



The effect of Probiotics on the Clinical, Histopathological and the laboratory Findings in a Female Patient with Oral Proliferative Verrucous Leukoplakia and Rheumatoid Arthritis (A Case Report).

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Abstract

Proliferative verrucous leukoplakia (PVL), has been segregated as a separate entity and defined as a diffuse white papillomatous lesion that occurs in multiple areas in the oral cavity at the same time. Despite the fact it starts as a minute keratotic area, it can expand in size in a slow clinical course. PVL also has a high-risk rate of malignant transformation in 60-70 % of the cases. It is also seen more in adult females. Rheumatoid Arthritis (RA) is a common autoimmune disease characterized by the inflammation of small joints of hands and feet together with other systemic complications including cardiovascular and renal disorders. Several immune mechanisms are involved in its pathogenesis. The use of probiotics has been widely spreading over the last few years. Their benefits have exceeded its frequent use in the regulation of intestinal health. Nowadays Probiotics have been used in several events as an anti-inflammatory and immune-modulatory therapy that proved to be potent and safe in many animals and in vitro studies, together with few human studies. In this case report the use of probiotics; Protexin Balance® 60 mg was given to a 58-year old female, twice a day after meal time taken for 16 weeks; proved to improve the clinical and histopathological findings in PVL through the reduction in size, keratosis and signs of epithelial dysplasia in the affected cells. Also, probiotics led to the change in the laboratory findings related to the activity of RA. This change was represented by the reduction of levels of C-reactive protein (CRP), Rheumatoid Factor (RF), and Erythrocyte Sedimentation Rate (ESR.). Also by the noticeable relief and the feeling of well-being that was experienced by the patient herself.

Keywords: Proliferative Verrucous Leukoplakia, Epithelial Dysplasia, Rheumatoid Arthritis, Probiotics.

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Introduction:

Rheumatoid Arthritis (RA) is a multifactorial (Genotype, environmental factors, and immunological factors interacting together) autoimmune disease characterized by symmetrical polyarticular arthritis of hands and feet primarily and then progress to other joints as the temporomandibular joint (TMJ) accompanied by the muscular pathology of the orofacial area. The incidence of RA ranges from 0.5–1.0% in adult population worldwide [1]. RA is associated with the

inflammation of the joints lining which is known as the synovium. The synovium is an acellular structure, but in RA it becomes infiltrated by B lymphocytes, CD4 cells, and macrophages. Hyperplasia of the intimal lining of the joints takes place as a result of a remarkable increase in macrophage-like and fibroblast-like synoviocytes. Neutrophils activation leads to the release of enzymes, like; matrix metalloproteinases, proteases and aggrecanases, leading to the degradation of the extracellular matrix and destruction of the articular structures [2]. The actual cause of RA is unknown; this explains the fact that RA is diagnosed according to the clinical phenotype [3].

Therefore, RA is usually diagnosed through the presence of the following features: synovial inflammation and hyperplasia; causing swelling, redness and tenderness of the joints, autoantibody production as rheumatoid factor (RF) and anti-citrullinated protein antibody [ACPA], and in addition to cartilage and bone destruction leading to there deformity, and systemic complications, such as cardiovascular, pulmonary, psychological, and muscular disorders [4].

Several studies have recognized that the risk of RA among women is more than among men. It is usually associated with adverse life incidence or exposure to a shock. These phenomena have been explained on molecular basis; which show a relation between the hypothalamic–pituitary–adrenal axis and cytokine production. The central nervous system regularly plays a role in immune regulation and homeostasis, together with neuro-immunologic interactions in arthritis as per studied in rodent models. These reactions can operate locally through the release of several neurotransmitters as in synovitis in rheumatoid arthritis) or centrally through the rapid up-regulation of cytokines in the hypothalamus during peripheral inflammation [5].

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Based on the several mechanisms that take place in the development of RA, various chemopreventive therapies are always studied to try to improve the quality of life of the patients and try to alleviate their pain, also, to try to prolong the time by which they develop a systemic disorder as a result of RA. Proliferative verrucous leukoplakia (PVL) has been recently defined as a diffuse, white, smooth or papillomatous patch that develops as a result of oral epithelial dysplasia. The most common affected areas are; the alveolar ridge, buccal mucosa extending till the buccal vestibule and also the lateral surface of the tongue. Although PVL has a very slow clinical progression from a mild keratotic lesion to a whole diffuse white patch; 60-70% of the cases reported malignant transformation into oral squamous or verrucous carcinoma [6,7]. The gingiva is the most common site that reported malignant transformation [8].

