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The relationship between the severity of gingival inflammation and the concentration of the gingival fluid proteins

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Abstract

The gingival fluid flow occur when the gingival tissue and vessels are irritated. In a chronically inflamed gingiva the blood-tissue barrier is no longer intact, an increased permeability exists which permits a leakage of plasma protein into the intervascular tissue, the affected blood vessels exhibits qualitative changes of the vascular wall these include the formation of non selective fenestrations which permit exudation of the whole plasma proteins. Most of these proteins are involved in the mechanisms initiating and controlling the inflammatory response.

The aims of this study is to provide information about some aspect of proteins in the gingival fluid during inflammation .Seven plasma proteins of different molecular weight chosen for assay: Haptoglobine, alpha-1 glycoprotien, orosomucoid, prealbumin, alpha-1 antitrypsin, IgG, IgA and IgM.

Thirty subjects selected for investigation, the gingival fluid collected by micropipettes from the gingiva adjacent to the upper anterior teeth, clinical assessment of the gingival condition made according to the gingival index GI of Loe and Silness then the patients divided into three groups. Concentration of the proteins determined by using laser nephelometry.

Demonstrate a remarkable difference in the rate of gingival fluid flow with higher flow rate in cases with chronically inflamed gingiva, the concentration of the proteins increase with the severity of the gingival inflammation . In conclusion the concentration of the proteins are not related to their molecular weight and laser-nephelometry seems to be a reliable technique for screening studies of gingival fluid proteins.

Introduction

A characteristic feature of inflammatory periodontal disease is the production of a fluid, termed the gingival fluid which flows into the oral cavity from the orifice of the gingival crevice or the gingival pocket [1,2]

The protein composition of oral fluid is modulated by environmental factors and physiological states, i.e. chemical, mechanical and pharmacologic stimuli, pathologic conditions, and psychological stress [3]

Numerous previous studies of the cellular, protein [4,5] , carbohydrate [6] , enzymatic [7,8,9,10] and ionic content of the gingival fluid lead to the concept that it is an altered inflammatory exudates.

Recent evidence [13,14,15] has suggested that the host response to the periodontal microbial flora is responsible for the pathological changes in periodontal disease.

The gingival fluid may influence the pathogenesis of the periodontal disease, its amount has been correlated with the clinical and histological severity of the inflammation [2,16].

Evidence shows that dental plaque differ widely in composition and metabolism [17.] A single stimulus such as an immune reactant may clearly trigger several different response mechanisms concomitantly in the host [18], the protein composition of the gingival fluid has received less attention.

All acute and/or chronic pathological processes resulting in tissue damage and destruction lead to an inflammatory response, the purpose of this response is haemostatic and its made up of local and systemic events, the signs of which taken as a whole, constitute the acute phase syndrome [19] . Among the metabolic changes occurring in this syndrome, the rise in the plasma concentration of a group of heterogeneous proteins known as the (Acute Phase Reactant Proteins) (APRP) is a very reliable and sensitive indicator of the presence of pathology [20]

Most of these proteins are involved in the mechanisms initiating and controlling the inflammatory responses [20,] some of them seem to establish a link between the body specific and non-specific defense mechanisms . APRP are indicators of the inflammatory response without being specific to a particular etiology [20,21] The aims of this study is to provide information about some aspect of proteins in the gingival fluid during gingival inflammation.

Seven plasma proteins of different molecular weight were chosen for assay, these include : alpha-1 antitrypsin, hepato globuline. Alpha-1 glycoprotein, orosomucoid, prealbumin. Some of these proteins themselves may be interesting from functional point of view; for example alpha-1 antitrypsin if present may inhibit proteolytic enzymes such as those needed to activate procollagenase in the gingival tissue [22,23] this may be active in the inhibiting the enzyme which will lead to the dissociation of the epithelial cells from each other and from the tooth surface.

Another aim of this study is to resolve apparently contradictory data from different sources concerning levels of IgG, IgA and IgM in the gingival fluid. Brandtzaeg [6] by using a semiquantitative method in three subjects determined that IgG, IgM are present in similar concentrations in the gingival fluid.

Holmberg and Killusion [24] by using the method of radial immune-diffusion, confirmed this conclusion for IgG, IgA and IgM, however Shillitoe and Lehner [25] by utilizing a similar method found that the gingival fluid contained these proteins in concentration equal to ½ to ¼ of those found in the serum. In another study[26] IgM was detected with greater frequency as gingivitis becomes evident, suggesting increased permeability in response to plaque accumulation.

