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Investigation of hemorheological parameters in periodontal diseases

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Abstract. Periodontal diseases are frequently associated with cardiovascular diseases (CVD). On the other hand, occurrence of CVD has also been related with increased blood viscosity. This study was planned to investigate four main hemorheological parameters contributing to blood viscosity –hematocrit, erythrocyte deformability, erythrocyte aggregation and plasma viscosity – and also some biochemical parameters (hs-CRP, fibrinogen, globulin etc.) in patients with periodontal disease. We hypothesized that poor periodontal health would be associated with deterioration of hemorheological properties. According to periodontal health status, subjects were divided into three groups as control (healthy), with plaque induced gingivitis and with chronic periodontitis. All groups included 15 males who had not received periodontal therapy in the last six months before the study, were non-smokers, had no systemic diseases and were not on any medication. Erythrocyte deformability and erythrocyte aggregation were measured with laser-assisted optical rotational cell analyzer (LORCA). Plasma viscosity was measured by a cone-plate viscometer. Data were analyzed with Kruskal-Wallis, Mann-Whitney U Test and Spearman Correlation Coefficient. Plasma viscosity (1.36 ± 0.01 mPa.s in the control group and 1.43 ± 0.02 mPa.s in the chronic periodontitis group, $P < 0.01$), erythrocyte aggregation tendency (aggregation index, amplitude and t½ were $58.82 \pm 1.78\%$, 20.22 ± 0.40 au, 2.80 ± 0.25 s respectively in the control group, and $67.05 \pm 1.47\%$, 22.19 ± 0.50 au, 1.84 ± 0.15 s in the chronic periodontitis group, $P < 0.01$), hs-CRP, fibrinogen and globulin levels were significantly higher, whereas HDL level was significantly lower in the chronic periodontitis group ($P < 0.05$) compared to the control group. All of these conditions may contribute to cardiovascular morbidity and mortality observed in people with periodontal disease, via increasing blood viscosity.

Keywords: Blood viscosity, hemorheology, plasma viscosity, erythrocyte aggregation, erythrocyte deformability, chronic periodontitis, plaque induced gingivitis

1. Introduction

Periodontal diseases are highly prevalent and widespread inflammatory diseases of the gum and other supportive tissues of the teeth, in the etiology of which a plaque biofilm is involved [29]. They frequently start with inflammation of the gingiva (gingivitis), and hence plaque induced gingivitis is the most commonly observed periodontal disease. Clinical features of plaque induced gingivitis include changes in gingival color and gingival contour, edema, bleeding on probing, presence of calculus and/or plaque. At this first stage there is no damage in other periodontal tissues. Plaque induced gingivitis can be

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reversed with an effective mouth care [8]. However, if not treated it can proceed to periodontitis and may permanently damage the gum and the alveolar bone which support the teeth. Clinical features of chronic periodontitis are gingival inflammation, bleeding on probing, periodontal pocket formation, clinical attachment loss and alveolar bone loss [17].

Systemic diseases are commonly observed in people with periodontal disease and among them cardiovascular diseases (CVD) are the most common [25, 26, 30]. Cueto et al have reported an association between periodontitis and acute myocardial infarction even after adjusting for well-known risk factors in acute myocardial infarction [10]. Beck et al have reported a relation between atherosclerosis/coronary heart disease and oral health, even after excluding the well-known risk factors statistically from the assessment [6]. Janket et al have reported that periodontal diseases were associated with a 19% increase in the risk of future cardiovascular disease. They have discussed that this increase in relative risk may have a profound public health impact, considering the high prevalence of periodontitis [16]. It has been reported that there is a relation between the level of bone loss around the teeth, number of affected areas in the mouth (i.e. the extent of the periodontal disease) and the incidence of coronary heart disease and strokes [5]. In a review, including three case-control study and 5 longitudinal studies, relation between oral health and coronary heart disease still existed when studied with different populations and with different assessment methods (i.e.: bone loss, pocket depth) [6].

High blood viscosity and deterioration of hemorheological parameters have been associated with cardiovascular diseases such as stroke and myocardial infarction [38, 39]. There are studies showing that increase in the blood viscosity is a risk factor as important as the LDL cholesterol and poses even a more potent threat than smoking for CVD [22, 40, 44]. In addition, high LDL [24, 36], fibrinogen [22, 41, 44], globulin [4, 9], hs-CRP [12] and low HDL [18, 19, 36] levels not only increase the risk of CVD but also increase the blood viscosity.

