Relationship between serum albumin as an antioxidant and periodontitis

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Abstract

Objective: The aim of this study was to assess the plasma antioxidant status in patients with periodontitis using serum albumin concentration as a criterion index. .

Materials and methods: This study was conducted on 20 individuals (10 healthy control and 10 with chronic periodontitis). Serum albumin level was detected by the bromcresol green albumin (BCG) colorimetric method.

Results: Patients with periodontitis had a significant decrease in the level of serum albumin than that of the healthy subjects ($p<0.05$).

Conclusion: The result of the present study demonstrated decreased level of serum albumin in patients with periodontitis, which indicates decreased antioxidant activity in patients with periodontitis on comparison with the healthy individuals.

Key words: Antioxidants, Serum albumin, Periodontitis.

Introduction

Periodontitis, an inflammatory disorder of the periodontium, affecting the alveolar bone and connective tissue that supports the teeth. The degree of marginal bone loss, the depth of periodontal pockets, the degree of attachment loss and the number of teeth with furcation development characterize the severity of periodontitis[1].

The presence of microorganisms in the oral cavity initiates a series of processes leading to the damage of healthy tissues. The damage of periodontal tissue results from a direct effect of the toxic products released by the bacteria, and from the action of the immune system stimulated by the bacterial infection [2,3]. Several reports have demonstrated the ability of periodontopathogens and their products to induce the generation of ROS by polymorphonuclear leukocytes (PMNs) which are recognized as a particularly rich source of ROS, which in the absence of suitable antioxidants in the crevicular space leading to tissue damage [4,5].

Following stimulation by bacterial antigen, PMNs produce $O_2^{\cdot -}$ via the metabolic pathway of the ‘‘respiratory burst’’, during phagocytosis[6-11]. Several inflammatory cells, fibroblasts, vascular endothelial cells and osteoclasts also produce ROS [12, 13, 14]. ROS serve as agents highly toxic to the internalized microbial agent; however, they can also lead to extracellular structure degradation[15,16]. Excess production of ROS and the resultant oxidative stress contribute significantly to tissue damage in many diseases [6,9,14,17]. Free radical-induced tissue injury and ROS have been implicated in the pathogenesis of periodontal disease and they are increased in individuals with periodontitis[18].

Several studies have shown increased generation rate of ROS from peripheral blood PMNs in rapidly

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progressive periodontitis (RPP) [19,20], juvenile[21], and chronic adult periodontitis[22-25]. Furthermore, Porphyromonas gingivalis lipopolysaccharide has been shown to cause a dose-dependent increase in $O_2^{\cdot-}$ production by stimulated neutrophiles from RPP patients[26]. Tissue injury due to free radical production has been suggested to be enhanced in individuals with periodontal disease due to a lack of adequate antioxidant defense[27,28]. The imbalance between the prooxidant and antioxidant systems may lead to a further oxidative attack and substantial deterioration of the periodontal tissue[29,30]. It is unlikely that oxidative processes play a causal role in the aetiology of periodontitis, but they are likely to contribute to disease progression unless abated through antioxidant action[31].

The body contains a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants as they form, or to repair damage caused by ROS in vivo. Antioxidants may be regarded as those substances which when present at low concentrations, compared with those of an oxidisable substrate, will significantly delay or inhibit oxidation of that substrate[32]. Antioxidants could be classified into tow major groups, enzymatic and non-enzymatic antioxidants[33]. The non-enzymatic antioxidants is further classified into (1) the low-molecular weight compounds which include some vitamins, glutathione and bilirubin,and (2) the metal binding proteins which include albumin, transferrin, and ceruoplasmin[34].

Human plasma is endowed with an array of antioxidant defense mechanisms. Important plasma antioxidants appear to be ascorbate, urate , α-tocopherol, albuminbound-bilirubin, and albumin itself [35]. Albumin synthesis takes place only in the liver. Albumin is not stored in the liver but is secreted into the portal circulation as soon as it is synthesized [36, 37]. The primary function of albumin is generally considered to be the maintenance of the colloid osmotic pressure in both the vascular and extravascular spaces. Also it acts as a transporting protein for a large number of compounds. These include free fatty acids, phospholipids, metallic ions, amino acids, drug, hormones, and bilirubin[38]. Metallic binding propriety of albumin makes it one of the most important antioxidant present in the plasma[39]. Its binding to copper helps in the inhibition of copper-ion dependent oxidants and $^{\cdot}OH$ radical formation and the copper ion-albumin complex represent a safe transit form that can be removed by the liver[40].

