertheless, while the SLD method showed better results than the group BG, both polishing methods significantly improved the enamel roughness compared to the control group C.

Sp describes the highest point of the defined area from the enamel surface (21). Therefore, the Sp value shows that the

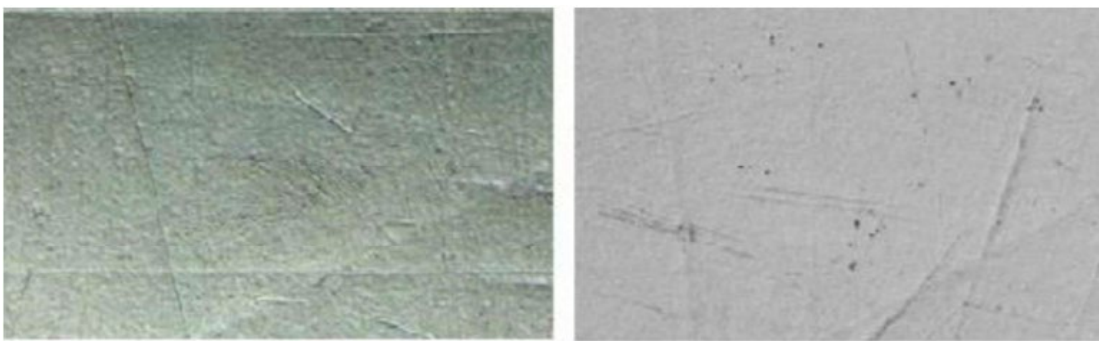

Fig. 1. *Electron microscopic images of enamel surface of the natural teeth, showing minimal scratching under 20x magnification.*

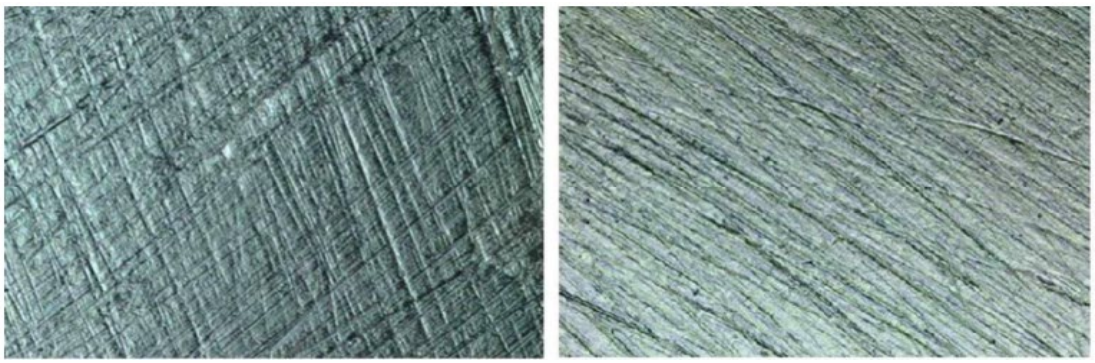

Fig. 2. *Electron microscopic images of enamel surface after adhesive remnant removal with SLD method, showing deep cracks and extensive scratching under 20x magnification.*

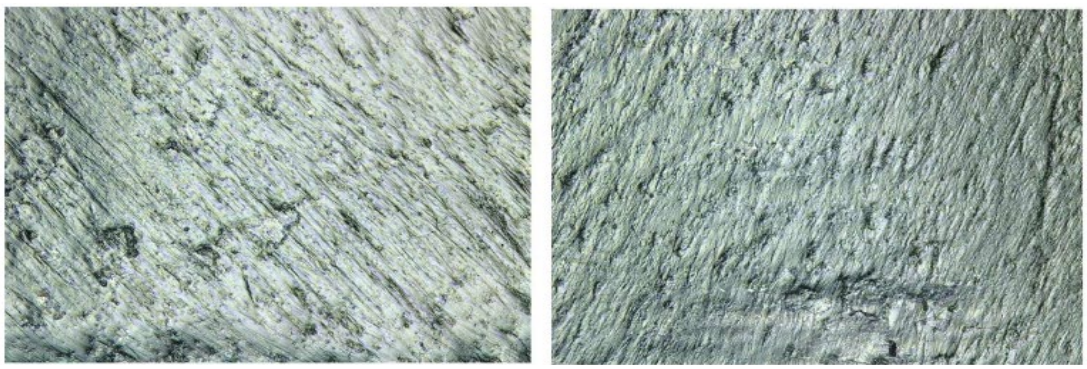

Fig. 3. *Electron microscopic images of enamel surface after adhesive remnant removal with BG method, showing spotting and dents under 20x magnification.*

roughness levels of groups SLD and BG are not significantly different ($p>0.05$). Furthermore, the Sp value for group C (control group) was lower than groups SLD ($p>0.05$) and BG ($p<0.01$).

Sv expresses the deepness of the pits from the enamel surface (21), and the value differs significantly in group BG ($p<0.01$). There was also a significant difference between groups SLD and C ($p<0.05$) for Sv. Due to the BG method's inability to smooth the pits and grooves on the surface evenly, the observed grooves and pits were caused mainly by mechanical damage during the treatment and polishing processes.

Sz is the sum of the Sp and Sv values within the defined area and it presents the value of the surface irregularity (21). As expected from the Sp and Sv results for group BG, this method has the maximum Sz value ($p<0.01$). In addition, a significant difference between groups SLD and C ($p<0.05$) for Sz was also observed.

Str explains the isotropy and anisotropy of the surface texture (21). When the Str values are near zero, this shows high texture uniformity. Contrarily, when the Str values are closer to one, this indicates a low texture uniformity. Group SLD had the lowest Str values in contrast to groups BG, and C. Group C did not show any significant differences from the other groups ($p>0.05$). Thus, group SLD showed the most recurring patterns on the surface.

DISCUSSION

We aimed to compare the effects of two adhesive remnant removal methods in an in vitro experiment using LSCM. The topic of adhesive remnant removal after bracket debonding remains relevant today since no consensus on the most suitable method of adhesive removal exists.

Similar studies have shown an increase in surface roughness regardless method used. A single study, however, has shown a decrease in the roughness after adhesive removal, which could be due to the operator's skills (22). Similar to other studies, we also showed a higher surface roughness after polishing with SLD and BG compared to the natural teeth surface. However, SLD seems to be the superior method, as it shows less surface roughness compared to BG. Other studies have adopted different methods of surface roughness evaluations. The methods include scanning electron microscopy (SEM) (17, 23-25), contact profilometry (26), or atomic force microscopy (27). These methods are destructive and abrasive, thus, making before and after comparisons impossible. They are also subjective assessments and do not provide the possibility of quantitative objective evaluations, thus creating bias in the results of these studies. LSCM, on the other hand, is a non-destructive method that makes quantitative measurements possible. Therefore, the risk of bias in our measurements is lower than in other studies.

CONCLUSION

Group SLD and BG differ significantly concerning their Sa values ($p<0.01$). The comparison of group C with group SLD revealed a significant difference for Sa ($p<0.05$), whilst group BG showed a highly significant difference ($p<0.01$) compared to group C. Under objective evaluation, the SLD method achieved superior polishing results, yielding a less rough surface than the BG method. This investigation also revealed a significant difference in surface roughness for both polishing groups compared to untreated teeth. Nevertheless, treated and polished teeth using SLD and a polishing brush can show a similar surface roughness to some untreated teeth.

Competing interests

The authors declare that they have no financial or non-financial competing interests.