It is reported in different studies that fibrinogen [1, 2, 45], globulin [4, 34] and hs-CRP levels [1, 11, 45] increase in both CVD and periodontal diseases. Such increases in fibrinogen [7, 15, 33], globulin [15] and hs-CRP [7, 20, 33] levels are known to increase plasma viscosity and erythrocyte aggregation. These findings suggest that high fibrinogen, globulin and hs-CRP levels in periodontal diseases may augment the onset of CVD via altering rheological properties of blood.

Alterations in hemorheological factors may be responsible for the development of cardiovascular complications in people with periodontal disease. However, in people with periodontal disease, hemorheological parameters have not been studied in detail. The present study was planned to assess 4 main hemorheological parameters (hematocrit, erythrocyte deformability, erythrocyte aggregation and plasma viscosity) and some secondary parameters known to affect the hemorheological parameters, in people with periodontal disease.

2. Subjects and methods

Plaque index, gingival index, periodontal index and gingival bleeding time index (GBTI) were used to assess periodontal condition. In addition, pocket dept (PD) and clinical attachment loss (CAL) measurements were also made. Diagnostic procedures and periodontal assessments were performed at Hacettepe University Faculty of Dentistry Oral Diagnosis and Radiology Department and Periodontology Department.

Following the assessment, subjects were divided into three groups as control (healthy), plaque induced gingivitis and chronic periodontitis according to their periodontal health status. Inclusion criteria were

being between 25–46 years-old, having at least 15 teeth, having received no periodontal therapy in the last 6 months, having no systemic disease, not being currently on medical treatment and being a non-smoker. Each group had 15 males with a total of 45 in the whole study.

Venous blood samples were drawn in the morning (9.00–10.00) from antecubital vein into tubes with EDTA for complete blood count, into tubes with sodium citrate for fibrinogen measurements and into tubes without any chemicals inside for hs-CRP, globulin, total protein, albumin, lipid profile and fasting blood glucose measurements. Another tube containing sodium heparin was used for hemorheological analysis. All hemorheological measurements were completed within 1 hour after the blood was collected. Hemorheological measurements were performed at Hacettepe University Faculty of Medicine Department of Physiology.

This study protocol was approved by the ethics committee of Hacettepe University (No. 09/31-2) and all subjects were provided with written consent before participating in the study. The procedures were in accordance with the ethical standards of the Ethics Committee of Hacettepe University and Helsinki Declaration.

2.1. Periodontal measurements

2.1.1. Plaque index

Plaque index, developed by Silness and Löe was used. Plaque Index in a scale from 0 to 3 was introduced. Absence of plaque deposits is scored as 0, plaque disclosed after running the periodontal probe along the gingival margin as 1, visible plaque as 2 and abundant plaque as 3 [35].

2.1.2. Gingival index (GI)

Gingival index, developed by Löe and Silness, was used. GI provides an assessment of gingival inflammatory status. Higher scores of gingival index indicate higher gingival inflammation. 0: Normal – healthy gingiva 1: Mild inflammation: slight change in color and slight edema. 2: Moderate inflammation: redness, edema and glazing 3: Severe inflammation: marked redness, edema and ulceration [21].

2.1.3. Periodontal index (PI)

Periodontal index, developed by Russel was used. The PI was intended to estimate deeper periodontal disease by measuring the presence or absence of gingival inflammation and its severity, pocket formation, and masticatory function. Russel's periodontal index, defines three important stages of periodontal destruction: Gingivitis (score 1 and 2), pocket formation (score 6) and last stage of destruction (score 8) [32].

2.1.4. Gingival bleeding time index (GBTI)

Bleeding time index, developed by Nowicki et al was used. This index was designed to measure the period between stimulation of gum with a sharp object and initiation of bleeding [27].

2.1.5. Pocket dept (PD)

PD is the distance between the base of the pocket and the gingival margin. It may change due to periodontal disease and/or changes in the position of the gingival margin. Higher scores of PD indicate periodontal destruction or gingival overgrowth [34].

