ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

Histopathological and immunohistochemical changes of the marginal periodontium in patients with Turner syndrome

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Abstract

Turner syndrome (TS) is characterized by partial or complete loss of a sexual chromosome, resulting in an incomplete development of the body, gonadic failure, cardiac and renal abnormalities, oro-dental changes, etc. In our study, we proposed to perform a histological and immunohistochemical (IHC) study of the periodontium changes in patients with TS. The biological material under study was represented by fragments of gingival mucosa harvested from 18 patients with TS who presented advanced periodontal lesions and required dental extractions. The fragments of gingival mucosa were processed by the classical histological technique of paraffin inclusion, subsequently the obtained sections being stained by the Hematoxylin–Eosin (HE) and examined under the optical microscope. For the IHC study, there were performed serial sections incubated with anti-cluster of differentiation (CD) 3, anti-CD20 and anti-CD68 antibodies for highlighting immune cells, as well as with anti-matrix metalloproteinase (MMP) 2 and anti-MMP8 antibodies for highlighting MMPs (MMP2 and MMP8) involved in the periodontal tissue lesions. In the present study, during the histological examination, there were observed morphological changes, both in the epithelium and in the gingival mucosa chorion. Epithelial changes consisted in the onset of acanthosis processes, in the thickening of the epithelium due to the increase of the spinous layer, as well as in the parakeratosis phenomenon. In the chorion, there was observed the presence of inflammatory infiltrates in various stages, presence of fibrosis (extended in some cases) and the presence of an important vascularization in some cases, with a high number of immunocompetent cells involved in the inborn immune response, but also in the adaptive one, as well as a more or less intense immunoexpression of MMP2 and MMP8. Our study suggests that TS may contribute to the development of some inflammatory processes in the marginal periodontium.

Keywords: periodontium, Turner syndrome, inflammatory processes, matrix metalloproteinases, immunohistochemistry.

Introduction

Turner syndrome (TS) affects women and is characterized by a complete or partial absence of an X chromosome. The present X chromosome has a normal structure and normal function [1]. TS is not hereditary [2, 3]. Data in the last years showed that in Europe the disease affects about one in 2000 women [4].

TS is characterized by an incomplete development of the body, gonadic failure, hormonal changes, cardiac or renal abnormalities, etc. [5, 6].

Described in 1938, by Henry Turner, the syndrome is characterized by the patients' small stature, primary amenorrhea, hypoplastic internal and external genital organs (uterus, infantile vagina), breasts with hypoplastic and distanced mammillae, ovary agenesis, *pterygium colli*, cubitus valgus and low hair line behind the neck [7]. Some of the anatomic changes may be minimal or even absent, thus not all patients present the same changes; only some of these pathological changes are associated with karyotype changes [8–13].

The studies performed in Romania showed that the patients diagnosed with TS most commonly present lymph edema, *pterygium colli*, aorta coarctation, spontaneous or complete puberty or an incomplete puberty [14].

The treatment with recombinant growth hormone (rGH),

obtained by genetic engineering, during childhood has irrefutable benefits, as GH plays an important part in maintaining the heterogeneity of tissues during normal development [15]. Moreover, the treatment with GH improves height growth, decrease of the body mass index (BMI), improves the lipidic profile – low-density lipoprotein (LDL), lowers cholesterol and triglycerides and gives a lower prevalence of high blood pressure [16].

The administration of rGH leads to important changes in the oral cavity, while the delay of its administration may lead to a high prevalence of retrogonadism in women diagnosed with TS. In these patients, there were also identified more types of dental cavities, edentations, changes of soft oral tissues [16–19].

Other studies showed that there are frequent oral changes in patients with TS, such as high palate disease, mandibular hypoplastic teeth, with short roots and an early eruption of permanent teeth [1, 7, 20–24].

Hugoson reported that female sexual hormones may affect the gum and periodontium by increasing the permeability of blood vessels, all the more in TS [25].

Some studies highlighted the presence of an aggressive periodontitis in patients diagnosed with TS, accompanied by the presence of some deep gingival bags, bone resorption and dental mobility, while other patients presented only gingivitis, with a marginal and papillary inflammation, associated with large calculus deposits [26, 27].

TS causes changes in the marginal periodontium manifested by marginal gingivitis and periodontitis. On the other hand, the mechanism of indirect bacterial aggression, represented by the host tissue reactions, cause the release of proinflammatory factors: prostaglandins (PGs), cytokines, and metalloproteinases [28].

Aim

Starting from these observations, we proposed that, by histological and immunohistochemical (IHC) examinations, to evaluate the morphological changes present in the gingival mucosa, in patients diagnosed with TS.