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Case report

LOW BACK PAIN: ALWAYS A NEUROLOGICAL PROBLEM? A CASE OF LERICHE SYNDROME IN A WOMAN

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ABSTRACT

Leriche syndrome is defined as a rare obliteration of the aortic bifurcation. It is characterized by a typical triad in male patients: claudication, erectile dysfunction and decreased distal pulses. The leading cause of this syndrome is atherosclerosis. Possible differential diagnoses include vascular and neurological diseases. We report a case of a 54-year-old woman whose main complaint was low back pain. The initial (wrong) diagnosis was spinal disc herniation, causing neuropathy. The use of computerized tomography led to the correct diagnosis: aortoiliac occlusive disease (Leriche Syndrome).

KEYWORDS: *Leriche syndrome, atherosclerosis, aortoiliac occlusive disease, arteriopathy, low back pain, neuropathic pain*

INTRODUCTION

Leriche syndrome, also known as Leriche disease or carrefour disease or aortoiliac occlusive disease, is a condition that affects the blood supply to the lower extremities, typically the legs. It is defined as an obstructive chronic peripheric arteriopathy. The obstruction is usually found at the level of the iliac bifurcation (1). It is a rare pathology; its incidence is 1 in 12000 patients with aortic occlusions in an autopsy study (2).

It is caused by the occlusion or blockage of the main blood vessels leading to the legs, including the aorta and iliac arteries. This results in the typical symptomatology in male patients: claudication, leg pain, impotence, decreased pulse in the legs and lower extremities pallor. In severe cases, it can cause gangrene, needing amputation (3). However, sexual

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dysfunction such as hypoactive sexual desire disorder, dyspareunia and vaginismus can also affect women; this is due to the obstruction of blood flow due to atherosclerosis involving the iliac vessels causes a reduction in vascular flow at the vulval, vaginal and clitoral levels. Another explanation for reduced sexual function in women with Leriche Syndrome is ischemic damage of the pudendal nerve responsible for the innervation of the genital organs (4). Again, the incidence is higher among male individuals. However, this disease may also interest women, even if at a more advanced age compared to men (5, 6).

This syndrome was described for the first time by Robert Graham in 1914. However, in 1940, French surgeon René Leriche (Fig. 1) described a syndrome of thrombotic obliteration of the aortic bifurcation. He also described the typical triad of symptoms: claudication, erectile dysfunction and decreased distal pulses (7, 8). The first cause of this pathology is the deposition of atherosclerotic plaque in the aortic bifurcation; rare causes may be the presence of emboli and vasculitis (9). Like all arteriopathies, Leriche syndrome also has other risk factors: hypertension, diabetes mellitus, dyslipidemia, coronary artery disease, atrial fibrillation, primary anti-phospholipid antibody syndrome, hemodialysis and smoking (10, 11).

The differential diagnosis includes other vascular diseases such as abdominal aortic dissection or neurological pathologies such as peripheral neuropathy, vertebral stenosis, spinal disc herniation and Guillain-Barré syndrome (12). Diagnosis is based on symptoms, measuring the ankle-brachial index and imaging. In particular, colour doppler ultrasonography generally shows a reduction or absence of flow in the lower limbs. However, computed tomographic angiography is considered the gold standard for diagnosis (13). The most modern techniques allow the identification of the atheromatous plaque (14).

In the past, the first choice of treatment for the aortoiliac disease was aortofemoral bypass surgery with excellent results: patient survival ranging from 64 to 95% at 5 years (15). However, more recently, revascularization with an endovascular method gained more importance, and it is now considered first-line therapy. This technique is associated with significantly lower peri-operative morbidity and mortality rates than bypass (13, 16). Moreover, the success rate of endovascular therapy ranges from 73% to 100% (11, 17, 18). Also, medical and pharmacological approaches to prevent the progression of Leriche syndrome exist. They target the main risk factors of atherosclerosis: hypertension, hyperglycemia, hyperlipidemia and homocysteine. In addition, the use of antiaggregant therapy such as aspirin is fundamental. Another extremely important behavioural change is quitting smoking (10, 19). In addition, a physiokinesitherapy program has been shown to improve walking ability and reduce leg pain by 50% - 200% (20).

Fig. 1. *René Leriche (1879–1955)*

CASE REPORT

This case report is of a 54-year-old post-menopausal woman with a previous medical history of anxiety-depressive syndrome untreated. She reported smoking about 20 cigarettes daily and being moderately active (amateur runner). She also reported having maternal familiarity with atherosclerosis. However, in the last year, she started complaining of low back pain with associated dysesthesias in both thighs, and she also reported constant cramping pains in her calves, preventing her from running. In addition, the patient complained of dyspareunia and vaginismus.

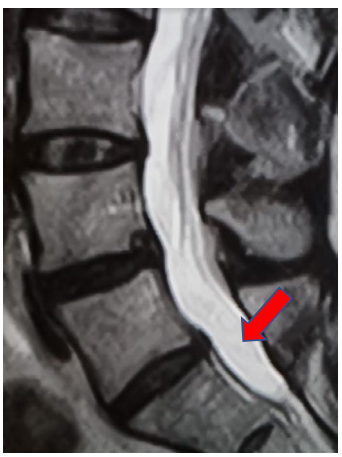

Fig. 2. *RM sagittal: little herniated disc in L5-S1 (arrow)*

For this reason, she was referred to her General Practitioner (GP) and treated with Thiocolchicoside and Ibuprofen. However, her GP sent the patient for an orthopaedic evaluation when symptomatology persisted. The orthopaedic, suspecting a possible disc herniation, prescribed her Magnetic Resonance Imaging (MRI) of the lumbar spine. The imaging investigation showed minimal protrusion in the left median paramedian site of the L3-L4 disc and at the level of L5-S1, the presence of an inter-somatic disc with signs of degeneration, reduction in height and the presence of minimal hernial focus below the right paramedian median ligament that imprints the ventral surface of the dural sac (Fig. 2). After an evaluation of the patient

and the imaging results, the orthopaedic prescribed her Oxycodone hydrochloride with Paracetamol and Ketoprofen.

The patient was prescribed physiotherapy, therapy with Etoricoxib 60 mg for 10 days and the possible use of a semi-rigid orthopaedic corset. However, despite the therapy, the woman continued to complain of the same symptoms. For this reason, she carried out a neurological examination. The neurologist prescribed electromyography, which documented chronic neurogenic suffering in the L4-L5 radicular competence area. Eventually, the patient carried out an ozone therapy session. However, after an initial benefit, the symptoms persisted. After evaluating the discrepancy between the symptomatology and the imaging, the neuroradiologist prescribed a computed tomography (CT) control. The CT scan documented the presence of a fibrocalcific atheromatous plaque at the level of the aortic bifurcation. In light of the CT scan results, the neuroradiologist suggested she investigate more with imaging techniques, more specifically abdominal ultrasound and doppler ultrasonography of aorta and iliac vessels (Fig. 3). The Abdominal Ultrasound also confirmed the presence of atheromatous plaque involving 60-65% of the aortic lumen.

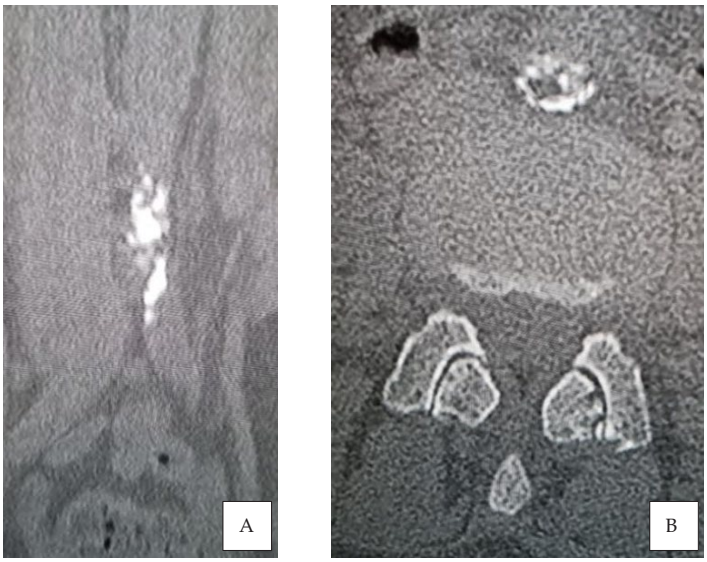

Fig. 3. *CT scan of fibrocalcified atheromatous plaque at aortic bifurcation level with a 60-65% stenosis: A) coronal view, B) axial view.*

Only at this point, a precise diagnosis of Leriche syndrome is formulated. Consequently, the patient performed a vascular surgical visit in which she was advised to make a CT-angiographic study, suspend the habit of cigarette smoking, and start therapy with an antiplatelet agent (aspirin). However, she refused to perform a CT-angiographic study, and no clear information about her smoking habit is present.