2.1.6. Clinical attachment loss (CAL)

CAL is the distance between the base of the pocket and a fixed point on the tooth crown such as cementoenamel junction. Changes in the level of attachment can be due only to gain or loss of attachment. Increased PD and loss of clinical attachment are pathognomonic for periodontitis. Therefore pocket probing is a crucial and mandatory procedure in diagnosing periodontitis [28].

All these periodontal measurements were made for each tooth and an average of all parameters for all teeth was taken into consideration.

2.2. Hemorheological and hematologic measurements

2.2.1. Plasma viscosity

Measurements were done by a cone-plate viscometer (Brookfield LVDV-II + PRO CP40) at 900 s^{-1} (120 rpm) shear rate and 37°C.

2.2.2. Erythrocyte deformability

Measurements were done by LORCA (Laser-Assisted Optical Rotational Cell Analyzer) (Mechatronics, Holland) at 9 different shear stress levels (0.3, 0.53, 0.95, 1.69, 3.0, 5.33, 9.49, 16.87, 30.0 Pa) and 37°C. For this purpose 25 μl of whole blood was mixed with 5 ml of a viscous PVP solution (polyvinylpyrrolidone, 360,000 MW, viscosity = 30.32 mPa) in Phosphate Buffered Saline (pH: 7.4). 1 ml of this suspension was placed into LORCA, into the gap between two concentric glass cylinders. The system has been described elsewhere in detail. As erythrocytes deform under shear stress, the diffraction pattern of the laser beam through which they pass becomes ellipsoid. The deformation is expressed by the elongation index (EI), derived from this ellipsoid diffraction pattern, a larger EI indicating greater deformation [14].

2.2.3. Erythrocyte aggregation

Measurements were done by LORCA at 37°C. For this purpose 1 ml oxygenized whole blood was placed into the gap between the cylinders. The system has been described elsewhere in detail [14]. Measurement of aggregation depends on the scattering of the laser beam back to measurement device. Recording and figuring out the difference in back-scattering light in time with a computer program is called as syllectogram. Aggregation parameters which indicate the aggregability of erythrocytes are obtained from the software of this computer via mathematical calculations conducted on this sylectogram. In this study aggregation amplitude (AMP, the total extent of aggregation), aggregation half time ($t_{1/2}$, time that elapses until the peak intensity is reduced by half, reflects the kinetics of aggregation), aggregation index (AI, a larger index represents quicker aggregation and/or a greater aggregation amplitude) and γ at ISC max (threshold shear rate needed to prevent aggregation) parameters were used [14].

2.2.4. Other measurements

Complete blood count was made for each sample. Hemoglobin (Hb), hematocrit (Htc), leukocyte, erythrocyte counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) were determined by an electronic blood counter (Coulter, USA). Blood count and fibrinogen, hs-CRP, globulin, total protein, albumin, lipid profile and fasting blood glucose measurements were performed at Hacettepe University Faculty of Medicine Laboratory of Hematology and Biochemistry.

2.2.5. Statistical methods

In order to determine the presence of any difference between groups, Kruskal–Wallis Variance Analysis was used. In order to determine the groups between which there was a statistically significant difference Mann-Whitney U Test was used after Bonferoni correction. Correlation between different parameters was determined by Spearman correlation test. While making correlation analysis the two periodontal disease groups were paired up. All statistical analyses were done using "SPSS 12.0 for Windows". $P < 0.05$ was considered as statistically significant. Data were given as mean ± SEM.

3. Results

3.1. Periodontal findings

Comparison of the groups according to the periodontal findings show that as the periodontal disease severity increases from control (healthy) to plaque induced gingivitis and lastly to chronic periodontitis group, all of the clinical parameters of periodontal disease increase significantly except plaque index and clinical attachment loss ($p < 0.001$). Values of plaque index were significantly higher in the chronic periodontitis and the plaque induced gingivitis groups compared to the control group whereas there was no significant difference between these two unhealthy groups. Clinical attachment loss did not reveal a significant difference between the control and the plaque induced gingivitis groups. In fact, gingivitis is a periodontal disease in which there is no attachment loss. All these observations show that our subjects were chosen correctly. Results concerning periodontal findings are given in Table 1.