Patients and methods

Thirty subjects between the age of 19-47 years of age and in good general health selected for investigation . All of them showed some degree of inflammation of their gingival and/or periodontal pockets. The gingival fluid was collected from the gingiva adjacent to the teeth of the upper anterior jaw. Clinical assessment of the gingival condition of this unit was made according to the criteria of

gingival index(GI) of Loe and Silness . The patients were divided into (3) groups as follows:

Group I with mean GI score between 0-0.9

Group II with mean GI score between 1-1.9

Group III with mean GI score between 2-3

Prior to collection the gingiva was thoroughly dried by gauze sponge and a gentle sream of compressed air and isolated cotton rolls were used to prevent contamination with saliva. The gingival fluid was collected by capillary with micropipettes of 2 microliter (Drummond Microcaps) placed at the orifice of the periodontal pocket. Micropipettes were held at the orifice of the pocket or inserted into the gingival crevices and care was taken not to traumatize the gingiva , if a fluid sample was contaminated with blood during collection the sample was discarded. The samples were transferred to plastic tubes and diluted in saline with 0.1 percent sodium aside, the specimens were stored at +4 c until subjected to tests.

The concentration of seven selected proteins were determined by using the laser nephelomerty which allows to determine quantitatively specific proteins in the biological fluids. The following antiserum were used to evaluate the concentration of the proteins:

1- Hepatoglobin antiserum special-laser
2- Alpha-1 acidglyocoprotein antiserum special laser.
3- Alpha-1 antitypsin antiserum special laser
4- Prealbumin antiserum special laser
5- Ig G antiserum special laser
6- Ig M antiserum special laser
7- Ig A antiserum special laser

Results

A remarkable difference in the rate of gingival fluid flow was noticed, with higher flow rate in cases with chronically inflamed gingival. The requisite amount was collected within 10 minutes, while for subjects with slightly inflamed gingiva 40 minutes were needed to collect the same amount . **Tables 1,2,3 and 4** list the concentration of the seven selected proteins in the gingival fluid within the three selected groups.

A reported gingival fluid immunoglobulins values varied within wide range from one group to another . IgG, IgM, Ig A shows a level increased with the severity of the inflammation . Ig A was the quantitatively dominating immunoglobulin in the gingival fluid in the three groups 306.6, 302.6, 378.3 mg/100 ml , while IgM and IgG levels were 227, 251.7 and 298.8 mg/100 ml for IgM and 95.8, 120 , 106 mg/100 ml for IgG.

The mean concentration of other proteins in the gingival fluid is demonstrated in **Tables 1,2,3 and 4** . It was shown that the level of these proteins increase with increase the severity of the inflammation. Data from the three groups were statistically analyzed by using t-test.

Discussion

The gingival fluid flow occur when the gingival tissues and vessels are irritated causing abnormal permeability of the dento-gingival blood vessels [27,28,29,30]

In a chronically inflamed gingiva the blood-tissue barrier is no longer intact, an increased permeability exists which permits a leakage of the plasma protein into the intervascular tissue [31,] the affected blood vessels exhibits qualitative changes of the vascular wall, these include the formation of

non-selective fenestrations which permit exudation of the whole plasma including immunoglobulins [32.]

The gingival fluid has been ascribed immunological properties by its contents of immunoglobulins [6]. The IgG , IgA and IgM molecules present in the gingival fluid may contain so. many specific antibodies to members of the microflora in the gingival crevice [33].

The antibodies in the gingival fluid were potentially of great biological importance in determining microflora and resistance to infections in the gingival area [34.]

This investigation gives no information of secretion of immunoglobulins in non-inflammatory gingival fluid. The immunoglobulin content of the gingival fluid in this study most closely resembles the results obtained from another study by Holmberg and Killander [24.]

The concentration of these immunoglobulins increases with the severity of the gingival inflammation; this may be due to increasing in the plasma cells which present in chronically inflamed gingiva, and are responsible for the formation of these immunoglobulins [35.]

The immunoglobulins were not statistically significance from one group to another. Alph-1 antitrypsin was found in the gingival fluid in relative concentration not different from other serum proteins.

The serum level of Alph-1 antitrypsin is known to rise during acute inflammatory conditions and it is the major antiproteolytic enzyme of the serum [23.] Weather it plays any protective role in the chronically inflamed periodontium is not known, it is known to function as an inhibitor of proteolysis , it has been shown however that human gingival crevicular materials contains elastase, neutrophile collagenase. In chronically inflamed tissue alpha-1 antitrypsin is bound to both proteolytic enzymes [36] , therefore it is possible that the protease inhibitors found in the gingival fluid may control proteolysis.

The increased level of this protein are an important component of the overall systemic response to local or generalized injury [21.] In our study the level of this protein is increased positively with the severity of gingival inflammation and it's not statistically significance from one group to another. Degradation of orosomucoid was consistently found on the gingival fluid immunograms, but never detected in plasma. The biological function of this protein is largely unknown, its rich in sialic acid and behaves as acute phase reactant, and it is relatively resistant to proteases.in another study samples of gingival crevicular fluid (GCF) were collected in 30 volunteers with inflamed gingiva, using either capillary tubes (cGCF) or Durapore strips (sGCF). They were examined, together with samples of serum from the same patients, by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and/or by two-dimensional electrophoresis, followed by silver staining. The results confirmed that the distribution of the major proteins in GCF is similar to that found in serum[37]

However, in vitro interaction with trypsin and lectins gave extensive degradation of orosomucoid as analyzed by crossed immunoelectrophorosis [38.] Increased excretion if found in pathological urine , where it forms crystalline deposits 39.. In our study orosomucoid level increased in correlation to GI score, statistical analysis demonstrate a significance differences of this protein.