Patients, Materials and Methods

Our study included 18 patients aged between 7–19 years old [average age 12.17±5.35 standard deviation (SD)], diagnosed with TS, admitted to the Clinic of Endocrinology within the Filantropia Hospital of Craiova, Romania, between 2016–2019. Of the investigated patients, 10 were from the urban area and eight from the rural area.

The study was approved by the Ethics and Scientific and Academic Deontology Committee of the University of Medicine and Pharmacy of Craiova, respecting the privacy of the patients (Approval No. 52/20.04.2018).

All the patients expressed their consent for participating in the study.

The TS diagnosis was decided based on the clinical aspects and confirmed by karyotyping, by using cytogenetic and molecular tests. The karyotype was determined by using the Leica CW4000 Karyo software, by studying 15 metastages obtained by banding techniques with Giemsa (G), being karyotyped in five metastages. In all studied metastages, there was highlighted chromosomal aneuploidy by the absence of one of the X chromosomes, with no changes in the chromosome structure detected by the G-banding technique. The conclusion was TS, with 45,XO karyotype.

Information on the health state of patients diagnosed with TS and a possible administration of GH were obtained from the medical records and from the medical history of every patient.

Besides the endocrinological condition, the patients presented symptoms specific to periodontal disease: changes of shape, color, gingival rash, dental mobility, bags of different depths, gingival overgrowth in various stages, gingival retractions, all causing discomfort to the patient.

Patients with TS were clinically and radiologically diagnosed with gingivitis and marginal periodontitis in a private dental practice, between 2017–2019.

Fragments of gingival mucosa were harvested during an extraction of periodontic teeth with second degree mobility or during a periodontal surgical treatment for gingival overgrowth, in patients diagnosed with gingivitis. Mucosa harvesting was performed in the interdental gingival papillae or at gingival growth level.

Harvesting of the biological material was performed after the patients expressed their informed consent.

Fragments of gingival mucosa were processed for performing histological and IHC studies.

The histological and IHC studies were performed in collaboration with the Department of Histology within the University of Medicine and Pharmacy of Craiova. The biological material, immediately after harvesting, was fixed in a 10% formalin solution with a neutral pH. After sectioning the material in the microtome, the sections were stained with Hematoxylin–Eosin (HE).

For the IHC study, there were used the following antibodies: anti-cluster of differentiation (CD) 3 (rabbit anti-human CD3 polyclonal antibody, Dako, 1:50 dilution) for highlighting T-lymphocytes; anti-CD20cy (mouse anti-human monoclonal antibody CD20cy, clone L26, Dako, 1:50 dilution) for B-lymphocytes; anti-CD68 (mouse anti-human monoclonal antibody CD68, clone KP1, Dako, 1:100 dilution) for macrophages; anti-matrix metallo-proteinase (MMP) 2 [anti-MMP2 (8B4) antibody, clone NB 200-114, Novus, 1:50 dilution] for the study of MMP2, and anti-MMP8 [anti-MMP8 antibody, purified mouse monoclonal immunoglobulin G2A (IgG2A), clone 100608, R & D Systems, 1:50 dilution] for the study of MMP8.

Results

The clinical examination of the oral cavity in patients with TS highlighted the presence of acute erythematous gingivitis, sometimes with gingival hypertrophy, especially interdentally (Figure 1), presence of some calculi deposits or of some periodontitis with bleeding at drill palpation (Figure 2).

The microscopic examination of the gingival mucosa in patients with TS, especially in those with the clinical

diagnosis of gingivitis, identified the presence of an important vascular congestion in the gingival chorion, with a high number of capillaries in the conjunctival papillae, but also in the deep chorion. Most often, there were identified angiogenesis vessels, characterized by the presence of a small lumen, lined by hypertrophied endothelial cells with large nuclei (Figure 3).

In most cases with TS, regardless of the clinical diagnosis of the periodontal disease, there were observed microhemorrhages in the periodontal conjunctive tissue, a sign for the fragility of small caliber blood vessels. Also, in the gingival mucosa chorion, in all the patients included in the study, we observed the presence of some lympho-plasmocytary and macrophage inflammatory infiltrates in various stages (Figure 4).

In the case of patients clinically diagnosed with marginal periodontitis, during the microscopic examination of the gingival mucosa, the epithelium appeared thickened (acanthosis), due to the growth of spinous layer and para-keratinization; in other patients, there were highlighted erosions of the covering epithelium with a direct exposure of periodontal tissues and gingival chorion to the microbial flora of the oral cavity. In the chorion, there was observed a massive fibrosis, with thick stripes of collagen fibers, extending over the conjunctive papillae and replacing the papillary lax conjunctive tissue with a dense conjunctive one. Among the thick collagen fascicles there was observed the presence of a high number of fibroblasts, a lympho-plasmocytary chronic inflammatory infiltrate, more or less developed, congested blood vessels and even micro hemorrhages (Figures 5–8).

