Nephelometric quantification of IgG in gingival blood from health to early disease (experimental assay on human)

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Abstract: We are dealing with the plaque-induced gingivitis as an experimental assay on young adult human volunteers. Creating gingivitis by withdrawn the habitual daily oral hygiene for 25 days, then re-establishit, no professional scaling done, for another 25 days to take three samples from circulating gingival blood as a closest point to the site of infection, the col-du-sac. Suggesting that this site is the field of antigen-antibody reaction. Analyses with immuno-nephelometric laser showed a positive correlation between BOP and IgG but insignificant. Our result suggest that the circulating gingival blood could be a source of studying the immunologic and inflammatory alteration during the gingival inflammation in reality as best as the gingival crevicular fluid.

Keywords: experimental gingivitis. IgG. Gingival blood. Nephelometer

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1. Introduction:
We are speaking about the plaque-induced gingivitis alone. Gingivitis is generally regarded as a site-specific inflammatory condition initiated by dental biofilm accumulation and characterized by gingival redness and edema; the absence of periodontal attachment loss (1). Gingivitis is commonly painless, could leads to spontaneous bleeding, and is often characterized by subtle clinical changes, resulting in most patients being unaware of the disease or unable to recognize it (2). Plaque-induced gingivitis is the complete reversibility of the tissue alterations once the dental biofilm is removed. Notwithstanding the reversibility of the gingivitis-elicited tissue changes, gingivitis holds particular clinical significance because it is considered the precursor of periodontitis.

Clinical methods to assess the presence and severity of plaque-induced gingival inflammation at the site level are based on the evaluation of crude macroscopic changes occurring in the marginal gingival tissues during the healthy-inflamed transition. The volume of the gingival crevicular fluid (GCF) has been largely adapted in clinical trials to assess the severity of gingival inflammation at site level. However, the most commonly used clinical measures for gingival inflammation mainly consist of qualitative or semi-quantitative indices based on visual assessment of gingival characteristics (edema/swelling, redness, etc.) and/or the evaluation of the tendency of the marginal gingiva to bleed upon mechanical stimulation exerted typically by a periodontal probe. These methods were first described more than 45 years ago and have not changed much since then (3). The gingival diseases are initiated by the bacterial challenges through their presence as an antigen and/or mitogen. Equally through the activation of immune system (4). The immune responses to dental plaque bacteria is of importance in the progression of periodontal diseases has been derived from the studies of the change which occurs in the inflammatory infiltrate during the course of the disease (5). As the Lymphocytes cell are present with the normal constituents of clinical healthy gingiva, the immunoglobulins are also present but in a negligible amount in both gingival tissues and fluid (6).

As the disease progress, the count of lymphocytes increases as well (7). Mostly of T type about 75%. During the progression of the disease, B cells, the precursors of plasma cells, becomes more predominant which mostly IgG producers (8, 9).
The single radial immunodiffusion studies of gingival crevice fluid showed that the concentration of IgG is varied according to the degree of inflammation. The crevicular fluid immunoglobulins are of blood plasma origin (10). Further immune florescent immunologic studies exhibited that IgG is a characteristic immunoglobulin of chronic type inflammation (11). Other evidences given suggested that the antigen-antibody reaction in gingival inflammation occurs in the gingival tissues just adjacent to the plaque inner wall. Thus the gingival blood seemed to be the closest source of plasma immunoglobulins could be incorporated in the gingival immune responses (12). The gingival immunoglobulin could be specific monoclonal as an antigen activation, or non-specific polyclonal as a result of mitogen activation (13).

2. The aim of this study:
Introduce the gingival circulating blood sampling as a studying material in experimental studies.
Looking for a correlation between the BOP and the IgG of gingival blood in health and disease.
Evaluate the IgG accounting changes.

3. Material and Methods
Twenty dental students (10 male and 10 female) as volunteers have been participate in this study, aged 20 up to 23 years old, regularly brushing their teeth since years, in good oral hygiene, haven't dental or gingival complains, systemically in good general health. All volunteers have been checked orally by others. Having clinically healthy gingiva, no signs of any other infection. Healthy gingiva, in color, volume and dentogingival relationship. Upper anterior region has been chosen as area of sampling the gingival blood. All volunteers have been asked to withdraw brushing and any other oral hygiene control aids next to the first sampling up to the next sampling, each one-week volunteers were asked for oral checking.