The incidence of PVL has been found to be more in females than in males and is usually associated with smoking and alcohol use in about two-third of the cases [7]. The etiology of PVL is still unknown, but in some cases Candidal infections and Human Papilloma Virus, sub-type 16, have been suggested to act as predisposing factors for PVL [8]. Clinically PVL can be differentially diagnosed from other white keratotic lesions, e.g. oral lichen planus, hyperplastic candidal leukoplakia. Oral lichen planus is usually associated with the presence of the Wickham's striae and usually associated with characteristic skin lesions. On the other hand, hyperplastic candidal leukoplakia has a very diagnostic feature; it is usually bilateral lesion occurring at the angle of the mouth, where its base is at the commissures and tapers towards the buccal mucosa in a triangular pattern [9]. In advanced stages, PVL cannot be clinically differentiated from verrucous carcinoma [10]. The definitive PVL diagnosis is established by taking multiple biopsies and determining different grades of epithelial dysplasia (Grade I, II and III) within the same lesion [11].

Surgical excision has not been the best approach for the treatment of PVL; as the lesion is diffuse and can affect multiple regions in the oral cavity, and its recurrence rate is very high. Several treatment modalities have been used like; laser, chemotherapy even in a study by Femiano et al., reported the use of Anti-viral (methisoprinol). In the treatment of PVL [12,13]. In(2003), Reichart and Philipsen determined that several studies should be carried out for studying the etiologic factors and other therapy modalities [14].

Probiotics have been widely researched and studied over the last few years. Probiotics are defined as "Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" [15]. The most beneficial microbes used in probiotics are Lactobacillus and Bifidobacterium. There are various mechanisms by which probiotic microorganisms perform their functions including competing with pathogens for nutrients or adhesion sites, degradation of pathogenic toxins, stimulating local and systemic immunomodulation together

with the release of antimicrobial substances [16]. In (2005), Oyetayo discussed the criteria of microbes that should be considered probiotic as follows; a) It should have a beneficial strain to the host by increasing its resistance against pathogens, b) it should be safe and non-toxic, c) Viable and preferably in large numbers, d) It can survive in the intestinal environment where it can survive in lower PH and bile, e) it can be stored under normal and convenient conditions [17].

Based on the previous data, it was intriguing to use probiotics in the case that was referred to the private practice to try to find new and unconventional methods to improve the patient condition and to try to get satisfying results without going through the dilemma of multiple surgical procedures and by using a safe and new treatment modality.

Case presentation:

A 58-year old female was referred to the private dental practice complaining from a white spread white course patch, started as a small white area related to the ridge then increased in size and expanded till the lateral surface of the tongue and buccal vestibule of the right surface of the oral cavity. The patient also complained of tenderness related to the temporomandibular joint (TMJ) whose onset started after being diagnosed with RA.

The patient underwent the following diagnostic procedures:

History of Chief Complain:

The lesion started as a minute keratotic area related to the right alveolar ridge one year before the examination. During one year period, it expanded as a white patch to include the lateral surface of the tongue till the buccal vestibule. The lesion was not painful or ulcerated, but it was rough in texture and was annoying to the patient.

Medical Condition:

The patient suffered from rheumatoid arthritis (RA) five years before. At the time of the oral examination, there was no swelling of the joints, i.e. no flaring, she was taking a maintenance dose of 5mg prednisone once/day and Hydroxychloroquine sulfate (Plaquenil)® 400mg once/day as an analgesic, in addition to multivitamins.

Lab. Investigations: The patient presented her latest Lab. Investigations where:

Hemoglobin level (Hb) 11.7 g/ dl, C-reactive protein (CRP) 96, Rheumatoid Factor (RF) 64, Erythrocyte Sedimentation Rate (ESR) first hour: 40, second hour 70.

Intraoral Examination:

Upon clinical diagnosis of the lesion, it was represented as a white diffuse papillomatous patch related to the lateral surface of the tongue, alveolar ridge, and extending to the buccal vestibule (Figure 1).