DISCUSSION

As it is known, Leriche syndrome is an abdominal aorta or bilateral iliac occlusive disease that is caused by atherosclerosis (21). This syndrome has a slow progression, which provides the development of collateral circles; therefore, symptoms are nonspecific and insidious (22). The most common symptoms include claudication, low back pain, decreased leg pulse, pallor of lower extremities and sexual dysfunction (3, 4). The last symptom is more common in men (25-39%) but can also involve female patients (4). The possible origin of these symptoms may be neurological, but all other causes need to be excluded. A deep and precise imaging and clinical study are needed in specific cases. This report highlights that the key to the correct diagnosis in this pathology was reached only thanks to an advanced imaging methods such as CT and doppler ultrasonography (23, 24). This case report shows a rare case of Leriche syndrome: first of all, the patient is female and therefore, the typical symptomatological triad is absent. Moreover, the anxious-depressive syndrome of the patient led to a delayed diagnosis.

CONCLUSIONS

Lower limb weakness and claudication are neurological symptoms that may be present in Leriche syndrome. However, they can be caused by other factors such as cerebrovascular injury, spinal disc herniation, peripheral nerve disease, neuromuscular junction disease, muscle pathology, or metabolic conditions (25-29). Therefore, in case of doubtful neurological symptoms refractory to pain-relieving medical therapy, positive anamnesis and presence of risk factors for atherosclerosis, presence of sexual disorders not due to pathologies of the genital organs, it is always necessary to perform a diagnostic deepening. For example, as in this case, CT was performed to evaluate the vascular compartment and the

possible presence of atheromatic plaques. In conclusion, in the case of bilateral and symmetrical symptomatology, non-typical spinal herniation and other non-neurological problems must be investigated.

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Review

GENETIC BASIS OF PIERRE ROBIN SYNDROME

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ABSTRACT

The clinical characteristic of Pierre Robin syndrome(/sequence) or PRS include glossoptosis, micrognathia, and blockage of the upper airways, commonly linked with a palatal cleft. It is a heterogenic pathogenic entity that can exist as an isolated disease or not syndromic nsPRS or in connection with some other syndromes or sPRS, with more prominent manifestations. Key phrases such as "Pierre Robin syndrome(/sequence)", OR "PRS", "genetic factor", "genetics", "mutations", "mutations in PRS", and "genetic relation" were used to search MEDLINE, PUBMED, and Google Scholar databases. The included methodological dataset was assessed using the EPPI (Evidence for Policy and Practice Information) Tool. The graphical depiction was produced using PRISMA flowchart generation. The data acquired from the systematic investigation showed that the deletion of chromosome 10q at the 4.34 Mb terminal and the microdeletion of gene 2q33 cause PRS. SOX9 is important in the development of illness. Comparing the amount and breakpoints of microdeletions as well as genotype-based associations led researchers to hypothesise that modulator genes near MN1 and NF2 may have an impact on the severity of cleft palate. It is yet unknown how SOX9 mutant protein causes the symptoms of PRS. This study emphasises the requirement for early genetic counselling and testing in this community of patients, in addition to research efforts to create genetic classifications to guide clinical therapy.

KEYWORDS: *genetic, syndromes, SOX9, Pierre Robin, palate, cleft*

INTRODUCTION

In a group of infants, Pierre Robin discovered a trio of clinical signs in 1923 (1). Glossoptosis, micrognathia, and obstruction of the upper airways were these markers. He originally discussed the connection between palatal cleft and common signs in 1934 (2). The Pierre-Robin syndrome/sequence, or PRS, is the official name for this condition in medicine. The first time the idea of the Pierre Robin sequence was proposed was by Carey et al. in 1982 (3). That

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the Pierre Robin trio is clinically demonstrated as an effective succession of pathogenic events (4). The Pierre Robin sequence exemplifies a heterogenic disease category. It can appear alone (i.e., not syndromic nsPRS) or in combination with other syndromes (sPRS), the former characterised by more intense symptoms and greater systemic involvement.

Epidemiological studies have shown that the Pierre Robin sequence is a very rare disease, occurring between 1 in 8,500 and 1 in 30,000 newborns (5). This wide range, in particular, is the consequence of studies conducted using very different individuals and nations at quite different times and with entirely different diagnostic techniques. With one instance for every 3120 live births, the United States of America has the high prevalence rate. Other nations have lower rates, with Germany having one case for every 8060 live births. One case per 8850 live births in Australia, 1 per 8,500 live births in the UK, 1 per 14,000 live births in Denmark, and 1 per 16,000 live births in Italy.

The Robin sequence is a remarkable organism in respect of its pathogenetic pathways and its phenotypic expressions (1). The aetiology of the condition is still poorly known, despite the significant advancements achieved in the subject over the previous few decades. It is important to distinguish between isolated cases of Pierre Robin syndrome and syndrome PRS cases where the etiopathogenesis has already been identified. According to a recent study (6), the Robin sequence can be linked to various factors. A genetic background with altered signalling pathways, airway disorders during the first three months of birth, brainstem malfunction, and/or neuromotor impairments can have an impact on mandibular growth and respiratory distress. An autosomal dominant and fully penetrant PRS locus was located on chromosome 17q24.3-25.1 in 2009 by Benko et al. (7) using genetic linkage analysis in 12 affected people from a four-generation PRS-affected family (the PRS locus).

Numerous studies suggested that the existence of mutated genes could explain Robin anomalies. It is probable that alterations in any of the mandibular development's four key genes—SOX9, KCNJ2, KCNJ16, and MAP2K6—could have contributed to the formation of isolated PRS (7-9). In reality, numerous studies on animals have shown that the in-issue mutations have a changed effect on the craniofacial phenotype. The SOX9 gene is a crucial part of the chondrogenic regulatory network; it performs a number of crucial functions throughout embryogenesis and is required for cartilage formation (4, 10-12). In humans, haploinsufficiency or loss of function of the SOX9 gene can result in campomelic dysplasia, a skeletal deformity condition. This condition and PRS are frequently linked. Deletions in the SOX9 enhancer and upstream of SOX9 can alter the gene's expression (17q24.3-q25.1). During the development of the craniofacial structure, several regulatory factors may be at play that helps SOX9 express correctly. Researchers hypothesised that the lack of these genes influenced the PRS phenotype. Milder phenotypes are brought on by disruptions that occur upstream or downstream of an intact SOX9 coding region, such as the non-syndromic version of PRS (7). Despite the absence of genes, these regions are abundant with HCNEF2 (highly conserved non-coding cis-regulatory elements) that operate as mandibular enhancers and are crucial for the proper development of the jaw, tongue, and palate.

The interaction bonding with MSX1, a chondrocyte-specific protein necessary for the development of the orofacial region, is altered specifically by the mutation of HCNE-F2. The OMIM database acknowledges a link between syndromic-PRS and distinct illnesses (13). A review of the literature on related Robin syndrome indicated the illness's well-established etiopathogenesis and the connection between PRS and these conditions. Mutations specifically characterise Stickler syndrome in type 2 and occasionally type 1 collagen genes such as COL2A1, COL11A1, COL9A1, or COL11A2 (14). While molecular research is useful, clinical outcomes usually offer the clearest proof of a diagnosis. Along with PRS, there is another syndrome called a velocardiofacial syndrome. An area of the 22q11.2 chromosome with a 3 million base pair deletion has been related to the aetiology of this disease (1). Therefore, this study has been designed to find the genetics roles in PRS.