3.1 Starting:
Capillary tubes of constant volume of 10 ul have been used to collect gingival blood from gingival papillae after picking the papilla at the inner site of gingival sulcus at col-du-sac area with a sterilized injection needle smoothly. Areas of sampling have been isolated with absorbent paper strips and a cotton wools inserted around the anterior dentition. Seven capillary tubes (total 70 ul) of blood from each volunteer have been collected, the diluted with physiologic solution (hanks) up to 1/40 in conical test tube, then centrifuged at 50c, speed of 3500 RPM in order to exclude the cellular elements. Each 70ul adding 2730 ul of transparent physiologic solution become 2800 ul. Then 10 ul of specific antiserum anti IgG added, incubated for 15 minutes in normal room degree (22oC), then entered in the nephelometric laser unit at room degree centigrade.

3.2 The immuno-nephelometric laser:
An auto meter unit (11) able to measure the agglutinated particles of antigen-antibody complex in a liquid media by means of a laser ray which defused in the medium and deflected in angle 0<8<90o then reflected to a sensible screen which can measure the difference in loss of laser ray. This difference gives the amount of immunoglobulin present in tested solution.

3.3 Sampling:
Three samples have been taken from each volunteer, first with average BOP was 0.202+/- 0.012. Second sampling when the clinical signs of gingival inflammation appeared 21 to 25 days later, the BOP recorded in average was 1.42+/- 0.23. The third sampling has been taken 25 days later after restoring the oral hygiene control as usual daily home care without scaling or any professional clinical interference. Antiseptic mouth wash (chlorhexiden 0.12%) once before bedding was advised. The average BOP was 0.76+/- 0.045.

4. Results:
The average BOP score of first sampling when the gingival condition showed a usual clinical healthy condition, was 0.202 +/- 0.012. Then significantly increased up to the average score of 1.42+/-2.3 indicating a mild inflammatory process has been occurred in the gingival tissues. The third sampling, when the healthy gingival condition was restored, showed an insignificant decrease in BOP to 0.761+/-0.046 (table1). The evolution of the immunoglobulin G count of gingival blood was significantly increased from 10.4323+/- 1.15G/L of the first sampling (when the gingiva is clinically healthy) up to the 10.7982+/- 1.13G/L. The third sampling showed, as the clinical healthy condition was restored, an insignificant decrease of IgG count as 10.354+/- 1.04 G/L (table1). Even this evolution is weak, it takes the same curve as the BOP does. Even the IgG counts were insignificant, it may exhibit that its evolution follows the progression and regression of the clinical
condition when the disease is reversible. This state may suggest a positive correlation between IgG of gingival blood and the BOP of clinical condition.

5. Discussion and Conclusion:
A comprehensive periodontal examination is generally based on the examination of all present teeth at its six surfaces of each tooth, mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual, to get the index score of one tooth, then by a simple mathematic calculation can get the average score by arch and then individual of both arches, as our result has been calculated. Bleeding from the sulcus is the earliest clinical symptom of gingivitis and precedes discoloration and swelling (14). Therefore, since gingival color changes are less obvious during the early stages of gingivitis and are most often overlooked, bleeding from sulcus can be considered a clear-cut sign of pathology (15). Bleeding on probing (BOP) is a widely used criterion to diagnose gingival inflammation. The classical signs of gingival inflammation associated with gingivitis are bleeding from the gingival sulcus upon gentle probing (BOP), increased gingival fluid flow rate, swelling, and discoloration of the gingival units. BOP may be used for discriminating between a healthy and gingivitis condition (16). The bleeding on probing (BOP) use the clinical sign as indicator of the periodontal condition and disease progression (17). Indicator of periodontal stability (18).