The IHC study had the objective of identifying the immune system cells to the gingival and periodontal inflammatory reaction. The anti-CD3 antibody immuno-marking highlighted the presence of T-lymphocytes, involved in the adaptive immune response, in all cases of gingivitis and periodontitis associated with TS. CD3+ T-lymphocytes were present in the chorion of the gingival mucosa in a variable quantity from one patient to another; sometimes, they were identified in a large number even in the deep chorion, especially around the collagen fascicles (Figure 9).

For highlighting the B-lymphocytes involved in the humoral adaptive immune response, there was used the anti-CD20cy antibody. B-lymphocytes were present in a large number in the inflammatory infiltrates of the chorion, in all the studied cases, regardless of the type of periodontal disease (Figure 10). B-lymphocytes were diffusely scattered in the chorion, in a larger or smaller number, according to the severity of the periodontal lesion. In severe cases of periodontitis, associated with necrosis of the covering epithelium, the number of B-lymphocytes was high. In these cases, there was observed the infiltration of gingival chorion and of the periodontal tissues with numerous plasmocytes, which shows an immune humoral, quite intense, reaction.

Highlighting of the macrophages was performed by immunomarking with anti-CD68 antibody. Similarly to the other types of immune cells, macrophages also had a diffuse, heterogenous arrangement in the periodontal conjunctive tissue (Figure 11).

Starting from the observations that during the periodontal inflammatory processes there synthesize and secrete many enzymes damaging and remodeling the periodontal tissue within the conjunctive matrix, in the present study we wanted to highlight the presence of MMPs. Of these, we chose to investigate the presence or absence of two of these enzymes, namely MMP2 and MMP8.

The IHC reactions for MMP2 were different in intensity in relation to the periodontal disease. Thus, in the patients with a clinical diagnosis of gingivitis, MMP2 presented a moderately positive reaction in the endothelial cells of the blood vessels, in the fibroblasts and monocyte elements in the chorion (Figure 12). In comparison, in the cases of periodontitis, the IHC reaction was more intense (Figure 13).

For MMP8, there was observed a similar reaction to MMP2, namely that in severe periodontal lesions MMP8 was intensely expressed in the conjunctive cells (fibroblasts, myofibroblasts), but also in the endothelial and inflammatory cells (Figure 14).

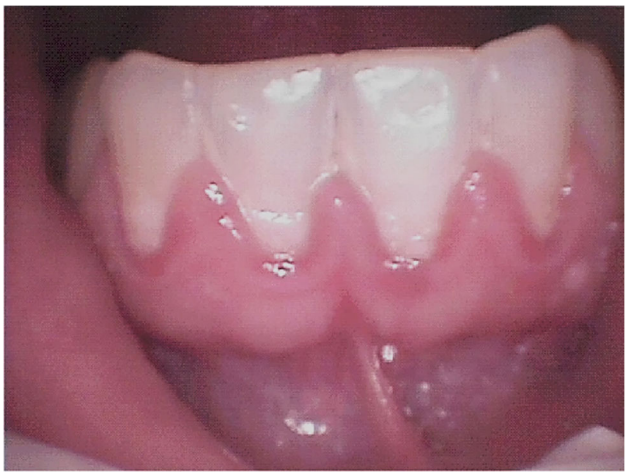

Figure 1 – ***Hypertrophic gingivitis caused by a dental biofilm. Patient with Turner syndrome.***

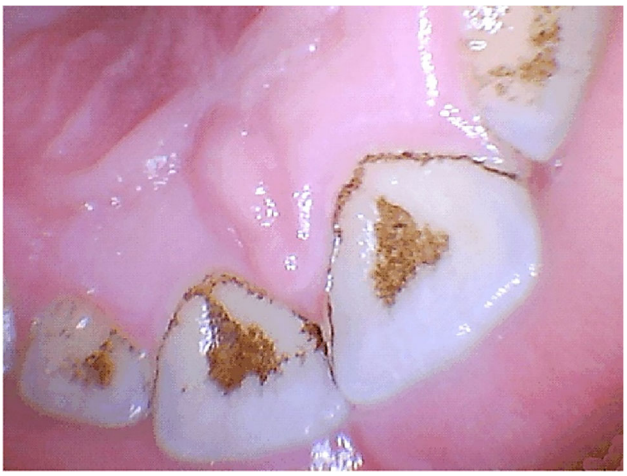

Figure 2 – ***Irritative gingivitis induced by dental calculus. Patient with Turner syndrome.***