MATERIALS AND METHODS

Data collection

In order to find relevant studies, we employed a variety of search techniques. Websites were utilised as data collection tools to gather studies and to find information. This approach involved using search engines like Medline, Google Scholar and Pubmed. Key phrases that were used are: "Pierre Robin syndrome(/sequence)", OR "PRS", "transcriptional factor", "genetics", "mutations", "mutations in PRS", and "genetic relation". About 20 articles were gathered using the following as the baseline statement: "Genetics of Pierre Robin syndrome."

Data cleaning and processing

Since the data was acquired from numerous websites, duplicates from the articles were removed. The automation software EPPI (Evidence for Policy and Practice Information) was selected for additional processing (15). The tool determined the articles that were incompatible with the platform or were outside of the domain it rejected them. Publications in English, journal articles of research documented within the past four years (2019-2022), authentic and adequate describing PRS, in particular, research papers addressing Pierre Robin Syndrome and genetics of PRS are the records inclusion criteria that are considered to be acceptable for conducting a systematic review. For this systematic review to be regarded appropriately, each and every one of these criteria must be satisfied by the records in question. Studies that are difficult to track down and do not contain references that may be relied upon or have an unjustified price tag are omitted from the review.

Systematic analysis

The results of collected studies are evaluated using PRISMA tools (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (16, 17), and a flowchart concentrated on the 2020 PRISMA checklist. Articles that do not fulfil the standards for identification, screening, authenticity, and, finally, accessing the necessary information are discarded; this guarantees that only the articles most pertinent to the discussion are accessed. On the body of work that has been chosen, a comprehensive study was undertaken (Fig. 1).

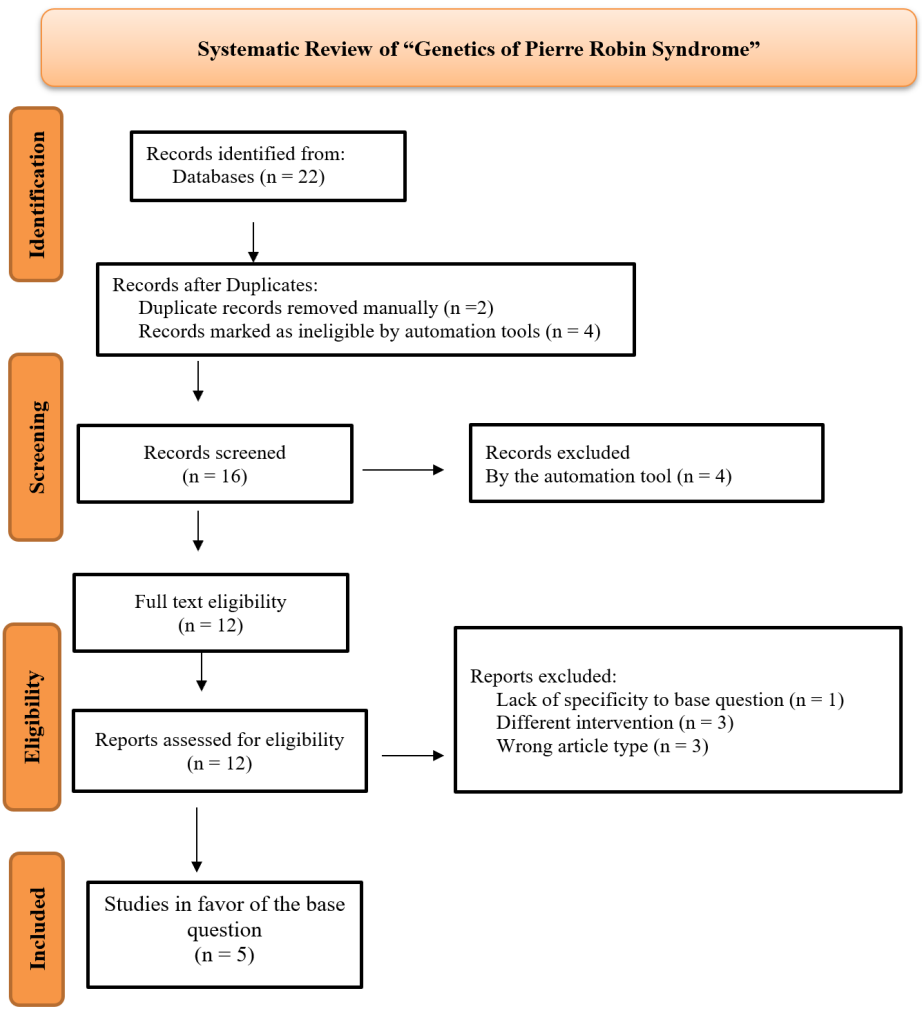

Fig. 1. *Graphical representation of systematic review process retrieved from PRISMA.*

RESULTS AND DISCUSSION

A study published by Resende et al. (18) describes research on a boy, age 29, with a history of right eye hypertropia from birth. In the English literature, more than 50 different cranial syndromes and deformities, many of which involve facial and ocular defects, have been linked to Pierre Robin syndrome (Table I). The hypothesis is that PRS-caused microdeletion of the gene 2q33 on the long arm of chromosome 2 is the root cause of such an occurrence (18). In another case study by Yekula et al. (19), a six-year-old with a history of premature 31st-week birth with observed diffuse hypotonia and developmental delay was observed. The findings declare that deletion of chromosome 10q at the terminal 14.34 Mb leads to PRS.

Murtaza et al. (20) reported the case of a male neonate who was four days old and had no family background of congenital abnormalities. Micrognathia and glossoptosis were symptoms the proband was experiencing, and eating and breathing were concerns. The study's findings revealed a strong correlation, indicating that the SOX9 gene may be involved. The SOX9 protein regulates how the facial structure should develop. The author, therefore, proposed that the haploinsufficiency of the SOX9 gene was the primary reason for PRS in this proband.

Another investigation by Al-Qattan et al. (21) claimed that SOX9 protein's positive regulator, SOX9, can specify the pathogenesis of PRS. SOX9 mutations are the primary cause of this autosomal dominant disease; a chromosome 22q12-based microdeletion that includes NF2 results in a condition that overlaps PRS and NF2. Saito et al. (22) present a patient with glossoptosis, micrognathia, a modest cleft palate form and severe early-onset NF2 that overlapped with PRS. In the patient, the author discovered a de novo chromosome 22q12 microdeletion in MN1 and NF2. The severity of the NF2 phenotypes in this example overlapping PRS and NF2 varied according to the severity of the cleft palate, as well as the size of the microdeletions. The size and termination of microdeletions have been compared, and connections between genotype and phenotype imply that several modifier genes proximal to MN1 and NF2 may be connected to the severity of cleft lip and palate.

Table I. *Average values obtained from the analysis.*

Author (REF)	Study characteristics	Findings
Resende et al. 2019 (18)	Case Report Gender= male Age=29 years old History=right eye hypertropia since birth	Microdeletion of gene 2q33 due to PRS
Yekula et al. 2020 (19)	Gender= Female Age=Six years old. History=diffuse hypotonia and delay development	Deletion of chromosome 10q. at 4.34 Mb terminal
Murtaza et al. 2021 (20)	Gender= male neonate Age= four-days old History= without congenital anomalies	SOX9 plays a role in PRS.
Al-Qattan et al. 2022 (21)	Pathogenic based systematic study	SOX9 is the positive regulator of PRS.
Saito et al. 2022 (22)	Patient with severe early-onset NF2. Patient exhibited showed micrognathia, glossoptosis, and cleft palate.	Genotype-phenotype based correlations and microdeletion size and breakpoint comparisons imply that modulator genes proximal to MN1 and NF2 may affect cleft palate severity.