Differential Diagnosis of the lesion was: Proliferative Verrucous Leukoplakia, Oral leukoplakia, Lichen planus, Chronic hyperplastic candida. An incisional biopsy was taken from the lateral surface of the tongue for histopathological examination and the confirmation of the diagnosis (Figure 2).



Figure1(a,b): A photograph of a Proliferative Verrucous Leucoplakia (PVL) as diffuse white patch related to the tongue, ridge and buccal vestibule.

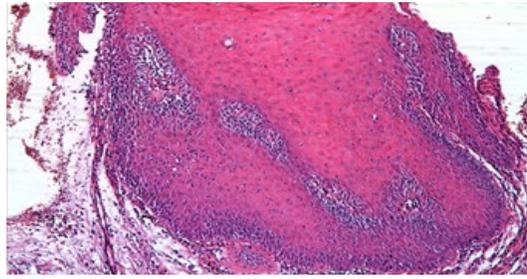


Figure 2: A photomicrograph of the epithelial dysplasia in the proliferative verrucous leukoplakia field showing basilar hyperplasia, mitotic figures and blunt elongated rete pegs in 2/3 of the epithelial layer(H&E×100).

Treatment plan:

After consultation with the patient's Rheumatologist, a regimen of probiotics for 16 weeks was suggested by the consultant to try to investigate its effect on the (PVL) lesion and also whether probiotics will lead to the improvement of the laboratory values of the patient or not.

The treatment was given in the form of Protexin Balance® 60 mg/ twice per day after mealtime for 16 weeks. The microbes in this probiotic included: Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophiles, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacterium longum, Lactobacillus bulgaricus. This probiotic was chosen as it contained the most well-known and studied microbes that are beneficial in the anti-inflammation process and boosting the immune system.

This regimen was given when the patient confirmed that she did not take any antibiotics or corticosteroids for the last 3 months before starting the probiotic therapy.

Before the beginning of this regimen, the patient signed a consent form that was revised and accepted by the Research Ethics Committee of Faculty of Dentistry, Cairo University and was given the code number 18430.

Also, the patient was strictly advised to check with her Rheumatologist twice a month for following up on her general systemic condition and the development of any possible complications.

After 16 weeks, another photographs of the lesion were taken, and another incisional biopsy to examine the histopathological changes in the lesion. Also, laboratory investigations were carried out to find any changes in RF CRP, ESR and Hb levels after therapy.(Figure 3,4).



Figure3: A photograph showing a noticeable reduction in the size of the keratotic lesion together with almost complete resolution of the lesion in the buccal mucosa.

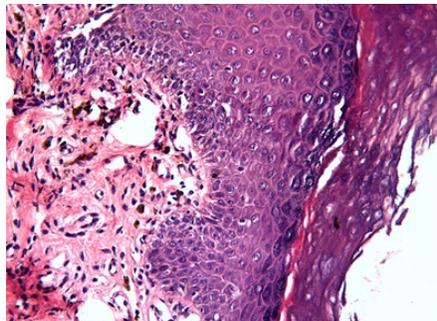


Figure4: A photomicrograph of the epithelial dysplasia in the lesion post-treatment showing thinning of the epithelial layers. Lesser rete pegs and no basilar hyperplasia (H&E×200).

Discussion:

After 16 weeks the patient was examined, and another incisional biopsy was performed to determine any cytological changes that took place in the tissues. Clinically there was a noticeable change in the size of the lesion where it reduced in size, and it also disappeared in other areas as in the buccal mucosa. Histopathologically, cells with signs of epithelial dysplasia were reduced, thinning of the epithelial layers, the rete pegs were less broad and became shorter with no basilar hyperplasia. Human researches that studied the effect of probiotics on oral premalignant lesions were very rare. The explanation of probiotics led to a reduction in epithelial dysplasia can be attributed to the fact that was studied in 2006 by Brzozowski et al. That study explained that *Helicobacter Pylori* – infection of the gastric mucosa in Mongolian gerbils enhanced apoptosis and mucosal proliferation.

Apoptosis was due to marked the expression of Bax protein was markedly enhanced and the inhibition of Bcl-2 protein in gerbils infected with *H. pylori*. [18]. A tumor to develop it needs the inactivation of the Bax and overexpression of Bcl-2, and thus an overall inhibition of apoptosis occurs [19]. That study also showed an impressive finding which is the use of anti-*H. Pylori* together with Lacidofil therapy were prominent in the reversal of the apoptotic cascade and elimination of the unnecessary cells [18].

