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Nephelometric Quantification of Complement C1, C3 and C5 Components of Gingival Blood, From Health to Early Disease. Experimental Study on Human

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ABSTRACT

The complements play an essential active role in the gingival inflammation, their roles have been studied in the connective tissues, in the crevicular fluid and in the peripheral blood as well. Unfortunately, the circulating peripheral blood cannot reflect a real picture of the immunologic reactions in the gingiva. In addition, the quantification of complement concentration in the gingival fluid or in the tissue could not been measured before. This study tries to quantify the amount of C1, C3 and C5 complement compounds in the gingival blood as a closest source to the site of inflammatory process of the gingiva. And to find a correlation between their concentrations compared to the inflammation severity. Twenty volunteers, dentistry students, sampled their gingival blood in three events, healthy gingiva, early disease, then returned to healthy state. The sera studied with the immuno-Nephelometer after a specific preparation. A specific antibody anti complements C1, C3 and C5 have been used to form a specific antigen-antibody complex in vitro. The results showed a significant increase in amount of C1, C3 and C5 (P<O.O5) plus a positive correlation with the progression or regression of the inflammatory process. These complements showed a positive significant correlation (P<0.05) with the GI of disease progression, but insignificant with regression. In conclusion, the gingival blood could give a close immunologic feature of immunologic changes of gingiva during health or disease.

Keywords: Complements, Immuno-Nephelometer, Gingival Blood, Experimental Gingivitis.

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INTRODUCTION

The immunologic reaction, general or local on a specific site, depends largely on the activation of the complement system. The complement orchestrate a critical events which include recruitment and activation of both, humoral and cellular immunity [1] Moreover, complement bridges innate to adaptive immunity by regulating the activation of both B cells and T cells, either directly or through effects on antigen-presenting cells [2]. On other hand, its protective role notwithstanding, complement may cause or exacerbate inflammatory tissue damage when over activated or deregulated, either by pathogens or due to inherent host genetic defects [3,]. Indeed, complement pathways constitute a major link between infection and various local or systemic inflammatory or autoimmune disease [4].

This communication reviews evidence implicating complement in periodontal inflammation and pathogenesis. Furthermore, it discusses interventional strategies that could complement current clinical periodontal treatment, which is often not sufficient by itself to reverse destructive inflammation [5]. On the gingiva, inflammation when occur, it will include the entire of gingival tissues, mainly, gradually, starting on the junctional epithelium and then forwarded to the gingival fibrous unit, and more, the entire periodontium is incorporated with inflammation including the local circulating blood system [6]. The gingival inflammation is a plaque induced infection, which means a bacterial antigen challenges going to take place. The entire immunologic elements are coming from the closest blood circulation to the site of infection where the adaptive immunologic process react [7]. Moreover, complement bridges innate to adaptive immunity by regulating the activation of both B cells and T cells, either directly or through effects on antigen-presenting cells [8].plaque-induced gingival inflammation is a bacterial infection initiated by the bacterial antigens and toxins started as gingivitis involving the gingival unit (epithelial and fibrous with their innervation and vascularization systems) that attract the immune elements from the nearby blood source, the specific and the nonspecific elements including the complements [9].

The complements are one of the most biologically complex chain of proteins implicated in the immune system, this chain includes 22 or more of an active biologic protein composing more than 10% of total plasma globulin. They play an essential role in matching and energizing the immunologic and inflammatory reactions [10]. The complement system is activated when C1componant is stimulated, in classic way, or when C3 is stimulated in, alternative way. The C3 plays a central role and have a noticed importance. The C5 acts as a cytolysing agent for all categories of cells [11]. C3a, C4a and C5a are anaphylactic proteins able to cause the contraction of smooth muscles, as well as to cause the degranulation of mastocytes to liberate histamine, which act on blood vessels to increase the vascular permeability [12]. On other hand, C3 and C5 act as intermediators for the chemotactic activity against the macrophages, they able to adhere and fix the immune complex on cell wall. The opsonisation of macrophages and

polymorph nuclear leucocytes cannot been takes place without the function of C3a, C5 and C5a [13].

The activation of complement system in the gingival tissues could be matched by the presence of immune complex in the epithelial layer of gingival sulcus, even the dental plaque of healthy gingiva able to activate the complement leading to the cleavage of C3 and C5 [14]. Bacterial Lipopolysaccharide (LPS) with the extract of Fusobactrium nucleatum where demonstrated as an activator factor of classic pathway [15]. Again, the mucopeptide of propionibacterium and the endotoxin of bacteroid melanenegenicus actinomyces viscus and other G negative plaque bacteria were able to activate the complement cascade and matching the immunologic reaction in the epithelial lining of the gingival sulcus [16] in addition, the sulcular plaque bacteria have been demonstrated as covered with immunoglobulin G and M. to gather with complement C1 and C4 [17]). Complement activation in the gingiva result in liberation of active proteins during the inflammatory process [18]. This activation exhibits cytotoxic, anaphylactic and chemotactic activities. Both, C3a and C5a stimulate the leukocytic phagocytosis [19] C5, C6 and C7 are chemotactic factors acting on the neutrophils and macrophages [20]. Other components showed a cytotoxic activity against the fibroblasts reducing the possibilities of fibrotic regeneration [21].