3.2. Demographical, blood count and biochemical parameters

Demographical, blood count and biochemical parameters of the groups are given in Table 2.

hs-CRP was 0.16 ± 0.05 mg/dl in the control group, 0.20 ± 0.05 mg/dl in the plaque induced gingivitis and 0.35 ± 0.06 mg/dl in the chronic periodontitis group. Fibrinogen values were 251.07 ± 9.87 mg/dl in the control group, 290.93 ± 13.86 mg/dl in the plaque induced gingivitis group and 298.33 ± 11.38 mg/dl in the chronic periodontitis group. Serum globulin values were measured as 2.51 ± 0.06 gr/dl in the control group, 2.68 ± 0.08 gr/dl in the plaque induced gingivitis group and 2.80 ± 0.05 gr/dl in the chronic

Table 1
Periodontal findings of the groups

	Control	Plaque induced gingivitis	Chronic periodontitis	*P* value
Plaque index	0.27 ± 0.06	0.98 ± 0.18	1.25 ± 0.17	<0.001
Gingival index	0.14 ± 0.03	0.88 ± 0.13	1.59 ± 0.22	<0.001
Periodontal index	0.22 ± 0.07	1.75 ± 0.07	5.16 ± 0.30	<0.001
Bleeding time index	0.11 ± 0.03	1.48 ± 0.16	2.35 ± 0.18	<0.001
Pocket depth	1.69 ± 0.04	2.24 ± 0.09	3.67 ± 0.16	<0.001
Clinical attachment loss	1.26 ± 0.21	1.44 ± 0.33	3.81 ± 0.35	<0.001

Values are presented as means ± SEM. There is statistically significant difference between all groups with Kruskal-Wallis Test ($p < 0.001$).

Table 2
Demographical, blood count and biochemical characteristics of the groups

	Control	Plaque induced gingivitis	Chronic periodontitis	*P* Value
Age (years)	32.13 ± 1.61	29.80 ± 1.01	36.13 ± 1.10#	<0.05
BMI (kg/m2)	25.95 ± 0.77	27.45 ± 1.01	26.99 ± 0.63	>0.05
Leukocyte ($10^3/\mu L$)	5.83 ± 0.22	6.85 ± 0.32	6.89 ± 0.32*	<0.05
Erythrocyte ($10^6/\mu L$)	5.15 ± 0.08	5.27 ± 0.08	5.25 ± 0.07	>0.05
Hemoglobin (g/dL)	15.42 ± 0.19	15.80 ± 0.20	15.49 ± 0.23	>0.05
Hematocrit (%)	44.49 ± 0.48	46.13 ± 0.65	44.72 ± 0.58	>0.05
MCV (fL)	86.59 ± 0.91	87.63 ± 0.58	85.30 ± 0.96	>0.05
MCH (pg)	30.01 ± 0.31	30.03 ± 0.28	29.56 ± 0.33	>0.05
MCHC (g/dL)	34.67 ± 0.31	34.28 ± 0.20	34.65 ± 0.27	>0.05
RDW (%)	13.03 ± 0.16	13.19 ± 0.11	13.11 ± 0.14	>0.05
Fasting plasma glucose (mg/dL)	81.40 ± 1.79	85.00 ± 2.36	88.20 ± 2.16	>0.05
Total cholesterol (mg/dL)	163.89 ± 9.09	181.11 ± 6.93	179.21 ± 8.20	>0.05
Triglyceride (mg/dL)	98.67 ± 15.69	118.13 ± 11.08	219.27 ± 45.48	>0.05
HDL cholesterol (mg/dL)	47.91 ± 2.51	44.14 ± 1.74	37.87 ± 2.13*	<0.05
LDL cholesterol (mg/dL)	95.93 ± 6.46	115.53 ± 6.13	104.53 ± 7.41	>0.05
VLDL cholesterol (mg/dL)	19.73 ± 3.14	23.63 ± 2.22	43.45 ± 8.94	>0.05
Protein (g/dL)	7.25 ± 0.05	7.37 ± 0.06	7.45 ± 0.07	>0.05
Albumin (g/dL)	4.74 ± 0.04	4.69 ± 0.06	4.65 ± 0.05	>0.05
Globulin (g/dL)	2.51 ± 0.06	2.68 ± 0.08	2.80 ± 0.05*	<0.05
Fibrinogen (mg/dL)	251.07 ± 9.87	290.93 ± 13.86	298.33 ± 11.38*	<0.05
hs-CRP (mg/dL)	0.16 ± 0.05	0.20 ± 0.05	0.35 ± 0.06*	<0.05