The aim of this study was to evaluate the antioxidant defenses status in human blood plasma in patients with periodontitis using serum albumin as an indicator.

Materials and Methods

Subjects:

Twenty non-smoker individuals, with an age ranging 35-45years, were involved in this study. Subjects were classified into two groups each comprised 10 subjects. Group one represents the healthy control and the other group represents individuals with periodontistis. Patients were evaluated as follows:

(1) Patients had at least 24 natural teeth.

(2) 6 teeth were affected by periodontitis as determined by clinical examination (probing pocket depth> 5 mm at approximal sites).

(3) Periodontal tissues were inflamed as assessed by generalized gingival redness,

edema, and gingival bleeding on probing.

(4) Patients had had no periodontal therapy during the 6 preceding months.

All subjects were systemically healthy, with no medical condition that would affect their participation in the study. Exclusion criteria applied were a course of anti-inflammatory or antimicrobial therapy within the previous 3 months, pregnancy, a history of previous or current smoking, inflammatory and infectious diseases, use of vitamin supplementation within the previous 3 months, and any special dietary requirements (e.g. Coeliac disease).

Samples

For laboratory analysis, blood samples of the patients and control were taken after overnight fasting (at least 8-hours) between 8 and 9 in the morning. For plasma isolation, a 4ml of blood sample was centrifuged at 3.000 rpm at room temperature for 5 min and plasma aspirated and stored into tubes at -20°c until analyzed for serum albumin.

Determination of Albumin (Colorimetric Determination):

Principle:

Albumin has the ability to bind dyes, so when bromocresol green binds to albumin, there will be a shift in the absorption wavelength. Serum is diluted with a buffered bromocresol green at (pH = 4.2). The measurements of the absorbance should be at 632nm (filter no. 607) within 30 seconds of mixing the sample and bromocresol green to avoid the problem of non-specific reaction of bromocresol green with globulin.

Reagents:

(Using Randox laboratories kits UK)

1-Albumin reagent.

2-Albumin standard 5%.

Procedure:

1-Prepare three sets of tubes, add:

Material	Test	Standard	Blank
Albumin reagent	3ml	3ml	3ml
Sample	20µl		
Albumin standard 5%		20µl	
Distilled water			20µl

2-Read the absorbance in spectrophotometer at 630 nm against the blank.

Calculation

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of sample - Absorbance of blank}}{\text{Absorbance of standard - Absorbance of blank}} \times 5$$

Statistical analysis

Quantitative variables are expressed as Mean ± S.D. Difference between the groups were analyzed using Student's t-test. Statistical significance was defined as $p< 0.05$.

Results

Table (1) shows the level of serum albumin of the studied healthy subjects and patients with periodontitis. The value of serum albumin of the healthy individuals is ranging from 4.5 g/dl to 5.5 g/dl, while for those of the patient the value of serum albumin is ranging from 3 g/dl to 4.1 g/dl.

Table (2) shows the mean ± SD value of serum albumin for the normal individuals (5.17 ± 0.39 g/dl) and that of the patients with periodontitis (3.57 ± 0.36 g/dl).

Figure (1) shows a significant increase ($P<0.05$) of serum albumin of patient with periodontitis when compared with that of control healthy subjects.

Discussion

The results of the present study demonstrated a significant decrease in the concentration of serum albumin in patients with periodontitis than that of the healthy control individuals. In humans, albumin is the most abundant plasma protein, accounting for 55-60% of the measured serum protein. The serum albumin concentration is a function of its rates of synthesis and degradation (consumption) and its distribution between the intravascular and extravascular compartments. The rate of albumin synthesis and degradation depends on the nutritional intake, consumption of this protein and the general health state[41].

The rate of albumin synthesis may be significantly reduced in inflammation. In inflammation, there is an increase in synthesis of the positive acute-phase proteins by the liver such as C-reactive protein, and as a reflex, there is a decrease in the rate of synthesis of negative acute phase reactant such as albumin.[42]. Several investigators had demonstrated a significant decrease in serum albumin level in many inflammatory conditions[43,44]. Periodontitis is an inflammatory condition in which the sequences of the basic stages of inflammation are the same as those of other inflammations occurring elsewhere in the body. So, part of the decreases in serum albumin in case of periodontitis could be explained on the bases of deceased synthesis of negative acute phase reactant in the liver.

Furthermore, in case of inflammation there is an increase in the vascular permeability. Increase in the vascular permeability leads to increased capillary leakage of plasma proteins (most important one is albumin) as result of endotoxin from Gram-negative bacteria and the cytokines released by the inflammatory cells leading to a decrease in level of serum albumin[45].