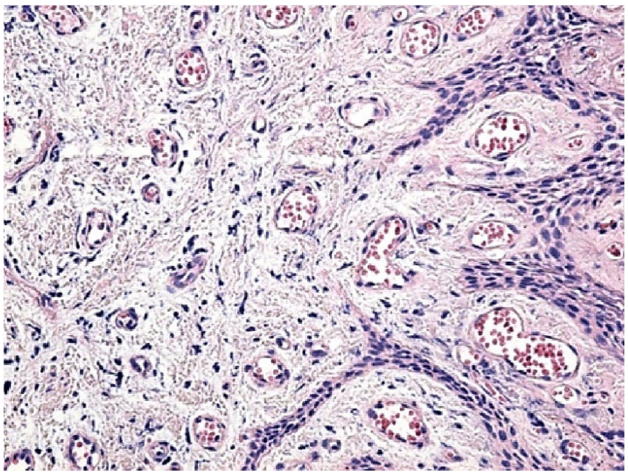

Figure 3 – ***Important vascularization in the chorion of gingival mucosa, with a high number of capillaries and vascular congestion. Deep and branched crests in the chorion with an aspect of rete pegs in a patient with gingivitis and Turner syndrome. Hematoxylin–Eosin (HE) staining, ×200.***

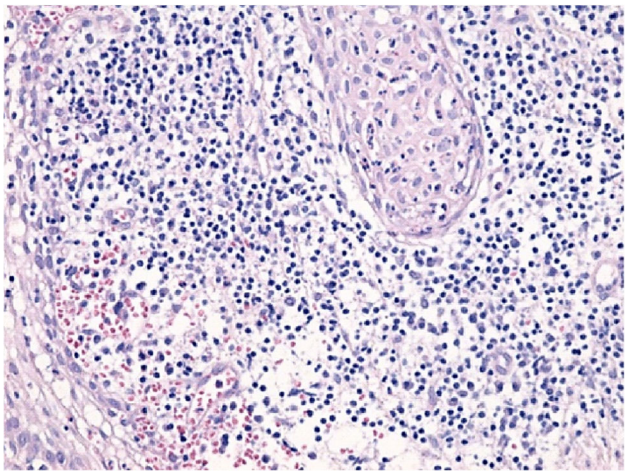

Figure 4 – ***Microscopic image of an area in the superficial gingival chorion, strongly infiltrated by lympho-plasmocytary and macrophage inflammatory cells. HE staining, ×200.***

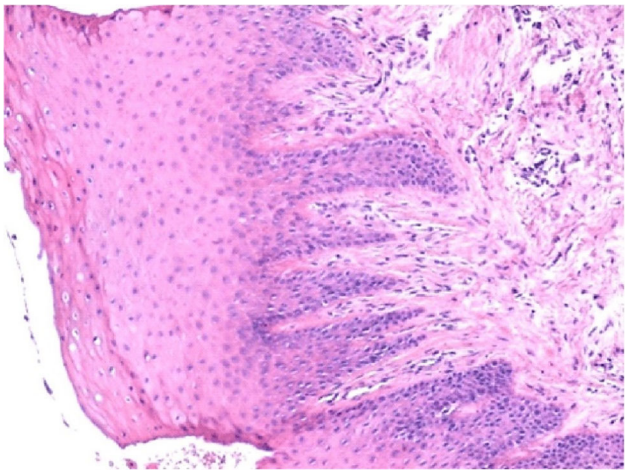

Figure 5 – ***Thick gingival epithelium, with acanthosis and parakeratinization. In the chorion of gingival mucosa, there is observed the presence of an important process of collagen fibrosis, characterized by the onset of thick fascicles of collagen fibers in the chorion papillae. HE staining, ×20.***

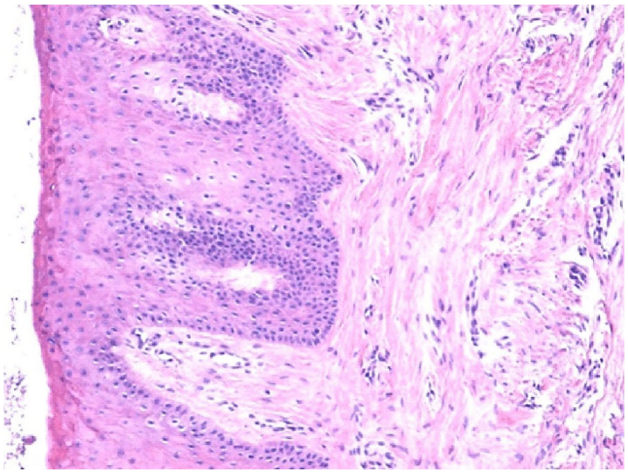

Figure 6 – ***Gingival epithelium with acanthosis and parakeratosis. In the chorion of the gingival mucosa, there is observed a massive fibrosis, with the presence of thick stripes of collagen fibers in the papillary crests of the chorion, as well as in its depth. HE staining, ×20.***