CONCLUSION

The data gathered from the systematic analysis revealed that PRS is caused by a microdeletion of gene 2q33 and deletion of chromosome 10q. at 4.34 Mb terminal. Moreover, SOX9 plays a crucial role in the occurrence of the disease. Microdeletion, breakpoint comparisons and genotype-based correlations suggest that modulator genes close to MN1 and NF2 may influence the severity of cleft palate. In addition to research attempts to develop genetic classifications to inform clinical therapy, this study stresses the necessity for early genetic counselling and testing in this population of patients.

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Review

EXTRINSIC EYE MUSCLE IMPAIRMENT IN BASEDOW'S DISEASE: A BRIEF REVIEW ON MOLECULAR MECHANISMS

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ABSTRACT

The thyroid gland is the main target of Basedow's disease or Graves' disease (GD), an autoimmune condition. It is the most typical cause of hyperthyroidism and affects people of all ages, notably fertile women. A brief review has been conducted to consider the impairment of eye muscles in Basedow's disease. A search operation was performed using PubMed, Science Direct and Research Gate databases. Some relevant articles were also collected from Google Scholars as well. Although thyroid-associated ophthalmopathy is an autoimmune disorder, the exact cause of the condition is unknown. GD links genetic variables with immune system dysregulation and the interplay between genetic and environmental factors. The pathophysiology of the disease involves autoimmune responses to suspected thyroid and orbital antigens. As a result, extraocular muscles, orbital connective tissues, and fat tissues have larger volumes.

KEYWORDS: *Basedow's disease, eye muscle, hyperthyroidism, exophthalmos, immune system dysregulation*

INTRODUCTION

One of the most frequent causes of exophthalmos is that caused by Graves' disease, an autoimmune condition that frequently affects the thyroid, skin, and eyes. The thyroid is a neck gland, a member of the endocrine system, a group of glands that release hormones able to control the body's metabolic processes and functions, along with blood pressure, core temperature, and heart rate. Goiter, an "abnormal enlargement of the thyroid", and excessive thyroid hormone production are symptoms of Graves' disease (1, 2).

Grave's disease is the most typical cause of hyperthyroidism and affects people of all ages, notably fertile women. In honour of Karl von Basedow, who first characterised an exophthalmic goitre in 1840, it is also referred to as "Basedow's

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disease" in German- and French-speaking nations. In English-speaking nations, it is referred to as "Graves' disease" in honour of Dubliner Robert Graves, who first described it in 1834 (3).

Thyroid eye disease can eventually manifest in certain Basedow's disease patients. People with or without an overactive thyroid (hyperthyroidism) less frequently develop thyroid eye disease. However, it can also happen to persons with hypothyroidism, including those who suffer from Hashimoto's thyroiditis. Long-standing mystery mechanisms are now acknowledged and linked to the emergence of auto-antibodies that stimulate thyroid proliferation and secretion. Its characterisation is not entirely clear, though (4).

Epidemiology

Men are more likely to experience severe ophthalmopathy, while women are 2.5–6 times more likely to have thyroid-associated ophthalmopathy (TAO). The disease often manifests at around 30 and 50 years of age, followed by a more severe course. According to reports, ophthalmopathy affects 25%–50% of Basedow's disease sufferers and 2% of "Hashimoto's thyroiditis" patients. These patients' rates of severe ophthalmopathy range from 3 to 5%. After 18 months of receiving a Basedow's disease diagnosis, most individuals experience ophthalmopathy. Nevertheless, development of ophthalmopathy can occur up to 10 years before and up to 20 years after the commencement of the thyroid disorder (5).

Pathogenesis

TAO is considered an autoimmune condition, although the pathophysiology is not fully understood. It is known that antigens shared by the thyroid gland and the orbit cause autoimmunity. Some researchers concur that the TSH receptor is a universal pathogenetic antigen. However, researchers discovered a 64-kDa protein shared by the orbit and the thyroid gland (5). According to recent investigations, the "cardiac calsequestrin gene" has been upregulated in TAO patients. They have hypothesised that autoimmunity to calsequestrin could be a pathogenic trigger for ophthalmopathy (6). Given a strong association between ophthalmopathy and TSH receptor antibodies, autoantibodies against the "orbital fibroblast membrane antigen collagen XIII" were also discovered.

The orbit and "extraocular muscle perimysium" are invaded by reactive T lymphocytes identifying thyroid-orbit common antigens. Circulating as well as local adhesion molecules that are activated by cytokines boost interaction. T-cell receptors on CD4+ T lymphocytes identify the common antigen succeeding T helper (Th) lymphocyte infiltration of the orbit. The immune response is strengthened by the cytokines that Th lymphocytes make, which stimulate CD8+ lymphocytes and B cells that produce antibodies (7). These cytokines encourage fibroblasts to produce and secrete glycosaminoglycans (GAGs) (8). GAGs cause swelling of the extraocular muscles, proptosis, and periorbital oedema due to their ability to collect water. The enlargement of the orbital contents is also aided by cytokine-induced fibroblast proliferation (9). Preadipocytes are found in orbital fibroblasts and are stimulated by hormones to become adipocytes. It has been established that these cells help to enhance the amount of retroorbital fat tissue (10).

Recent research has shown that immune system genetics and thyroid autoantibodies play a significant part in determining the beginning of ophthalmopathy and defining its severity after it has occurred. In cases of ophthalmopathy, frequencies of anti-Thrombopoietin (TPO) antibody and anti-Tyreoglobulin (TG) positive of 90% and 50%, correspondingly, have been recorded (11).

Genetic and environmental elements are reported to have a role in the etiopathogenesis of thyroid ophthalmopathy in combination with autoimmunity.

Genetic factors

Numerous research has looked into how genetics may play a part in the onset of ophthalmopathy (12). In research analysing the ocular and palpebral results of first and second-degree relatives of individuals diagnosed with TAO, Basedow's disease, and Hashimoto's thyroiditis, 33% of euthyroid relatives had TAO symptoms like upper eyelid retraction. It has been estimated that 79% of the chance of getting Basedow's disease is determined by heredity, and environmental variables influence 21%. Twin studies have revealed that perhaps the incidence of Basedow's disease is up to 30% in monozygotic twins (13, 14).

Numerous investigations have documented polymorphisms in the genes that encode thyroid-specific proteins such as TG and immune system protein genes like interleukin (IL)-2RA, PTPN22, CD40, CTLA 4, FCRL3, and IL-23R. Patients

with TAO have been found to have "single-nucleotide polymorphisms (SNPs)" in the "tyrosine phosphatase gene" that regulates the TSH receptor, as well as the genes for the inflammatory cytokines IL-13, IL-21, and IL-23. The progression and starting age of ophthalmopathy has been linked to NF-κB1 gene polymorphism, a transcription regulator (15).

The HLA-DRB1 allele was shown to be associated with extraocular muscle engagement in a study examining the link between "MHC class II human leukocyte antigen (HLA) alleles" and ophthalmopathy (16). Twenty-four SNPs found in the NRXN3 and ARID5B genes have been linked to Basedow's disease and may also control fat deposition (17). It has been demonstrated that individuals with autoimmune thyroid illness were more likely to have a nucleotide alteration in a TG gene promoter linked to interferon-alpha (IFNα). The attachment of "IFN regulatory factor-1" to the "variant TG promoter" by IFNα directly influenced the gene expression driving thyroid autoimmunity (18). A genetic marker for TAO was suggested for the "calsequestrin-1 gene" in additional studies (19, 20).