The Aim of this Study is to

1. The use of local gingival blood circulation as a source of immunologic data in gingiva.
2. Quantification of C1, C3 and C5 in correlation with gingival index (GI).
3. Follow the variations in quantity of C1, C3 and C5 in correlation with disease progression and regression.

MATERIAL AND METHOD

Volunteers Groups

Twenty dental students, 10 male and 10 females, aged 19-22-year-old, have been chosen with good oral hygiene, systemically healthy and healthy gingiva according to the clinical examination based on gingival index (GI) [22]

Three samples taken, first sample of gingival blood, when the gingiva seemed healthy with GI score mean was 0.202+/- 0.012, all volunteers advised to practice a perfect oral hygiene control, for two weeks, followed with antiseptic mouth wash (chlorhexiden 0.12%) once daily before going to bed immediately. Second sampling of gingival blood, taken from the same sites, two weeks later, and volunteers advised to give up home care completely during these two weeks. The mean of Gingival Index (GI) score was 1.42+/- 0.23.

The third sampling, all volunteers advised to perform a perfect home care with brushing the teeth, chlorhexiden 0.12% mouth wash once daily at night. The mean GI score was 0.761+/- 0.045, the clinical signs of gingiva returned to healthy. No professional care was performed.

At the same time, three samples of general circulating blood have been taken from arm vein, 1ml at each time (S1, S2 and S3) processed as done with gingival samples.

Sampling Protocol

Upper anterior segments have been chosen as site of local circulating blood sampling. A capillary tube of constant volume of 10ul has been used to for blood collection directly from gingival papillae, after picking the papillae at the col-du-sac. The site, from canine to canine of maxilla, has been cleaned, disinfected with povidon iodine 3% and dried, isolated with cotton roles from both sides. Seven capillary tubes of 10^{ul} of gingival blood were gathered, diluted up to 1/40, centrifuged at 5 ^{0}C to exclude the cellular elements.70^{ul}+2730^{ul} of transparent physiological solution= 2800^{ul}. A specific antiserum Anti C1, C3 and C5 have been added in constant volume on each separated equal third of supernatant of the nominated component. Incubation for one hour results to get a measurable immune complex. The antibodies are specific antisera anti complement of human.

With nephelometric laser, the immune complex measured according to the degree of reactional turbidity of the mixture (13), where the complement considered as antigen. (Immuno-nephelo-meter of Hyland, USA. Antiserum laser, Travenal laboratories, S.A, USA).

RESULTS

The mean average of first sampling (S1) was C1=0.432 +/- 0.1 $^{G/L}$, C3= 0.442 +/- 0.12 $^{G/L}$ and C5= 0.0724 +/- 0.08 $^{G/L}$. The second sampling (S2) was as follow; C1=0.786 +/- 0.04 $^{G/L}$, C3= 0.661 +/- 0.03 $^{G/L}$, C5= 0.162 +/- 0.02 $^{G/L}$. The third sampling (S3) was as; C1=0.494 +/- 0.21 $^{G/L}$, C3= 0.552 +/- 0.15 $^{G/L}$, C5= 0.100 +/- 0.06 $^{G/L}$.(table1).

The gingival index scores (GI) showed results as follow; S1 mean = 0.202 +/- 0.012, S2 mean = 1.42 +/- 0.23. S3 mean = 0.761 +/- 0.046. (table2).

The average ratio of complements in general circulating blood is as follow; C3> C4>C3PA>C5.

There is a significant evolution of the GI ($P<0.05$) when compare the mean of S1 with S2, as well the evolution of complement amounts C1 C3 and C5. In comparing the mean of SI and S2. The amounts of complements showed a significant decrease ($P<0.05$) in S3, while the regression of GI was insignificant when compared with that of S2.

C1 seemed to be greatly influenced with inflammatory process, the increase in amount from S1 to S2, and the regression from S2 to S3, both was significant ($P<0.05$). C3 appeared to be increased in less amount than did the C1. This result may suggest a classic pathway of activation. The C5, almost doubled in S2. The evolution of complements C1, C3 and C5 appeared to be positively correlated in significance with the degree of inflammation severity ($P<0.05$), their increase or decrease in concentration is significant, while the evolution of GI appeared significant in progression but insignificant in regression, even though, it takes the same curve design.

The complement ratio within S1 and S3 appeared in resemblance with each other, and with the ratio of the general circulating blood. But, it inverted in S2 when the C1showed increased concentration.

General blood specimens didn't show any differences in amounts or complements ratio to each other in the three samplings (S1, S2 and S3), the comparisons were all insignificant, and all reading were almost constant. The

compare of these reading vs the gingival blood in S1, and S3 showed insignificant relations.in exception of S2, where there is a significant difference in favor of gingival blood for CI and C3 (Table 3).