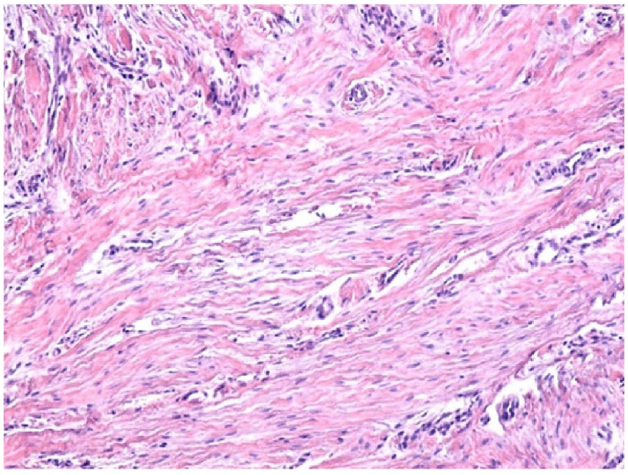

Figure 7 – ***Massive collagen fibrosis in the deep chorion of the gingival mucosa, in a 17-year-old patient with Turner syndrome. HE staining, ×200.***

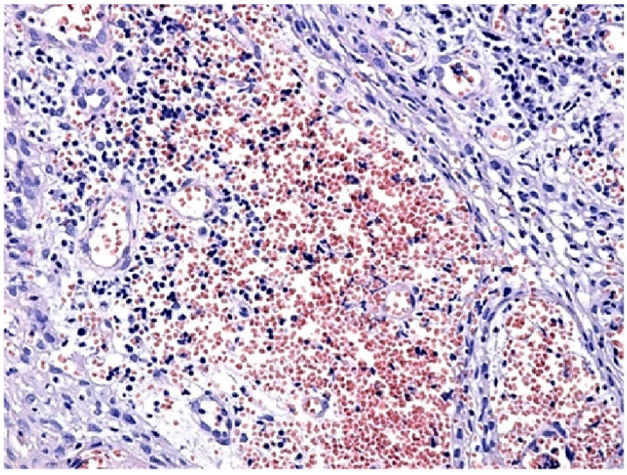

Figure 8 – ***Epithelial crests with congested blood vessels, microhemorrhage and a low lympho-plasmocytary inflammatory infiltrate, in a patient with gingivitis. HE staining, ×200.***

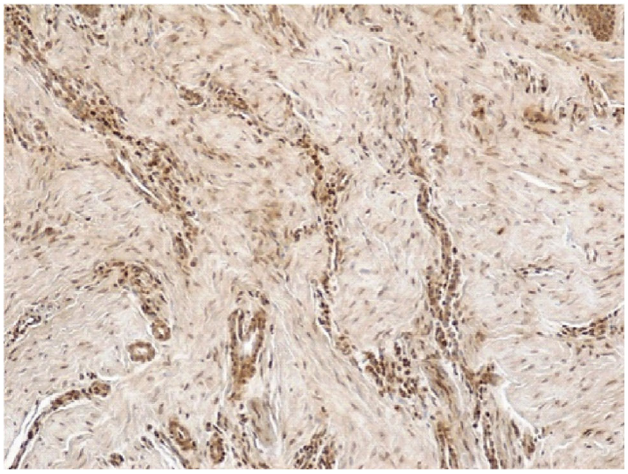

Figure 9 – ***High number of CD3+ T-lymphocytes situated among the collagen fiber fascicles in a patient with Turner syndrome and marginal periodontitis. Immunomarking with anti-CD3 antibody, ×200. CD3: Cluster of differentiation 3.***

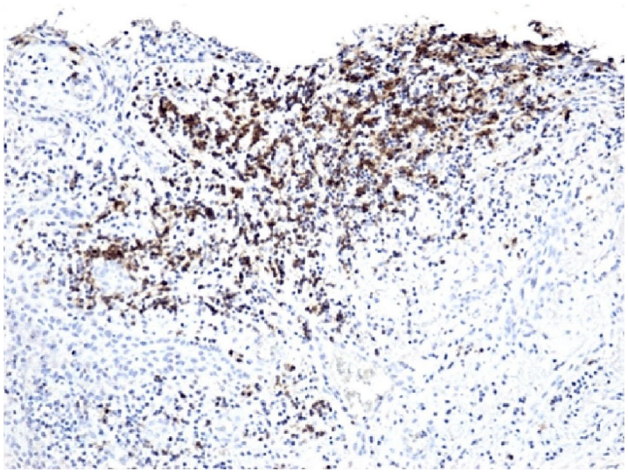

Figure 10 – ***Abundant inflammatory infiltrate, with a high number of B-lymphocytes, in a case of periodontitis associated with surface epithelium necrosis. Immunomarking with anti-CD20cy antibody, ×100. CD20cy: Cluster of differentiation 20 (cytoplasmic epitopes).***