Environmental factors

Environmental triggers such as stress, viral diseases, iodine, IFN and interleukin therapy, and sex hormones may cause ophthalmopathy in people who carry the relevant genes (21).

Infections

By promoting the expression of "costimulatory molecules" like MHC class II or by changing how their own proteins are presented, bacteria can cause an inflammatory response. Even though there are findings in the literature connecting "Yersinia enterocolitica infection" and "human foamy virus" to Basedow's disease, causative linkages could not be shown (22).

Smoking

Each patient's TAO progresses differently. Severe illness is more likely to affect men and smokers. In a trial of 59 untreated individuals with mild conditions who were followed for a year, 13.5% of patients experienced worsening of their clinical condition. Additionally, it is well-known that extraocular muscle augmentation is more common in older patients, while orbital fat expansion is more common in younger individuals (23).

A proposed mechanism by which smoking may contribute to disease progression and potentially serious clinical manifestations is that "hypoxic cell culture conditions" enhance adipogenesis in ocular fibroblasts (24). Additionally, studies on children with TAO have revealed that passive or second-hand smoking exposure can exacerbate Basedow's disease and may exacerbate ophthalmopathy (25, 26). The link between smoking and poorer clinical outcomes, including an increased chance of blindness, should be explained to all smokers. Additionally, during clinic visits, the significance of quitting smoking should be highlighted. Furthermore, smoking causes the treatment for ophthalmopathy to be delayed and to work less effectively (5).

Standard treatments and therapies

A group of experts, general endocrinologists, ophthalmologists and surgeons may need to work together to provide treatment. They must develop and offer a treatment plan in a methodical, thorough manner. Support on the psychosocial front is also crucial.

Medical devices

Some patients with mild thyroid eye conditions may get supportive care in the form of artificial tears, ointments, dark shades to reduce light sensitivity or prisms that are fitted to spectacles. Double vision can be fixed with prisms. To prevent double vision, some patients may use eyepatch.

Teprotumumab

Teprotumumab, the first medication authorised to treat thyroid eye disease, was given FDA approval in January 2020. The protein "insulin-like growth factor-1", which is thought to be a key player in the emergence of the disease, is inhibited (or blocked) by teprotumumab. When consuming teprotumumab, affected people have demonstrated a considerable improvement in double vision, proptosis, and general quality of life.

Corticosteroids

Corticosteroids, which do not decrease diplopia and proptosis but decrease inflammation and oedema, may be administered to patients with moderate-to-severe conditions. Prednisone is a typical corticosteroid often used to treat people with thyroid eye disease.

Surgery

Surgery might eventually be needed for some people with moderate to severe illnesses. Surgery is typically postponed until after the disease's aggressive phase has passed; however, if doctors believe that a patient's vision is in danger due to the disease's development during the active period, surgery may be required.

Orbital decompression, lid surgery, and motility are among the surgical alternatives. Proptosis, or protruding eyes, and retraction of the eyelids can both be improved surgically. In order to lessen or get rid of double vision, motility surgery requires moving muscle attachments around the eyes.

CONCLUSION

A condition that impairs eyesight and is debilitating is thyroid-related orbitopathy. The pathophysiology is not yet fully understood. TAO, however, is thought to be an inflammatory condition. The symptoms and indicators of TAO should be discussed with patients who have thyroid problems. Surgery is still necessary for serious diseases that endanger eyesight and are resistant to medical treatment and restorative care while the illness is dormant.

A person's facial features may change noticeably due to thyroid eye disorder, which is not totally curable. Individuals with the disease frequently experience depression, and cosmetic changes can significantly worsen mental trauma. People with thyroid eye illness are advised to include a psychologist in their treatment plan to work with the afflicted people both during and after treatment.

The management of TAO has changed a lot and will keep changing. With the introduction of forthcoming biologic medicines and targeted therapy, we predict considerable progress in the care of TAO patients.

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Review

WINCHESTER SYNDROME: A SHORT REVIEW

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ABSTRACT

Winchester syndrome (WS) comprises dwarfism, coarsening of facial features, corneal opacities, leathery complexion, and hypertrichosis. It is an inherited osteolysis syndrome and is considered a bone syndrome. WS is inherited autosomally recessively, and pathological characteristics include time-worsened cutaneous, ocular, and bony-articular changes, multiple bony articular abnormalities, and cataracts. Although clinical symptoms vary, carpal and tarsal bone disintegration, generalized osteoporosis, increasing joint contractures, low height, peripheral corneal opacities, and coarse facial characteristics are prevalent. Skin characteristics include hypertrichosis, gingival enlargement, leathery skin in an annular or linear distribution, subcutaneous nodules, and extensive progressive multilayered symmetrical limited banding. Mucopolysaccharidosis with enzymatic abnormalities is suspected to be the cause of this disease. WS is now recognized as a homozygous missense mutation (E404K) in matrix metalloproteinase-2. Specialists, including paediatricians, orthopedists, radiologists, ophthalmologists, rheumatologists, neurologists, stomatologists, dermatologists, and psychologists, evaluate patients with symptoms suggesting this pathologic syndrome to make a diagnosis and arrange treatment.

KEYWORDS: *Winchester syndrome, vanishing bone, skeletal-articular system, mucopolysaccharidosis*

INTRODUCTION

Winchester syndrome (WS) includes dwarfism (caused by problems with the skeletal-articular system), coarsening of facial features, corneal opacities, leathery complexion, and hypertrichosis. It is one of the inherited "vanishing bone" syndromes, or osteolysis (1). WS is a genetic condition that runs in the family and is inherited in an autosomal recessive manner. The pathologic alterations' underlying molecular causes are not fully known. The aberrant activity of the fibroblasts, which contributes to some of the pathologic alterations in this condition, was investigated (2, 3). Winchester et al. (4) initially identified pathologic alterations in two sisters, ages 3.5 and 12, who were first cousins, in 1969. These

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sisters were said to have "a novel acid mucopolysaccharidosis" and rheumatoid arthritis-like skeletal abnormalities. Later, Brown and Kuwabara (5) obtained a corneal biopsy; the outcomes were typical of the mucopolysaccharidoses. Three occurrences of this disease were reported in Mexico by consanguineous relatives in 1974 (2). The WS was the name that authors gave to the collection of observations. They specifically identified skin alterations in the extremities and trunk that Winchester et al. (4) did not address. A 3-month-old Iranian was the sixth patient described in 1977 (6), while a 4-year-old in Bombay, India, was diagnosed with a similar instance in 1978 (7). The parents were first cousins in both situations. Dunger et al. (8) reported 2 more occurrences in 1987, while Lambert et al. (9) discussed two examples involving siblings in France, a 13-month-old girl and a 12-year-old girl.

CLINICAL PRESENTATION

One of the hereditary osteolysis syndromes, this rare genetic illness is characterized by the breakdown and resorption of afflicted bones, leading to skeletal abnormalities and functional disability (10). Although the clinical symptoms differ, it is common to notice the dissolution of the carpal and tarsal bones, generalized osteoporosis, progressive joint contractures, low stature, peripheral corneal opacities, and coarse facial features. Hypertrichosis, areas of hyperpigmented, gingival enlargement, hypertrichosis leathery skin in an annular or linear distribution, subcutaneous nodules, and extensive progressive multilayered symmetrical restricted banding of the skin are examples of cutaneous characteristics. The patient's first year of life is often when the first pathologic alterations occur (1, 4, 6). Inflammatory disorders with painful joints and restricted mobility first occur, which is suggestive of rheumatoid arthritis. These inflammatory alterations frequently affect the interphalangeal, metacarpophalangeal, and carpal joints. Both bilaterally and symmetrically were altered. Large joints like the knee and spinal joints may also undergo alterations simultaneously (10).