Inflammatory cells such as macrophages are the main source of cytokines of which is IL-1 had been demonstrated to cause hypoalbuminemia in case of inflammation. Hypoalbuminemia during the inflammatory reaction can be partially ascribed to a specifically decreased and for some extent inhibition of synthesis of albumin by the liver as the result of monocytic (macrophagic) products, including IL-1[42].

Albumin is considered as the main and the most important extracellular antioxidant[45]. The antioxidant activity of albumin is due to the following properties of albumin[46].

- Albumin has the ability to bind to serum free fatty acid protecting them against lipid peroxidation.
- Albumin has the ability to bind to bilirubin. This albumin-bound bilirubin is an efficient free radical scavenger.
- Albumin is the major extracellular source of the reduced sulphydryl groups, termed thiols. Thiol groups are avid scavengers of reactive oxygen and nitrogen species, especially the superoxide hydroxyl and peroxynitrite radicals.
- Albumin can also limit the production of these reactive species by binding free copper Cu^{2+}, an ion known to be particularly important in accelerating the production of free radicals.

Because albumin itself is damaged when it acts as an antioxidant, it has been viewed as a sacrificial molecule

that prevents damage occurring to more vital species[47].

As a sequence of the damage a and increased consumption of albumin as an antioxidant there will be a decrease in the level of serum albumin.

All the factors affecting on the synthesis and degradation of serum albumin are summarized in figure (2)

So, the decrease in the concentration of serum albumin in periodontitis could be attributed to the following:

-Periodontitis is an inflammatory disease in which there is increased lipid peroxidation.
- The inflammatory process in periodontitis leads to decrease in albumin synthesis (negative acute-phase reactant) and increase in albumin extravascular leakage (due to bacterial toxins and cytokines).
- The increased free radical production due to lipid peroxidation in periodontitis increases the damage and consumption of albumin as an antioxidant.

Regarding the relation between albumin and periodontitis, most of the previous studies focus on the level of salivary albumin as antioxidant neglecting its level in serum. While in the present study serum albumin viewed from two aspects, one as a protein that is affected by inflammation and in which it is considered as negative acute-phase reactant and the second aspect is the antioxidant activity. In inflammation, the decreased level of serum albumin due its extravascular leakage accompanied by decreased synthesis will lead to adverse consequence, presented by the decreased antioxidant activity due to the decreased in the level of serum albumin.

Conclusion

Periodontitis like all other inflammatory process could be affected and could affect the systemic health status of the affected individuals. This is clear as shown in the result of the present study, which shows the systemic affect through the reduction in the level of serum albumin, which is considered as vital plasma component because of its antioxidant activity.

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Table 1. The value of serum albumin of each individual involved in the study

	Serum albumin (g/dl)	
	Control	**Periodontitis**
1	5.4	3.6
2	4.7	3.8
3	4.5	3
4	4.9	3.9
5	5.2	4.1
6	5.1	3.7
7	4.8	3.8
8	4.9	3.1
9	5.5	3.2
10	5.2	3.5

Table 2. Comparison of serum albumin between periodontitis and control healthy groups

Group	n	Mean±SD (g/dl)	Min	Max
Periodontitis	10	$5.17 \pm 0.39^{*}$	4.5	5.5
Control	10	3.57 ± 0.39	3	4.1

Significantly different from healthy individuals *P< 0.05

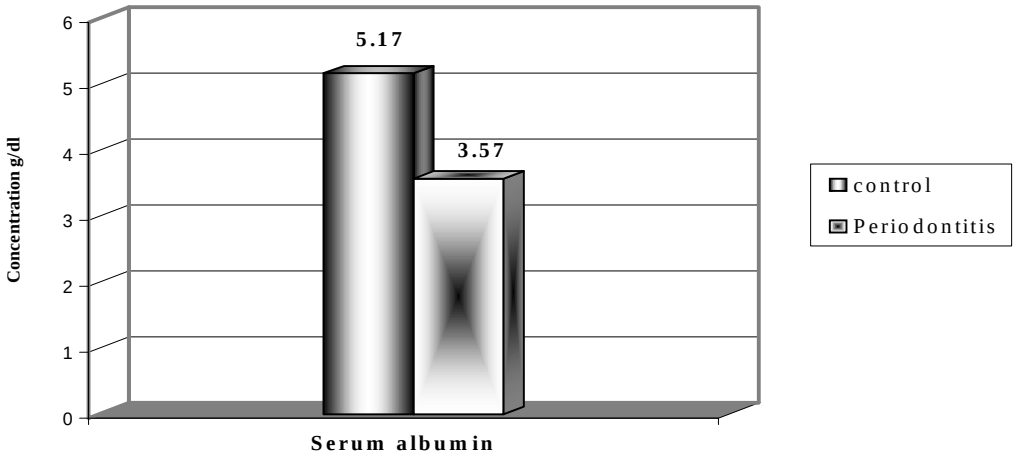

Fig.1 Mean concentration of serum albumin in healthy individuals and in patients with periodontitis

