Evaluation of salivary total proteins, albumin, globulin, and A/G ratio among healthy individuals and patients with chronic periodontitis

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ABSTRACT

Aim: The aim of this study is to estimate the level of salivary total proteins, albumin, globulin, and A/G ratio among healthy individuals and patients with chronic periodontitis. Materials and Methods: The saliva samples were collected from a total of 20 individuals where 10 samples from normal individuals as a control group and 10 samples from chronic periodontitis patients. Salivary protein estimation was done based on the biuret method. Salivary albumin was estimated using the bromocresol green method. Results: The results of our study showed higher levels of total protein, albumin, and globulin in chronic periodontitis patients as compared to healthy individuals. The increased protein levels can be due to the inflammatory process that activates the sympathetic system to enhance the synthesis and secretion of some proteins, thereby increasing the protective potential of saliva against the diseases. Conclusion: Useful diagnostic information can be obtained by determining the total proteins, albumin, globulin, and A/G ratio. Significant changes in these values can pinpoint specific changes. As saliva is one of the major constituents of the oral cavity, estimation of total salivary proteins helps in the diagnosis of chronic periodontitis, thus facilitating early prognosis and treatment.

KEY WORDS: A/G ratio, Albumin, Globulin, Periodontitis, Total protein

INTRODUCTION

Periodontal diseases are chronic inflammatory disorders comprising of many diseases, of which gingivitis and periodontitis are the most common. These occur as a result of the interaction between a pathogenic bacterial biofilm and host-derived inflammatory cells and molecules.[1,2] Gingivitis is a reversible inflammatory condition while periodontitis is a chronic irreversible inflammatory state of the supporting structures leading to the destruction of connective tissue and alveolar bone.[3,4] The traditional methods for the diagnosis of these diseases involve clinical measurements and radiographic assessments which are often poorly tolerated by the patients and are also subjected to measurement errors.[2] Hence, today, various researches are being conducted to evaluate possible compounds in the oral fluids through which it may be possible to assess the presence and severity of these diseases as well as to identify the patients at risk for these diseases.[3]

Saliva is an oral fluid which can be easily and rapidly collected and does not require any specialized equipment or techniques.[3] Saliva is secreted by the submandibular gland, the sublingual gland, and the parotid gland. In mouth, saliva gets mixed with mucus, inorganic substances, glycoprotein, enzymes, secretary immunoglobulins and lysozymes and so on. The enzyme in saliva is a must for starch and fat digestion in few parts.[5] These enzymes break down in the tooth gap. Food pellets play an important role in protecting the teeth from germ damage.[6] Saliva mixes with food and helps in swallowing it, also keeps the oral cavity moist and lubricated.[7,8] There are many ingredients that play an important role in disease development. Saliva made for the body’s own secretion of a physiological substance and the composition of complex, and more there are more than 90 kinds of

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proteins that can be stably present in saliva. More than 40 kinds of saliva proteins have been isolated before, including albumin and globulin. A variety of oral diseases are known to survive in saliva total protein in the serum which is made up of albumin and globulin. The globulin, in turn, is made up of α1, α2, β, and γ globulins. These fractions can be quantitated using protein electrophoresis, but the total protein test is a faster and cheaper test that estimates the total of all fractions together. The traditional method for measuring total protein uses the biuret reagent, but other chemical methods such as Kjeldahl method, dye binding, and refractometry are now available. The measurement is usually performed on automated analyzers along with other laboratory tests. Serum albumin is the most abundant blood plasma protein, is produced in the liver, and forms a large proportion of all plasma protein. The human version is human serum albumin, and it normally constitutes about 50% of human plasma protein. Serum albumins are important in regulating blood volume by maintaining the oncotic pressure (also known as colloid osmotic pressure) of the blood compartment. They serve as a carrier for molecules of low water solubility, by masking their hydrophobic group. Albumin also acts as a carrier for lipid soluble hormones, unconjugated bilirubin, free fatty acids etc. The globulins are a family of globular proteins that have higher molecular weights than albumins and are insoluble in pure water but dissolve in dilute salt solutions. Some globulins are produced in the liver.

In the oral cavity, proteins, especially albumin, are considered as a serum ultrafiltrate to the mouth. Salivary proteins have been shown to be increased in medically compromised patients whose general conditions get worse. Elderly subjects usually show less-effective immune response than the young ones. Gingivitis and periodontitis are oral diseases that are characterized by chronic inflammation. Here, salivary protein and albumin concentrations were determined as markers for plasma protein leakage, occurring as a consequence of the inflammatory process.

So far, there is sparse literature regarding the relationship between total protein, albumin, globulin, A/G ratio, and periodontitis. Hence, this case–control study was designed to assess and compare the salivary levels of total protein, albumin, globulin, and A/G ratio in healthy and chronic periodontitis subjects.