Values are presented as means ± SEM. *Statistically significant compared with the control group. #Statistically significant compared with the plaque induced gingivitis group.

periodontitis group. HDL values were 47.91 ± 2.51 mg/dl in the control group, 44.14 ± 1.74 mg/dl in the plaque induced gingivitis group and 37.87 ± 2.13 mg/dl in the chronic periodontitis group. hs-CRP, fibrinogen, globulin and HDL values were compared and, difference among the groups were found to be significant ($P < 0.05$). Further statistical evaluation revealed that hs-CRP, fibrinogen and globulin values of the chronic periodontitis group were significantly higher than the values of the control group ($P < 0.017$), however HDL value was significantly lower than the control group ($P < 0.017$) (Table 2).

3.3. Plasma viscosity

Plasma viscosity was measured as 1.36 ± 0.01 mPa.s in the control group, as 1.40 ± 0.01 mPa.s and 1.43 ± 0.02 mPa.s in the plaque induced gingivitis and the chronic periodontitis groups respectively. Statistical comparison of the groups all together by Kruskal–Wallis Variance Analysis revealed a significant difference among the groups ($P < 0.01$). Further statistical evaluation by Mann-Whitney U Test revealed that plasma viscosity of the chronic periodontitis group was significantly higher than that of the control group ($P < 0.017$).

Table 3
Aggregation values of control, plaque induces gingivitis and chronic periodontitis groups (AMP, AI, t 1/2 and γ at ISC max)

	Control	Plaque induced gingivitis	Chronic periodontitis	*P* value
AMP (au)	20.22 ± 0.40	21.42 ± 0.44	22.19 ± 0.50*	<0.01
AI (%)	58.82 ± 1.78	64.28 ± 1.38	67.05 ± 1.47*	<0.01
$t_{1/2}$ (s)	2.80 ± 0.25	2.12 ± 0.15*	1.84 ± 0.15*	<0.01
g at ISCmax(s^{-1})	109.64 ± 6.96	126.79 ± 8.61	147.50 ± 11.07*	<0.01

Values are presented as means ± SEM. *Statistically significant compared with the control group.

3.4. Erythrocyte aggregation

AMP was 20.22 ± 0.40 au in the control group, 21.42 ± 0.44 au in the plaque induced gingivitis group and 22.19 ± 0.50 au in the chronic periodontitis group. AI was 58.82 ± 1.78% in the control group, 64.28 ± 1.38% in the plaque induced gingivitis group and 67.05 ± 1.47% in the chronic periodontitis group. $t^{1/2}$ was 2.80 ± 0.25 s in the control group, 2.12 ± 0.15 s in the plaque induced gingivitis group and 1.84 ± 0.15 s in the chronic periodontitis group. γ at ISC max was 109.64 ± 6.96 s^{-1} in the control group, 126.79 ± 8.61 s^{-1} in the plaque induced gingivitis group and 147.50 ± 11.07 s^{-1} in the chronic periodontitis group. Comparison of AMP, AI and γ at ISC max values, showed significant difference among the groups. ($P < 0.01$). Further statistical evaluation revealed that AI, AMP and γ at ISC max values of the chronic periodontitis group was significantly higher than the control group ($P < 0.017$), whereas $t_{1/2}$ value was significantly lower than the control group ($P < 0.017$) (Table 3).

3.5. Erythrocyte deformability index

Erythrocyte deformability index at 30 Pa was 0.60 ± 0.02 in the control group, 0.60 ± 0.03 in the plaque induced gingivitis and 0.60 ± 0.03 in chronic periodontitis group. There was no significant difference between erythrocyte deformability indices of the groups at any of the 9 shear stresses ($p > 0.05$) (Table 4).