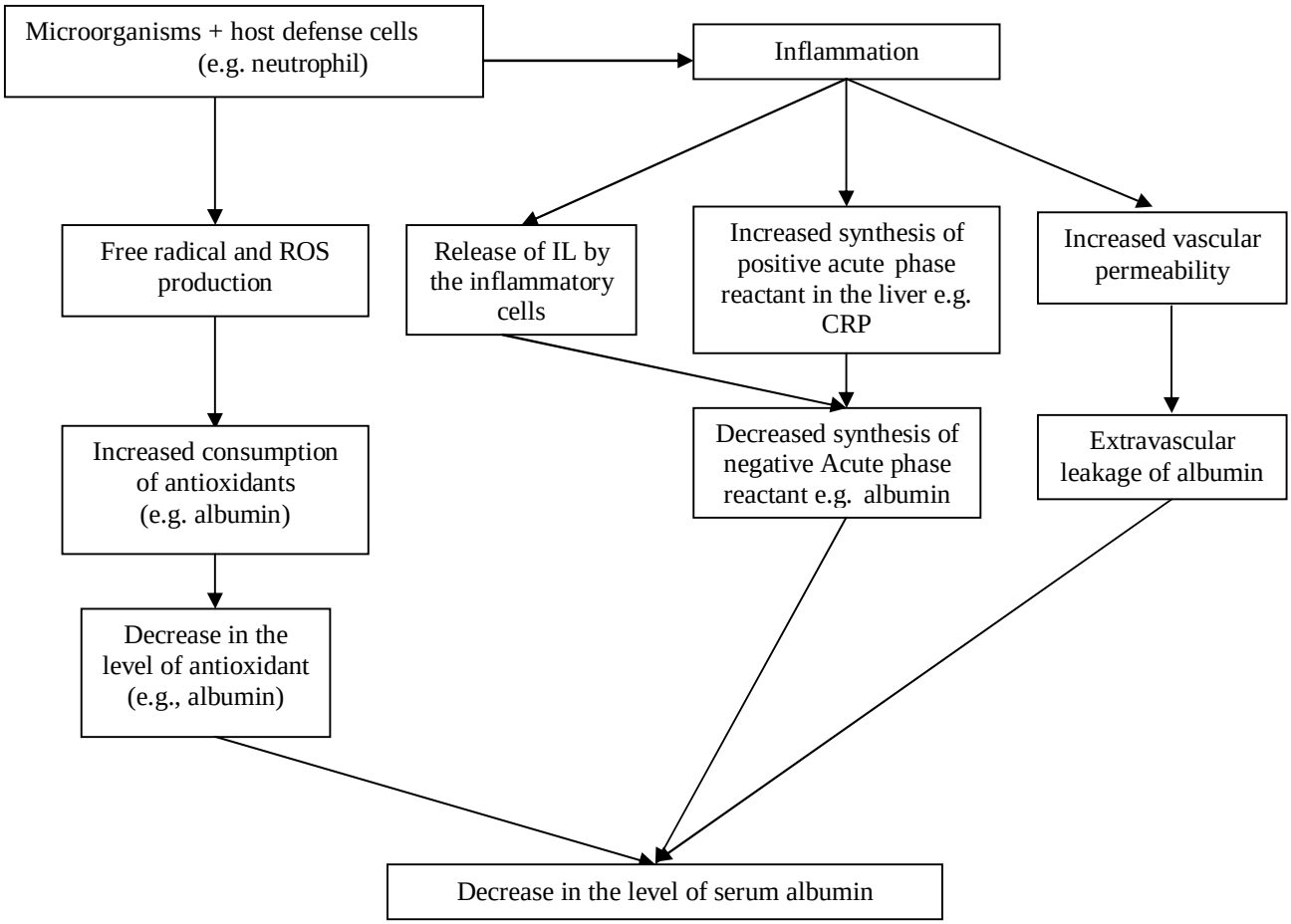

Fig (2): shows the pathway of reduction in the concentration of serum albumin in case of inflammatory diseases of infectious background