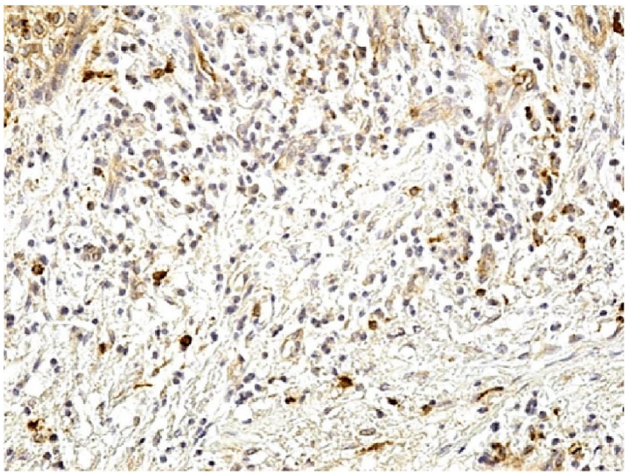

Figure 11 – ***CD68-positive macrophages scattered diffusely in the chorion in a case of gingivitis. Immunomarking with anti-CD68 antibody, ×200. CD68: Cluster of differentiation 68.***

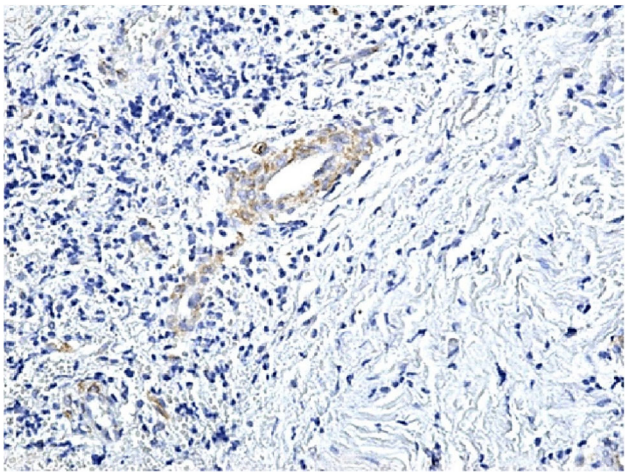

Figure 12 – ***Positive reaction for MMP2 in the blood vessels of the chorion, in a diagnosed case of gingivitis. Immunomarking with anti-MMP2 antibody, ×200. MMP2: Matrix metalloproteinase 2.***

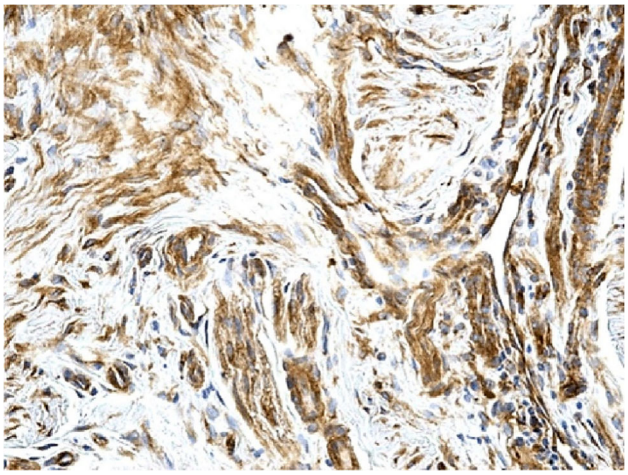

Figure 13 – ***Intensely positive immunohistochemical reaction to MMP2, in the fibroblasts, monocyte–macrophage cells and the blood vessels endothelium, in a patient with periodontitis. Immunomarking with anti-MMP2 antibody, ×200.***

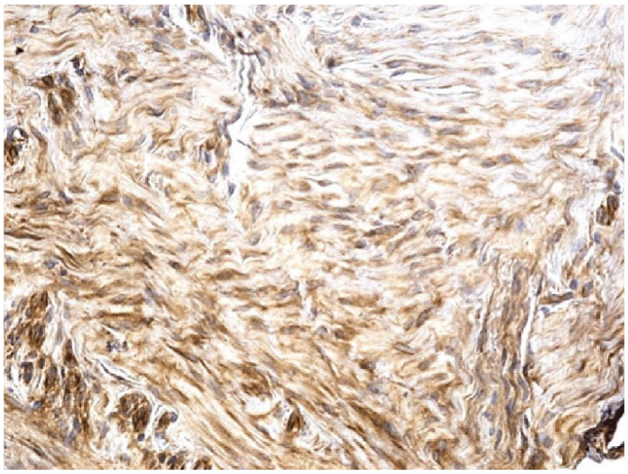

Figure 14 – ***Intensely positive reaction to MMP8 of the periodontal conjunctive cells. Immunomarking with anti-MMP8 antibody, ×200. MMP8: Matrix metalloproteinase 8.***

Discussions

In the patients with TS, there were observed changes of the marginal periodontium and, because this is connected to the rest of the body through vascular, lymphatic, and nervous structures, the periodontal lesions may influence the pathology of other organs. Also, this connection may explain the periodontal damage caused by the inflammatory reactions in other organs, and, thus, the onset and progression of periodontal diseases.