Winchester reported findings in the second instance involving a 3.5-year-old child with joint abnormalities resulting in rigidity of the spinal column and limbs (4). The patient had this stiffness before turning 20 months old. Permanent flexion contractures in the tiny joints of the hands and feet and in the knee, hip, elbow, and shoulder joints are caused by the disease's gradual course, which may endure for many years. The spinal column may experience similar alterations. Failure of motion could be the outcome of inflammatory changes in the joints. Patients with peripheral corneal opacities develop them between 2 and 5 years, or they are discovered later. The child's eyesight declined over time. Cataracts are not discovered in 5 documented cases involving children 3 months, 13 months, 4 years, 12 years, and 16 years of age (9, 10).

Prognosis and diagnosis

The condition progresses, causing worsening cutaneous, ophthalmic, and bony-articular alterations. In these people, managing anaesthesia can be difficult (11). Destructive changes occur in the joints of the hands, wrists, tarsus, and foot due to intensified osteoporosis, including osteolysis of the carpal and tarsal bones. Backbone compression fractures can result from severe osteoporosis of the vertebral bodies. Strong contractures in the knee and hip joints can make movement difficult (3). Multiple bony articular alterations may result in lifelong impairment. As a result of exacerbated cataracts, visual loss may ensue. Skin and gum biopsy samples should be obtained for histological and ultrastructural analysis to confirm the diagnosis of WS (11).

Pathophysiology

It appears that WS is inherited autosomally recessively (4). The abnormalities that take place in this syndrome are thought to be the result of problems with glycosaminoglycan metabolism. In the urine of two WS patients, Dunger et al. (8) discovered an aberrant oligosaccharide composed of one fucose and two galactose molecules. Hollister et al. (2) and Cohen et al. (3) studies did not find morphological proof of lysosomal storage. These authors contend that uronic acid is also found in 27% of healthy individuals, making metachromasia of the fibroblasts and a 2-fold increase in the uronic acid content in these fibroblasts insufficient evidence for the diagnosis of mucopolysaccharidosis.

The authors contend that WS should not be considered an acid mucopolysaccharidosis but rather a nonlysosomal connective-tissue disorder. Their findings imply that this spectrum of pathologic alterations is mainly mediated by fibroblasts. Anomalous fibroblast functions are likely to manifest as contractures, leathery skin, aberrant collagen in the dermis, and

corneal opacities. The authors advise additional research to understand the pathophysiology of this autosomal recessive illness. A 21-year-old woman with a severe case of osteolysis who was diagnosed with WS had a homozygous missense mutation (E404K) in the active region of matrix metalloproteinase 2 (MMP2) (12). In addition, an MMP2 homozygous new mutation was found in a WS family. Allelic disorders include WS, nodulosis-arthropathy-osteolysis, and Torg syndrome (13). MMP2 deficiency and MMP 2 gene mutations are linked to these two disorders (14, 15). In 13 people with multicentric osteolysis nodulosis and arthropathy, five unique MMP2 mutations were found in India (16). One patient had homozygous mutations in membrane type-1 metalloproteinase (MT1-MMP or MMP14), an inactivating homoallelic mutation that decreased MT1-MMP membrane localization and impaired pro-MMP2 activation as a result of the hydrophobic-region signal-peptide substitution (p.Thr17Arg) (1). The MMP2 gene has a homozygous frameshift variation that has been described (17). The WS phenotype appears to be determined mainly by MMP14 catalytic activity (18).

The following test results and distinctive symptoms of the pathologic alterations should be taken into consideration when diagnosing WS is hypothesized:

the recessive autosomal inheritance pattern in a case history involving siblings and cousins;

arthritic changes that start when the patient is around a year old, along with symptoms resembling rheumatoid arthritis;

joint abnormalities and chronic damage in older individuals, together with short height;

an actual absence of the carpal and tarsal bones is one of the skeleton's distinctive radiologic alterations of multifocal osteoporosis;

the opacity of the cornea's periphery;

the agglomeration of facial characteristics.

With leathery skin, hyperpigmentation, and hypertrichosis, there may be focal or diffuse thickenings. Winter proposed WS diagnostic standards in 1989. These consist of the skeletal radiologic features mentioned above and at least two traits: short stature and progressive articular contractures, corneal opacities, thickened hyperpigmentations or hirsutism of the skin, hypertrophy of the gums, and coarsened facial features (10).

Both small and big joints, as well as the spinal column, experience pathologic alterations. The fundamental characteristic of this pathological syndrome is the involvement of these joints and the spinal column. Changes in the bones that make up the joints and oedema of the periarticular tissues may result in deformities. The joints of the hands and feet, the toes, the wrists, and the metatarsals all show these modifications. Similar changes can affect the backbone, knee, elbow, shoulder, and hip joints (18).

Management

Patients exhibiting signs suggestive of this pathologic illness should be examined by numerous specialists to determine the definitive diagnosis of WS. Pamidronate does not reduce peripheral osteolysis in nodular arthropathy and multicentric osteolysis brought on by matrix metalloproteinase 2 gene mutations (19).

Patients with findings suggestive of this pathologic syndrome involve evaluations by specialists, including paediatricians, orthopedists, radiologists, ophthalmologists, rheumatologists, neurologists, stomatologists, dermatologists, and psychologists, in order to determine the precise diagnosis and plan further medical care. In addition, for appropriate genetic counselling and consultations, families with this syndrome should be referred (20).

The pathologic changes in the spine and limb joints impair the patient's ability to be active. Therefore, exercise as a treatment is suggested. Orthopaedic devices are necessary for some WS patients. However, future therapies are needed for a long-term treatment plan (21).

CONCLUSION

WS includes hypertrichosis, corneal opacities, leathery skin, and coarsening of the facial features. It is an inherited bone syndrome called osteolysis syndrome. The homozygous missense mutation (E404K) in the active site of MMP2 currently causes WS. MT1-MMP and MMP14 also cause this condition. Families with this syndrome should be referred for proper genetic counselling and consultations. It is advised to exercise as a treatment. Some patients with WS need orthopaedic equipment. For a long-term treatment strategy, nevertheless, further therapies are required.

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Letter to the Editor

MUCOUS MEMBRANE PEMPHIGOID AFFECTING THE ORAL MUCOSAE: A BRIEF REVIEW

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ABSTRACT

A mucous membrane pemphigoid is a group of immune-mediated mucocutaneous diseases characterized by the formation of blisters whose rupture leaves an erosive area. It is classified as a rare disease with an unknown aetiology, although some agents may be considered causative. The pathogenesis consists of a subepithelial detachment caused by autoantibodies against basement membrane proteins. The diagnosis is made by integrating clinical appearance, histopathology, and direct immunofluorescence. Other diagnostic aids are indirect immunofluorescence and enzyme-linked immunosorbent assay. The main treatment is systemic and/or topical corticosteroids; in non-responsive patients, there are innovative alternative treatments with immunosuppressants and rituximab.

KEYWORDS: *Pemphigoid, oral, vesicle, disease, medicine, pathology*

INTRODUCTION

The term mucous membrane pemphigoid (MMP) refers to a variety of chronic, immune-mediated, vesiculobullous disorders that are heterogeneous in nature. They affect the skin and oral mucosa with vesicles, blisters, and erosion due to the formation of self-antibodies against the basal membrane and subsequent subepithelial attachment loss (1). MMP is the most common acquired autoimmune bullous dermatosis, with an incidence ranging from 6 to 14 new cases per year per million population (2). It occurs without gender predilection during the sixth decade of life, although rare cases of MMP in children and adolescents have been reported. The aetiology remains unknown, although several studies have shown a genetic predisposition with the involvement of the HLA-DQB1*0301 allele (3). Physical triggers (radiotherapy, ultraviolet radiation), burns, trauma, drugs such as vaccines, or even chronic use of spironolactone and phenothiazines have been found in 15% of patients diagnosed with MMP (4).