Another criterion indicator, and marker, is the crevicular fluid flow, there is a positive correlation between the flow and the gingival inflammation (19). GCF is composed of serum and locally generated components such as tissue breakdown products, inflammatory mediators and antibodies in response to oral microorganisms present in the dental biofilm thus it offers great potential to reflect the response that the cells and periodontal tissues promote to attempt regaining homeostasis and also how certain periodontal-pathogens incorporated these response mechanisms to promote bacterial survival within the gingival crevice and pocket.

Previous studies demonstrated that the quantification of GCF volume is a reliable and accurate indicator of gingival inflammation (20). Although the importance of GCF has been recognized for decades, historically the origin and function of this fluid has been a subject of controversy. Most of the controversy relied on whether this fluid is the result of a physiological or pathological process. The gingival crevicular fluid originates from the vessels of the gingival plexus of blood vessels and flows through the external basement membrane and the junctional epithelium to reach the gingival sulcus. (21). The saliva, also, incorporated within the inflammatory indicator, some studies have characterized the salivary proteomic profile of gingivitis compared to periodontal health (22). The analyses showed that gingivitis was associated with significantly increased amounts of blood proteins (serum albumin and hemoglobin), immunoglobulin peptides and keratins, and more than double the amounts of MMP-8, MMP-9, and IL-6. In periodontal health, salivary cystatin appeared to be more abundant. Similarly, to GCF proteomics, the use of salivary proteomics to identify a patient with gingivitis has substantial limitations, mainly due to the heterogeneity in gingivitis definition among studies, as well as the methodology used for proteomic profiling.

Nephelometric has been applied to the quantitative determination of various protein -and other antigens in blood serum, urine or cerebrospinal fluid such as lipoproteins, immunoglobulins, complement factors, rheumatoid factors and immune complexes (23). Measurement of light scattering has also been used for the determination of cell size, e.g. in the fluorescence-activated cell sorter (FACS). A recent development is the so-called micro particle-enhanced nephelometric immunoassay where antigen or antibody is conjugated to spherical, hydrophilic micro particles in the size range of 50–300 nm. This approach seems to provide higher sensitivity and lower reagent consumption as compared to conventional immunonephelometric.

It has been showed at least part of the damages occurs in the periodontium during the inflammatory process is due to the immune responses against the biofilm bacterial antigens (24). It might be presumed that antibody producing plasma cells directed against the biofilm antigens and that within the gingival crevice (25). Studies applied on the immunoglobulins of peripheral blood in relation to local gingival AG could not demonstrate the real influences of these AG on the evolution the immunoglobulins clearly (26). May be due to the heavy interferences with the systemic conditions (27). We suggest this technic of sampling the gingival circulating blood to open the way in front of the immunologic studies taking the gingival circulating blood as a closest source of informations of gingival inflammatory reactions. The gingival blood could represent a close site of active reaction (28). The gingival sulcus epithelium represent the weakest site and most susceptible, delicate and sensible barrier that separates the sulcular bacteria from the circulating gingival blood (29). This thinnest barrier is the site of our sampling.

The Ag-Ab reaction has been detected positively in the gingival crevice as well as within the gingival tissues (31). Ag-Ab-complement reaction has been approved to be founded on the basal membrane of the crevicular epithelium (32). On other hand, a local production of immunoglobulin and complements has been observed in the gingival tissues and crevice fluid during the evolution of gingival inflammation (33). The regression and the reversibility of gingival inflammation after restoring the oral hygiene proofs again that the decrease of IgG amount in the 3rd sampling is clearly related to the decrease in the amount of bacterial Ag, this study suggests a positive correlation between the presence of bacterial Ag and the amount of IgG in the circulating gingival blood taking in consideration that no professional therapy applied in this study, that is means, the home care alone is a therapeutic measure able to relieve the gingival inflammation and, in addition, it explain the normal findings of IgG within the healthy condition of gingival tissues. The non-significance of the evolution in amount of IgG could be explained by the limitation in area sampling which is the papilla of upper anterior segment. On other side, the fact that gingivitis is a T lymphocyte cell predominantmore than a B lymphocyte cell (34).

We suggest that the gingival circulating blood could give a close, clear picture of immunoglobulin evolution during the gingival inflammation and the immune response evolve positively with the disease progression and/or regression.

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Note: immunoglobulin measured in g/L. normal systemic value 20g/L.

References:


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