Some studies mention that the destruction of periodontal soft tissues is the result not only of the pathogenic microorganism action, but also of an immune response of the host. As a result, the purpose of therapy is to reduce the periodontal tissue lesions by diminishing the inflammatory reaction mediated by neutrophils and monocytes/macrophages and to re-establish the periodontal tissue health [29–31].

TS is often associated with serious diseases, like Hashimoto thyroiditis, Graves' disease, celiac disease, ulcerative colitis, Crohn's disease, juvenile rheumatoid arthritis, Sjögren's disease, sarcoidosis, psoriasis, thrombocytopenic purpura [32]. We observed that also the patients included in our study presented associated diseases: Hashimoto thyroiditis – 11 (61.1%) cases, Graves' disease – one case (5.55%), celiac disease – three (16.6%) cases, juvenile rheumatoid arthritis – one case (5.55%), and psoriasis – two (11.1%) cases.

The environment factors, infections and senescence in the autoimmune disorders may play an essential part in determining the clinical manifestations of periodontitis in patients with TS, through the disorders of the immune system [33–35].

Hashimoto thyroiditis was recognized as one of the most associated pathological states in the patients with TS [36, 37]. In our study, the women diagnosed with TS presented this autoimmune disease in a large number, as well (10 cases – 55%).

The risk for autoimmune disease in the patients with TS is approximately twice higher than in the individuals without TS [38].

Studies performed on Brazilian female patients diagnosed with TS showed that they have a genetic change that may be a predisposing factor for the development of an autoimmune disease [39].

Another study in the USA showed that the susceptibility of patients with TS to also have an associated autoimmune disease is due to a change of the forkhead box P3 (*FOXP3*) gene expression connected to chromosome X. The *FOXP3* gene controls the activity of T-cells and the complete loss of the *FOXP3* gene expression leads to the onset of a severe autoimmune disease [40].

Ever since 1948 Atria *et al.* reported that there is an association between autoimmune thyroid disease and TS and the subsequently performed studies showed that in many situations, hyperthyroidism accompanies TS [41, 42].

Other research studies showed that celiac disease may frequently be associated with TS, the disease incidence being 11% [43].

Moreover, there was observed that most patients diagnosed with TS who present a growth delay do not respond to the GH therapy if they also have an associated celiac disease.

Still, there are also patients diagnosed with a celiac disease in whom, despite the treatment, there persists an insufficient growth of the patient, and also hypogonadism. Cytogenetic studies showed the existence of an associated TS [44].

Of the dermatological diseases, there is observed a high frequency of the association between TS and the pigmented nevi and vitiligo [45].

On the other hand, the systemic changes that accompany TS may influence the periodontitis progression and may change the immune response of the host [46, 47].

Besides carries, periodontal disease or gingivitis may accompany TS [19, 48]. TS may be diagnosed before birth by the chromosomal study of the cells obtained from the amniotic fluid. Still, a third of the girls suffering from TS are diagnosed at birth [2, 49].

There are studies showing that dentists may diagnose first the TS, because they may recognize the morphological changes associated to this disease [50]. The early diagnosis of TS associated with oral conditions is extremely important for applying a specific treatment for TS that may also improve the progression of lesions in the oral cavity [2].

The treatment of dental hygiene for the patient with TS is quite challenging. The identification of odonto-periodontal changes may allow the decision for an individualized treatment [51].

Periodontitis is a chronic inflammatory disease that affects many individuals, especially in the underdeveloped countries [52]. The causes for periodontitis onset are multiple ones, involving both a bacterial component and some influences of the systemic or hormonal changes [53–55].

However, a special attention should be given to the immune system. Carranza & Cabrini showed the presence of mast cells in the periodontium and highlighted that the severity of gingivitis is correlated with the increase of mastocyte cell number [56].

Other studies showed that inflammation and oxidative stress maintain the periodontal disease, being associated with the presence of periodontal bags and bone necrosis. The administration of some antioxidative and anti-inflammatory products, like Resveratrol, considerably reduce the periodontal inflammatory phenomena [57].