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Pathogenesis and the auto-antigens

MMP is characterized by antibodies directed against self-antigens of the hemidesmosome plaque known as BP180 (180 kDa) or BPAG2 and BP230 (230 kDa) or BPAG1. Both antigens are key hemidesmosome components responsible for the adhesion between epithelium and underlying connective tissue (1). Other target antigens are laminin 332, α6/β4 integrin subunits, laminin 311 and type VII collagen.

Antibodies directed against the α6 subunit are frequently associated with mucosal lesions, while autoantibodies against the β4 subunit are generally associated with ocular involvement. Antibodies directed against laminin 332 are associated with a more severe disease involving multiple mucosal sites (5). IgG (subtypes IgG1, IgG3, and IgG4), IgA and IgE (rarely) are the primary autoantibodies implicated. The binding of these autoantibodies to the basement membrane triggers complement activation that culminates in the release of metalloproteinases and cytokines responsible for dermal-epidermal detachment.

Clinical manifestations on the mucosal sites

Desquamative gingivitis, which occurs in 85% of instances of MMP, is the most common manifestation, followed by conjunctivitis in 65% of cases. Less frequently, the vaginal, nasal, pharyngeal, laryngeal, and oesophageal mucosa are affected. Less than 30% of patients develop skin lesions (6). In addition to the gingiva, lesions may involve the buccal mucosa, palate, and tongue. Sometimes vesicles may appear brownish-red in colour when blood extravasation is involved. However, the blisters quickly burst, resulting in an erosive area. The erosions are very painful and take a few weeks to heal with the simultaneous formation of other lesions, while ocular lesions are less frequent. They begin as chronic conjunctivitis of the sclera with fibrosis outcomes, which can lead to symblepharon (fusion of the sclera with the palpebral conjunctiva), entropion (inversion of the palpebral margins) or ankyloblepharon (fusion of the eyelids) up to blindness.

A challenging diagnosis

In most cases, the diagnosis of MMP is complex; the symptoms and signs are non-specific and vary from one form to another (7). The diagnosis is based on the evaluation of three criteria:

- Clinical manifestations
- Histological examinations: histological examination and direct immunofluorescence (IFD)
- Serological examinations: indirect immunofluorescence (IFI) and enzyme-linked immunosorbent assay (ELISA).

An examination is the starting point for making a diagnosis. The presence of vesiculobullous and erosive lesions at the level of the oral mucosa and/or the skin level are clinical findings shared by several autoimmune diseases. Nikolsky's sign (8) is helpful during the examination. It can be evocated directly or indirectly. The direct method consists in applying pressure, e.g. by blowing air, directly on an already present lesion; if it is a bullous lesion, this gesture will cause the lesion to expand.

On the other hand, the indirect method involves applying pressure, using a blunt instrument, directly on the healthy mucosa; in the case of a vesiculobullous pathology, this action results in the formation of a blister. Given its lack of specificity, this clinical examination is only helpful in directing the pathologist during the diagnostic procedure. It allows us to highlight an epithelial detachment but not its nature.

The mucosal examination is fundamental for an early differential diagnosis; a negative Nikolsky's sign and the absence of white reticular lesions can be helpful to differentiate pemphigoid from lichen planus; the clinical way the ulcerative lesions appear can lead to the differentiation of the pemphigoid from recurrent aphthous stomatitis or ulcerative cancer lesions. Thus, an accurate physical examination and a highly experienced and trained clinician are essential for an adequate second-tier analysis.

Biopsy: a crucial exam

The histological examination provides an incisional biopsy (9). In order to perform an adequate histological analysis, it is crucial to take a sample of perilesional tissue, i.e. at the edge of the lesion, where healthy tissue is present. Biopsy sampling in cases of suspected bullous pathology is extremely delicate and complicated, as simple tweezing or mishandling of the surgical specimen carries the risk of iatrogenic epithelial detachment, thus preventing adequate anatomopathological evaluation.

The technique currently used to perform the biopsy is the stab-and-roll technique (10). It involves inserting the scalpel

blade perpendicularly to the gingival mucosa; the blade is rotated within the cutting margins before reaching the bone surface. This way, the forces are directed in a single direction without causing epithelial detachment of the specimen, unlike the direct technique. However, the latter requires a deeper incision resulting in a more significant movement of the scalpel blade.

Sampling can also be performed using a 6-mm diameter punch, which makes the size of the piece homogenous and standardized and allows for less handling of the surgical piece. After sampling, the surgical piece is fixed in formalin to avoid tissue alterations that could affect the microscopic analysis. Moreover, it is not sufficient to assess the presence of an epithelial detachment, which is common in different vesiculobullous diseases.

The pathological mark in MMP is the presence of sub-epithelial vesicles with an inflammatory infiltrate represented by lymphocytes, eosinophils, and neutrophils. There are no Tzank cells, and the epithelium does not have a tombstone pattern. Sometimes, however, a reparative process at the level of the basement membrane can make it difficult to differentiate between intraepithelial and subepithelial detachment, making the histological exam not diriment. For this reason, a histological examination is completed with immunofluorescence analyses to confirm the diagnosis.

Direct immunofluorescence represents the diagnostic gold standard in the context of vesiculobullous pathologies and is essential for diagnosis in doubtful cases. Moreover, immunofluorescence can be considered more sensitive than conventional histological examination because autoimmune deposits generally precede the appearance of epithelial detachment. This diagnostic method involves a biopsy of perilesional tissue, performed simultaneously with the histological examination. According to Gilvetti et al. (11), the optimal sampling site for direct immunofluorescence analysis is the gingiva, which is thus optimal in the case of patients with desquamative gingivitis. It also appears that the optimal technique is punch sampling.

Direct immunofluorescence provides quantitative information about target antigens, immunoglobulin subclass and binding type. In pemphigoid, direct immunofluorescence microscopy reveals the intercellular binding of immunoglobulins within the epithelium giving the typical linear deposition of immunoglobulins at the basement membrane.

Indirect immunofluorescence is a serological method that tests for autoantibodies in the patient's serum. Indirect immunofluorescence microscopy uses a substrate of various kinds, including monkey skin, rabbit, and human oesophagus, which is incubated with the patient's serum (12). Then fluorochrome-labelled antibodies are added and directed against the patient's autoantibodies. Indirect immunofluorescence, although performed during the diagnostic procedure for bullous disorders, is not considered sufficient to make a definitive or differential diagnosis.

Enzyme-Linked Immunosorbent Assay (ELISA) is an enzyme immunoassay that identifies and quantifies autoantibodies directed against specific antigens in each sample. ELISA is a method used for diagnosing and monitoring disease following therapy. This method involves a primary binding between the antibody in the patient's serum and a specific antigen, thus forming a primary complex. Next, a specific antibody conjugated with an enzyme is introduced, which binds to the primary complex, resulting in a coloured product. Finally, analysis by spectrophotometer allows evaluation of the response, which correlates with the intensity of the signal.

For the diagnosis of pemphigoid, currently marketed kits use recombinant forms of the NC16A portion of BP180, the C-terminal and N-terminal sequence of BP230, and type VII collagen (13). The ELISA method is performed on serum; recent studies also apply this method to the patient's saliva. Such studies aim to use saliva as a diagnostic method, as it is less invasive and troublesome for the patient than a blood sample.

Treatment

The severity of MMP has a significant impact on how it is treated. Patients with modest risk factors may initially need topical treatment, but high-risk patients may also need effective systemic therapy. Systemic corticosteroids have been proven to have an effective result in treating MMP, yet they have adverse effects when used long-term. Therefore, other medications, such as immunosuppressants, biological agents, inflammatory-reducing medications, and antibiotics, are also used (14).

CONCLUSIONS

MMP is a defined nosological entity which needs an clinical and laboratory diagnosis. It is caused by several factors and therapy is mainly based on the use of an immuno-suppressant agent.

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