Tollefsen and Saitvedt [40] demonstrated that the protein composition of the gingival fluid in its features mirrors that of the serum. A

quantitative assay by using crossed mmunoelectrophorosis in serum and gingival fluid, they found a reduced concentration of haptoglobin in most gingival fluid, it is also clear that the local milieu has a modifying effect.

Calculation of the quantity of haptoglobin in our patients demonstrate a lower concentration of this protein in group I and group II in comparison to group III, this observation indicates no significant differences between these groups.

The protein composition of the gingival fluid may be related to the degree of inflammation [40]. Our patients selected by one clinical criteria showed an increase in the prealbumin concentration from one group to another, that means with the severity of gingival inflammation, statistical analysis demonstrates significant differences of this protein. This comes in agreement with the results of other study [41], in which β_2 -microglobulin (β_2-m), lysozyme and protein concentrations in gingival fluid were analyzed in 19 patients with severe periodontitis and in 19 controls devoid of any clinical signs of inflammation. A significant increase of the total protein and β_2-m levels was found in periodontal subjects. In contrast, lysozyme concentration did not reflect the inflammatory status of the periodontium. And also agrees with another study[42], in which 3 acute phase proteins, from the local gingival inflammatory response, were examined for their ability to distinguish healthy, gingivitis and periodontitis sites. Indirect competitive immunoassays were developed for the quantification of alpha 2-macroglobulin (alpha 2-M) and transferrin (TF), and for alpha 1-antitrypsin (alpha 1-AT), the results showed that Higher GCF absolute amounts of alpha 2-M, alpha 1-AT and TF were consistently obtained from diseased (gingivitis and periodontitis) sites than healthy sites.

In conclusion, we could demonstrate that the concentration of the proteins in the gingival fluid are not related to their molecular weight and laser nephelometry seems to be a reliable technique for screening studies of the gingival fluid. The simultaneous assay of many proteins in one run increases the precision of this method.

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Table 1: The Concentration of the proteins in group I mg/100 ml

Patient no.	Age	G.I	IgM	IgG	IgA	Alpha-1 Antitrypsine	Hepatoglobine	Orosomucoide	Prealbumin
1	32	0.40	234	68	306	612	306	158	34
2	25	0.43	216	80	292	606	246	150	34
3	38	0.51	258	80	290	598	254	154	34
4	34	0.52	188	128	288	588	240	146	-
5	24	0.52	222	138	294	592	258	152	32
6	40	0.53	204	32	310	592	254	148	-
7	19	0.53	272	46	298	596	312	156	36
8	28	0.55	236	74	404	588	312	146	-
9	30	0.55	178	204	296	596	288	164	-
10	42	0.55	264	108	288	614	380	152	-
Mean	34	0.56	227	95.8	306.6	598	285	152.6	19.7

Table 2: The Concentration of the proteins in group II mg/100 ml

Patient no.	Age	G.I	IgM	IgG	IgA	Alpha-1 Antitrypsine	Hepatoglobine	Orosomucoide	Prealbumin
1	28	1.56	218	18	294	534	266	150	-
2	29	1.56	212	220	310	592	314	152	40
3	22	1.58	192	12	288	592	276	148	-
4	24	1.58	212	90	302	592	274	156	34
5	47	1.59	212	82	288	596	254	152	34
6	32	1.59	232	90	292	592	270	156	34
7	25	1.60	230	70	292	594	246	150	36
8	19	1.67	262	278	338	524	362	160	38
9	30	1.69	232	120	320	592	266	158	36
Mean	28	1.59	251.7	120	302.6	556.4	323	153	28

Table 3: The Concentration of the proteins in group III mg/100 ml

Patient no.	Age	G.I	IgM	IgG	IgA	Alpha-1 Antitrypsine	Hepatoglobine	Orosomucoide	Prealbumin
1	40	2.72	220	52	294	594	274	154	36
2	23	2.75	278	60	294	592	258	156	38
3	28	2.75	235	92	292	594	246	156	32
4	26	2.75	218	130	294	590	244	154	38
5	23	2.75	240	104	300	592	250	156	40
6	26	2.75	242	62	300	604	300	170	38
7	26	2.75	224	94	294	592	256	154	38
8	31	2.78	222	140	294	590	246	158	40
9	19	2.83	476	52	604	1208	516	308	76
10	34	2.90	468	128	600	1188	512	348	80
11	22	2.95	464	252	596	1176	492	716	80
Mean	28	2.79	298.8	106	378.8	747.2	326.7	202.7	48.7

Table 4 The mean Concentration of the proteins in the three groups

Group	Age	G.I	IgM	IgG	IgA	Alpha-1 Antitrypsine	Hepatoglobine	Orosomucoide	Prealbumin
Group I	34	0.56	227	95.8	306.8	598	285	152.6	19.7
Group II	28	1.59	251.7	120	302.6	556.4	323	153	28
Group III	28	2.79	298.8	106	378.8	747.2	326.7	202	48.7