Studies performed on girls diagnosed with TS showed a high number of lymphocytes, granulocytes, and monocytes in the reference intervals specific to age, but the report of CD4+/CD8+ T-lymphocytes was lower than expected [58].

The correlations between tissue damaging and the role played by inflammatory cytokines, through the effect these exert on the MMPs expression or on their balance, were intensely investigated in the periodontal disease. The host response because of the bacterial factor persistence may affect the homeostatic mechanism, thus determining the release of inflammatory mediators, including of pro-inflammatory cytokines, of MMPs (proteases) and of PGE2, which may promote the destruction of the extracellular matrix (ECM), of the lamina or fibronectin to stimulate alveolar bone resorption and remodeling of the conjunctive tissue [59].

MMPs are primary proteinases involved in the damaging

of the periodontal tissue through the degradation of ECM. They are secreted in a latent or inactive way by a multitude of inflammatory or conjunctive cells. Proteases capable of activating MMPs are represented by bacterial enzymes (such as chymotrypsin, a protease produced by *Treponema denticola*), as well as by neutrophils. Other activators of MMP are inorganic substances (metallic ions, thiol reagents and oxidants), enzymes (trypsin, tryptase, kallikrein), and other local factors produced by the tissues [insulin-like growth factor-1 (IGF-1), tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), transforming growth factor-beta (TGF-β)], glucocorticoids, retinoic acid, etc.

MMPs are inactivated by some macroglobulins that are found in the gingival crevicular fluid and serum [60, 61]. MMPs also play an important pathogenic role in other diseases (arthritis, hepatitis, periodontitis, glomerulonephritis, etc.) [62]. The cells in the human body that secrete MMPs are neutrophils, macrophages, mastocytes, epithelial cells, endothelial cells, fibroblasts, osteoblasts and even osteoclasts.

After the triggering of gingival inflammation by the bacterial microorganisms in the oral cavity, these cells synthesize MMPs in excess, thus leading to the destruction of the gingival conjunctive tissue and to the subsequent damage of the deep periodontal tissues [63].

It was observed, though, that the number of cells expressing MMPs is much higher in the inflamed periodontal tissue, in comparison to the healthy gingival tissue [64–66].

In the presence of a bacterial infection, polymorphonuclear (PMN) cells release large quantities of MMP to neutralize the pathogens invading the periodontium. Still, this overproduction of MMPs leads to collagen degradation, a main component of the periodontium.

In our study, we proposed to evaluate the IHC expression of MMP2 and MMP8 in the inflamed gingival mucosa and we observed that MMP2 and MMP8, respectively, are intensely expressed in the inflamed periodontal tissues or during the remodeling process. Still, the IHC reactions for MMP2 were different in intensity related to the diagnosis of periodontal disease. Thus, in the patients with a clinical diagnosis of gingivitis, MMP2 presented a moderately positive reaction in the endothelial cells of the blood vessels, in the fibroblasts and monocyte elements in the chorion, thus suggesting the fact that these enzymes play an important role in the damaging of the marginal periodontium.

The high number of cells expressing MMP8 found by us in this study explains a high possibility of the alveolar bone damaging. The intense reaction to anti-CD68 antibody in some cases from our study show an accumulation of macrophages capable of phagocytize cellular and tissular debris resulted from the inflammation of the periodontal tissues and to modulate alveolar bone resorption present in periodontitis. That is why we consider that the modulation of the immune response might be a potential therapeutic approach, but also of the resorption process of the alveolar bone.

As a matter of fact, other studies also showed that, starting from the role of MMPs in the damaging of periodontal tissues, there were promoted lots of antibiotic therapies addressed to periodontal inflammatory diseases, which, besides the antimicrobial properties, they may also reduce the levels of MMPs [61, 67].

Relatively recent studies showed that the patients with TS respond to surgical and periodontally regenerative treatments in the same manner as the patients with a normal phenotype [27].

Conclusions

The inflammatory lesions found in patients with TS suffering from periodontal disease were reduced, moderated or severe in intensity, mainly manifesting through moderate papillary and marginal inflammation, microhemorrhages, vascular congestion, angiogenesis, and collagenous fibrosis in the periodontium. The IHC expression of MMP2 and MMP8 indicate the involvement of these endogenous enzymes in the lesions of the marginal periodontium. The varied changes observed in the patients with TS may be caused by associated comorbidities, but also by the introduction of hormonal therapy in due time, which means that these patients should receive a close monitorization from various medical specialists. To maintain an appropriate oral health, it is important that the dentist should recognize the clinical signs of TS and its impact on the oral cavity and especially, on the marginal periodontium.

Conflict of interests

None to declare.

Authors' contribution

Smaranda Adelina Preda and Nina Ionovici equally contributed to this article.

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Received: March 3, 2021 *Accepted: August 17, 2021*