Table 4
Erythrocyte deformability index (EI) of control, plaque induces gingivitis and chronic periodontitis groups

	Control	Plaque induced gingivitis	Chronic periodontitis	*P* Value
EI at 0.30 Pa	0.062 ± 0.003	0.055 ± 0.005	0.057 ± 0.004	>0.05
EI at 0.53 Pa	0.070 ± 0.001	0.066 ± 0.002	0.064 ± 0.002	>0.05
EI at 0.95 Pa	0.115 ± 0.003	0.112 ± 0.005	0.111 ± 0.005	>0.05
EI at 1.69 Pa	0.209 ± 0.003	0.207 ± 0.006	0.204 ± 0.006	>0.05
EI at 3 Pa	0.318 ± 0.003	0.314 ± 0.005	0.312 ± 0.006	>0.05
EI at 5.33 Pa	0.419 ± 0.003	0.414 ± 0.004	0.414 ± 0.006	>0.05
EI at 9.49 Pa	0.499 ± 0.003	0.496 ± 0.003	0.498 ± 0.005	>0.05
EI at 16.87 Pa	0.560 ± 0.002	0.556 ± 0.003	0.560 ± 0.004	>0.05
EI at 30 Pa	0.600 ± 0.002	0.598 ± 0.002	0.602 ± 0.002	>0.05

Values are presented as means ± SEM.

Table 5
Correlation coefficients and statistical evaluation of interrelation between hs-CRP, fibrinogen, globulin and HDL values and clinical parameters of periodontal diseases are presented

	PD	CAL	GI	GBTI	Plaque I	PI
ED	0.085	0.138	0.40	0.29	0.058	0.105
PV	0.506***	0.309*	0.540***	0.467**	0.388**	0.502***
AMP	0.361*	0.338*	0.251	0.226	0.075	0.333*
AI	0.521***	0.406**	0.485**	0.442**	0.356*	0.506***
$t_{1/2}$	–0.514***	–0.390**	–0.484**	–0.442**	–0.368*	–0.509***
γ at ISCmax	0.406**	0.342*	0.337*	0.299*	0.192	0.373*

*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

3.6. Correlation analysis results of periodontal and hemorheological findings

Correlation coefficients and statistical evaluation of interrelation between clinical parameters of periodontal disease and hemorheological parameters are given in Table 5.

All clinical parameters of periodontal disease (pocket depth, clinical attachment loss, gum bleeding time index, gingival index, periodontal index, plaque index) were positively correlated with plasma viscosity, aggregation index and γ at ISCmax values, whereas they were negatively correlated with aggregation half time. In addition, pocket depth, clinical attachment loss and periodontal index were positively correlated with AMP values.

3.7. Correlation analysis of biochemical parameters and periodontal findings

Correlation coefficients and statistical evaluation of interrelation between hs-CRP values and clinical parameters of periodontal diseases are presented in Table 6.

PD, CAL, GI and PI were positively correlated with hs-CRP, fibrinogen and globulin whereas they were negatively correlated with HDL. In addition GBTI and Plaque-I were positively correlated with globulin.

3.8. Correlation analysis of biochemical parameters and hemorheological findings

Correlation coefficients and statistical evaluation of interrelation between hs-CRP, fibrinogen, globulin, HDL values and hemorheological parameters are given in Table 7.

Table 6
Correlation coefficients and statistical evaluation of interrelation between hs-CRP, fibrinogen, globulin and HDL values and clinical parameters of periodontal diseases are presented

	PD	CAL	GI	GBTI	Plaque I	PI
HsCRP	0.482**	0.327*	0.357*	0.373*	0.197	0.407**
Fibrinogen	0.431**	0.295*	0.311*	0.285	0.169	0.395**
Globulin	0.492**	0.308*	0.543***	0.479**	0.516***	0.420**
HDL	–0.405**	–0.482**	–0.320*	–0.251	–0.288	–0.321*

*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

Table 7
Correlation coefficients and statistical evaluation of interrelation between hs-CRP, fibrinogen, globulin, HDL values and hemorheological parameters

	ED	Plasma Viscosity	AMP	AI	$t_{1/2}$	γ at ISCmax
hsCRP	0.132	0.571***	0.169	0.470**	–0.451**	0.464**
Fibrinogen	0.454**	0.569***	–0.057	0.770***	–0.768***	0.394**
Globulin	0.087	0.743***	0.153	0.632***	–0.621***	0.329*
HDL	–0.052	–0.316*	–0.282	–0.199	0.185	–0.403**

*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

There was positive correlation between plasma viscosity, aggregation index, γ at ISCmax and hs-CRP, fibrinogen, globulin. On the other hand there was negative correlation between plasma viscosity, γ at ISCmax and HDL, and negative correlation between aggregation half time and hs-CRP, fibrinogen, globulin. All these correlations were statistically significant.