DISCUSSION

The interest in studying the gingival diseases from the point of immunologic view appeared in three essential points, 1st, the huge micro organismal species present permanently covering the entire oral cavity organs which make the tissues embedded in a contaminated field, 2nd, the barriers of tissue defense are so weak to point of a continuous penetration and invasion of bacterial Antigens is possible this allow a continuous activation of tissue chemicals including the complements, 3rd, the bacterial enzymes and toxins act to activate immune elements capable to increase the tissue destruction, thus facilitate the antigenic factors more deeply and emerges the humoral elements through the defected tissue barriers making the immunologic process in the gingiva as a double blade knife.

This situation making the inflammatory and the immunologic processes interlinked with each other with no clear-cut line between health and disease [23]. Histological studies could show a feature of pristine state with other different feature of clinical healthy gingiva with the permanent presence of inflammatory and immunologic cells [24]. The immunologic elements of general circulating blood could give a real clear and close feature about the presence of a systemic diseases and could reflect a real hematologic and serologic changes that leads to a spot diagnosis [25]. Unfortunately, the general circulating blood couldn't give the diagnostic features of gingival and periodontal conditions. Earlier, few studies suggested a potential role of the complement's proteins in the inflammatory process of gingiva and suggested an initiative role in tissue destruction [26]. In addition to IgG, IgA and IgM, the C3, C4 and C5 have been detected in the gingival fluid [27], it has been demonstrated a local biosynthesis of complements C3 and C5 in the tissues of a chronically inflamed gingiva [28].

The increase in C1 amount and the inversion of complements ratio in S2 could support the earlier findings and suggest a local biosynthesis of C1, C3 and C5, and suppose that the activation of cleavage of complements in early gingivitis is mainly classic pathway due to the predominance of Gram positive bacteria in the dental plaque and the high possibility of antigen antibody complex formation [29].the C3 and C4, have been extracted from the gingival fluid of healthy gingiva as well from diseases gingiva. The concentration of C3 and C4 have been found higher in case of inflamed gingiva than that of the healthy [17]. In young adult patients having chronic gingivitis, C3 and C4 concentrations appeared elevated, that of C3was higher than that of C4 [30]. Some studies showed the cleavage of C3 into C3a and C3b increases with the progression of the disease. A positive correlation have been found between the C3 concentration and the regression of the inflammation with treatment [31]. These studies, including this one, suggest that there are a local production and local activation of complements compounds during the inflammatory process in the gingiva which supplied with local blood [32].

Indeed, it should be noticed that the cleavage of C1, C3 and C5 could occur due to the proteolytic action of certain bacterial enzymes [18].earlier studies concluded that the complements are actively existed in gingival tissues and suggested a local biosynthesis [32]. Their production varies, depending upon the disease severity and according to a clinical entity [18, 33].The ratio of C1, C3 and C5 of inflamed gingiva (S2) appeared varied from those of general circulating blood, this result seemed to be related to a local production of complement, this, suppose that the gingival blood could be a good witness to exhibits a clear and close local immunologic picture of a pathologic changes at the different stages of the gingival disease progression. C1 acts as a starter for the complement cascade, C3a and C5a are anaphylatoxic, they have the ability to degranulate the mast cells and liberate histamine which increases the vascular permeability [34].

The neutrophil diapedase increases as the intercellular spaces of the endothelial cell enlarged. Both, the diapedeses and the extra vascular exudation of plasmatic fluid are the major cause of the oedematic appearance of active gingival inflammation [35]. neutrophils, could showed a hyper function in active aggressive periodontitis as a response to increased complements activity and amount [32, 36]. The insignificant differences in amounts of C1, C3 and C5 when comparing data of S1 vs S3, means that the regression in inflammation accompanied with the regression in complement activation, this result could confirm that complements fragments evolve with positive correlation in its concentration in the gingival blood ($P<0.05$). In conclusion, studying the gingival blood could give a close immunologic and inflammatory feature of gingival inflammatory process high lighter than gingiva crevicular fluid.

Table 1: Means of gingival index scores (GI)

S1	S2	S3
0.02 +/- 0.012	1.42 +/- 0.23	0.761 +/- 0.046

Table 2: Amounts of complements in gram/litter (G/L) in gingival circulating blood

S1			S2			S3		
C1	C3	C5	C1	C3	C5	C1	C3	C5
0.432+/-O.10	0.441+/-0.10	0.724+/-0.08	0.786+/-0.04	0.661+/-0.03	0.162+/-0.02	0.494+/-0.21	0.552+/-0.15	0.998+/-0.06

Table 3: complement values in general circulating blood

S1			S2			S3		
C1	C3	C5	C1	C3	C5	C1	C3	C5
0.422+/-0.08	0.396+/-0.090	0.705+/-0.032	0.744+/-0.06	0.669+/-0.08	0.173+/-0.06	0.481+/-0.04	0.567+/-0.07	0.894+/-0.05

Table 4: Ratio of complements in circulating blood is constant in health and diseases as well the amounts

Ratio in general circulating blood	Ratio in gingival circulating blood
S1,S2,S3/ C3>C1>C5	S1/ C3>C1>C5 S2/ C1>C3>C5 S3/ C3>C1>C5

CONFLICT OF INTEREST

None

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