Another study was done by Yan et al., [20] studied the probiotics potential to prevent Intestinal Dowel Disease (IBD), by the use of soluble *Lactobacillus rhamnosus* GG (LGG) soluble factors which lead to the regulation of cell survival signaling and inhibit cytokine-induced apoptosis in intestinal epithelial cells [20]. Probiotics were also proved to activate the intestinal epithelial cell protective responses through synthesis and secretion of mucin, enhancing the epithelial function as a physiologic barrier, and promote cell survival [21,22,23,24,25].

Several studies conducted on animals and humans also proved that probiotics play a significant role in enhancing both specific and non-specific immune response; through the activation of macrophages, increasing natural killer cells activity, increasing cytokines and immunoglobulin levels. Activation of the immune responses also result in inhibition of pathogen growth and reducing the chances of infection from pathogenic microorganisms [26].

Human Studies that discussed the effect of probiotics on prevention of cancer are very rare. Only one clinical study showed an increase in the recurrence-free period in subjects with bladder cancer and was taking probiotics. Also, animal models and in vitro studies indicated that probiotics could reduce colon cancer risk via the reduction of its incidence and growth of tumor cells. [27].

The laboratory investigations of the patient concerning the activity of Rheumatoid arthritis after 16 weeks were: Hb 11.7 g/dl, C-reactive protein (CRP) 6, Rheumatoid Factor 30, Erythrocyte Sedimentation Rate (ESR) First hour 26, Second hour 45. By comparing these findings to those done before therapy; there was no change in Hb level, the reduction in RF and ESR values were 47 % and 65 % respectively. The apparent reduction was in the value of CRP that changed from 96 before treatment to 6 after probiotics. This means that inflammation was reduced by 93.5 %. On the clinical level, there was marked relief of the TMJ tenderness, and the patient was satisfied with the results. Also upon regular examination of the patient by her Rheumatologist, there were no flaring or swelling of any of the joints.

These results came in accordance with the pilot clinical study that evaluated the long-term use of *Lactobacillus rhamnosus* GG (LGG) on the symptoms of RA. This double-blind study included 21 patients suffering from RA that were randomized and given two capsules of LGG and placebo twice daily for 12 months. There was a reduction in a mean number of tender and swollen joints from 8.3 to 4.6 in the LGG

group while the placebo group the reduction was from 5.5 to 4.8. The activity of RA was reduced by 71% in LGG group, and the majority of the patients reported a better feeling of well-being [28].

In 2010 A clinical study was done by Mandel et al. proved that RA patients, when treated with *Bacillus, coagulans* GBI-30, 6086 for 60 days experienced improvement in the Patient Assessment Score ($p=0.052$) statistically, and Pain Scale ($p=0.046$). Also, there was a significant improvement in the patient self-assessed disability together with the reduction in CRP levels [29]. This reduction in inflammation was because *Bacillus coagulans* GBI-30, 6086 once gets in the intestine it becomes activated and releases anti-inflammatory molecules. *Bacillus coagulans* GBI-30, 6086 also produce bacteriocins and lowers intestinal pH leading to the eradication and the dislodgement of microbes that can initiate an inflammatory response [30].

In 2011, Amdekar et al. they conducted an animal model study and proved that *Lactobacillus casei* had an anti-inflammatory effect on Collagen-Induced Arthritis (CIA) via the inactivation of COX-2 and NF- κ B which are potent inflammatory mediators associated with RA [31].

This current study was one of the very few studies that tried to prove the impact of probiotics use on one of the aggressive premalignant lesions like PVL and at the same time its effect as an anti-inflammatory in an autoimmune disease like RA.

This study experienced limitation due to the short period during which the study was conducted; 16 weeks are not enough. Also, the probiotics have to include more numbers of patients suffering from oral premalignant lesions and autoimmune diseases like RA separately to try to document accurate results regarding each disorder.

Conclusions:

Probiotics can be a useful and safe therapy that can improve the inflammatory symptoms and modify laboratory findings in RA patient. Probiotics also can promote apoptosis of cells with epithelial dysplasia through their immuno-modulatory effect. Probiotics lead to clinical improvement of proliferative verrucous leukoplakia (PVL) through reducing the size of the lesion, in addition to changing both texture, and the intensity of keratosis of the lesion.

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