4. Discussion

To our knowledge the present study is the first research on the interrelation of periodontal diseases with the main hemorheological parameters. Our results revealed that as the severity of the periodontal disease increased (gradually from control to plaque induced gingivitis and finally to chronic periodontitis), all clinical parameters of the periodontal disease, plasma viscosity, aggregation index (AI), aggregation amplitude (AMP), γ at ISCmax, hs-CRP, globulin, fibrinogen levels and leukocyte values increased; whereas HDL and aggregation half time ($t_{1/2}$) values decreased. Periodontal diseases did not affect erythrocyte deformability.

High hs-CRP, fibrinogen and globulin levels which are accepted as risk factors for CVD were also related with the periodontal diseases [45]. Many studies have reported high fibrinogen [1, 2, 45], globulin [4, 34] and hs-CRP levels [1, 11, 45] both in the CVD and in the periodontal diseases. On the other hand plasma viscosity is affected by the level and composition of the plasma proteins and higher fibrinogen, globulin and hs-CRP levels all result in higher plasma viscosity [20, 23, 37].

In our study in addition to the rise in plasma viscosity, plasma fibrinogen, serum globulin and serum hs-CRP levels were all increased in both of the disease groups in comparison to the control group and the difference reached significance in the subjects with chronic periodontitis. In accordance with these findings there was also a significant and positive correlation between fibrinogen, globulin, hs-CRP levels and the parameters used in assessing the severity of the periodontal disease. Correlation between the severity of the disease and plasma viscosity was positive as well. Our findings suggest that the increase in fibrinogen, globulin and hs-CRP levels may be the cause of the increase in plasma viscosity. Supporting this idea the correlation between plasma viscosity and each of fibrinogen, globulin, hs-CRP levels was significant and positive.

In the present study, AMP and AI were higher while $t_{1/2}$ was shorter in both of the disease groups in comparison with the control group and these differences reached significance in the subjects with chronic periodontitis. High AMP points to a greater extent of erythrocyte aggregation and short $t_{1/2}$ signifies an increase in the speed of aggregation. Increase in AI can be related with both the increase in AMP or speeding of the aggregation. It has been reported that in the cases where fibrinogen and globulin levels

increase, erythrocyte aggregation also increases [3, 13, 37, 40, 43]. In addition, there are studies presenting an increase in erythrocyte aggregation when there is an increase in serum hs-CRP levels [3, 7, 31, 42]. Our results revealed significant positive correlation between AI and γ at ISCmax with the fibrinogen, globulin and hs-CRP levels. Fibrinogen, globulin and hs-CRP levels were negatively correlated with $t_{1/2}$ significantly. These results support the idea that the increased erythrocyte aggregation observed in subjects with chronic periodontitis may be related with fibrinogen, globulin or hs-CRP or with all of them. In addition, a significant positive correlation was also observed between the fibrinogen, globulin, hs-CRP levels and severity of the periodontal disease. Similarly intensity of the disease was negatively correlated with $t_{1/2}$ whereas positively correlated with AI, AMP and γ at ISCmax.

Erythrocyte deformability indices measured at 9 different shear stress levels did not reveal any change in the patient groups. Mean corpuscular volume, mean corpuscular hemoglobin concentration which may affect erythrocyte deformability, did not reveal any difference either.

5. Conclusion

In the periodontal disease subjects who were involved in the present study, erythrocyte deformability and hematocrit, which are among the parameters determining the blood viscosity, did not differ from the values of healthy subjects; however plasma viscosity and erythrocyte aggregation was found to be increased. Increases in both plasma viscosity and erythrocyte aggregation are possibly due to the changes in plasma protein levels (especially fibrinogen, globulin and hs-CRP).

It is well known that blood viscosity is an important risk factor for cardiovascular diseases. It is also clear that there is an important link between periodontal and cardiovascular diseases. Our study has revealed an increase in plasma viscosity and erythrocyte aggregation in periodontal diseases being especially prominent in chronic periodontitis. Alterations in the rheological properties of blood may be the link between the periodontal diseases and the cardiovascular diseases. Identification of such a relationship may help prevent the cardiovascular diseases in periodontal diseases.

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