**MATERIALS AND METHODS**

**Collection of Saliva Sample**

Human whole unstimulated saliva was collected by spitting method without swallowing, with the subject seated in an upright position between 9 am and 11 noon, after they had refrained from oral intake, tooth brushing, and smoking for 2 h before saliva collection. Approximately, 5 mL of saliva was collected. The saliva samples were collected from a total of 20 individuals where 10 samples from normal individuals as control group and 10 samples from chronic periodontitis patients.

Salivary protein estimation was done based on the biuret method. Protein in plasma or serum sample forms a blue-colored complex when treated with cupric ions in alkaline medium. Salivary albumin was estimated using the bromocresol green method.

**Estimation of Total Protein**

Total protein was estimated colorimetrically by Biuret method. Protein in plasma or serum sample forms a blue-colored complex with cupric ions in alkaline solution. The intensity of the blue color is proportional to the protein concentration.

**Estimation of Albumin**

Albumin was estimated by Bromocresol green method, and the reaction between albumin from serum or plasma and the dye bromocresol green produces a change in color that is proportional to the albumin concentration.

**RESULTS AND DISCUSSION**

The mean total protein level in the control group was 2.26 g/dl, and the mean total protein level among the chronic periodontitis patients was 10.69 g/dl [Figure 1 and Table 1]. The mean albumin level in the control group was 0.83 g/dl, and the mean albumin level among the chronic periodontitis patients was 3.26 g/dl [Figure 2]. The mean globulin level in the control group was 1.71 g/dl and the mean globulin level among the chronic periodontitis patients was 8.59 g/dl [Figure 3]. The mean A/G ratio in the control group was 0.527 and the mean A/G ratio among the chronic periodontitis patients was 0.481 g/dl [Figure 4]. From the results obtained, it is clearly inferred that there is a significant increase in the total protein, albumin, and globulin levels in the saliva of patients with chronic periodontitis when compared to that of normal individuals, and there is a decrease in the A/G ratio ($P < 0.05$). Low A/G ratio is associated with liver damage, where as increased A/G ratio is generally due to excessive body water loss.

Protein concentration changes by a variety of factors. Due to the salivary protein buffering, the rate of flow

| Table 1: Mean value of control and chronic periodontitis samples |
|------------------|------------------|-----------------|
| (g/dl)            | Control          | Periodontitis   |
| Total protein     | 2.26             | 10.69           |
| Albumin           | 0.83             | 3.26            |
| Globulin          | 1.71             | 8.59            |
| A/G ratio         | 0.527            | 0.481           |
protein is a vital component of saliva and is responsible for most of its functions such as lubrication, physical protection, cleansing, buffering, maintenance of tooth integrity, taste and digestion, and antibacterial activity.\[2,22\] The results of our study showed higher levels of total protein in chronic periodontitis patients as compared to healthy individuals which were statistically significant. The increased protein levels in our study in the test groups could be due to the inflammatory process that activates the sympathetic system to enhance the synthesis and secretion of some proteins (as evidenced by increased amylase levels), thereby increasing the protective potential of saliva against the diseases.\[21,23\] The increased levels could partly be also due to an increased leakage of plasma proteins into saliva due to inflammation as suggested in a study done by Basu et al.\[19\] Our results are in agreement with other studies which showed similar results.\[6,23\]

The hypothesis that periodontal microbes trigger inflammatory response and result in higher levels of salivary albumin and total protein is well known. Therefore, proteins are considered potential markers for plasma protein leakage.\[24\]

In our study, a significant positive correlation was also observed among total proteins, albumin, and globulin, indicating that they are implicated in host response. This is in accordance with other studies which also showed a positive correlation among total proteins, albumin, and globulin. Thus, these values can be used as a diagnostic aid in indicating the presence of chronic periodontitis.

**CONCLUSION**

Useful diagnostic information can be obtained by determining the total protein, albumin, globulin, and A/G ratio. Significant changes in these values can pinpoint specific changes, and thus, saliva is being one of the major constituents of the oral cavity; therefore, the estimation of total salivary proteins helps in diagnosis of chronic periodontitis, thus facilitating early prognosis and treatment.

A very highly significant rise in the salivary total protein and albumin concentration using the biuret and bromocresol green methods suggests the role of these simple methods in assessing these parameters as markers for gingivitis and periodontitis, where plasma protein leakage occurs as a consequence of the inflammatory process. However, a longitudinal study would be required to draw definite conclusions and prove the role of saliva as a prognostic indicator.

The mechanism of the changes in the composition and the relationship remains to be further studied. In recent years, the research on saliva has gradually
attracted the attention of scholars in developing a non-invasive detection methods for disease diagnosis and prevention and further studies must be encouraged in this field.

REFERENCES


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