IMMUNOLOGY OF CHRONIC GENERALIZED PERIODONTITIS. 2. ESTIMATION OF TOTAL HEMOLYTIC COMPLEMENT (CH 50) AND ITS FRACTIONS C3 AND C4

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ABSTRACT: The total hemolytic complement activity (CH50) and its fractions C3 and C4 were determined in forty patients with chronic generalized periodontitis (CGP). The values were compared with an equal number of age and sex matched periodontally healthy controls. Total hemolytic complement activity was expressed as number of CH 50 units per ml of blood, while C3 and C4 levels were assessed by radial immunodiffusion and expressed as mg%. The observations showed a significant increase in CH50, C3 and C4 levels in the sera of patients with CGP. The study reveals the role of complement system in the pathogenesis of chronic generalized periodontitis.

INTRODUCTION

Chronic Generalized Periodontitis (CGP) is the most common form of periodontal disease found in human beings. The advanced lesion seen in overt periodontitis includes further collagen loss, osteoclastic activity and an inflammatory infiltrate of plasma cells, lymphocytes and macrophages. Complement activation may occur consequent to an antigen-antibody reaction or it may be brought about by several alternative mechanisms. Activation of the complement system may cause chemotactic attraction of polymorphonuclear leukocytes, which then release enzymes that cause tissue damage1,2. Complement activation can stimulate osteoclastic bone resorption3 and production of lymphokins by B-lymphocytes4. In addition complement facilitates many reactions which may provide protection such as enhanced phagocytosis and bacteriolysis. Several investigators have attempted to demonstrate the role of complement mediated reactions in periodontal pathogenesis by demonstrating it in periodontal tissues, serum and gingival crevicular fluid. The relative protective and destructive roles of complement have been studied in other infectious disease by systemic analysis of complement levels and function. So far few workers have tried to correlate serum complemnt activity and periodontal disease.

The present study is undertaken to estimate total hemolytic complement activity and the levels of immunorechemically detectable C3 and C4 fractions in the sera of patients with CGP and to compare it with periodontally healthy controls.

MATERIALS AND METHODS

Forty patients with CGP were included in the study. The diagnosis of CGP was made from the history, clinical features and radiographical appearances. The mean periodontal disease index (PDI)5 in CGP patients were >5.24. Forty age and sex matched subjects served as control group. Individuals having a PDI score <2.34 were included in the control group. All the subjects were screened clinically, biochemically and biophysically to exclude the possibility of any infection, parasitic infestation, allergy, asthma or liver involvement.

Five ml of venous blood was obtained from all the subjects, labelled appropriately and sera separated. The serum samples were centrifuged twice at 0°C at 3000 rpm for 10 min. The sera were frozen and stored in aliquots at 70°C until the time of analysis.

Total hemolytic complement was estimated by the method of Mayer. In short, sheep erythrocytes (SRBC) are sensitised with the antibody. The sensitised SRBC and Barbitone buffer were taken in different test tubes, serum diluted 1:30 in the same buffer was added at varying against the serum concentration. The percentage lysis was plotted against the serum concentration. From the sigmoid curve obtained, the CH50, value was calculated as the quantity of complement necessary for 50% lysis of the red cells. Total serum complement activity was expressed as the number of 50% hemolytic units (CH50) per ml undiluted serum. The immunochemically detectable C3 and C4 levels were estimated by radial immunodiffusion (RID) technique of Mancini and Co-workers. Commercially available radio immunodiffusion plates were used (Behringwerke AG, Marburg, FRG).

Total serum complement activity was expressed as number of 50% hemolytic units (CH50) per ml undiluted serum. Statistical analysis was done by students 't' test (two-tailed p-values are quoted).

**RESULTS**

The mean age and sex distribution of all the subjects studied are given in Table 1. Total hemolytic complement activity (CH50), C3 and C4 levels in all the subjects studied are shown in Table 2. The CH50 values of patients with CGP were significantly higher than that of normal control.

<table>
<thead>
<tr>
<th>Table - 1 Age and Sex distribution of subjects.</th>
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<tbody>
<tr>
<td>Normal(Controls)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Number Of Subjects</td>
</tr>
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<td>Age (mean ± SD)</td>
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**Table - 2 Serum Levels of CH50, C3 and C4 in Patients and in Controls.**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>CH50(U/ml)</th>
<th>C3 (mg%)</th>
<th>C4 (mg%)</th>
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</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>98.99 ± 2.54</td>
<td>104.89 ± 2.34</td>
<td>40.01 ± 1.89</td>
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<td>(n = 40)</td>
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<tr>
<td>Patients with CGP</td>
<td>169.07 ± 2.21</td>
<td>124.17 ± 3.32</td>
<td>71.29 ± 3.18</td>
</tr>
<tr>
<td>(n=40)</td>
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All values are mean 2SE 'n' stands for number of subjects

of the control subjects (p < 0.01). The C3 and C4 levels in the serum assessed by single radial immunodiffusion technique showed significantly higher levels in CGP patients compared to the control subjects (p > 0.01).

DISCUSSION

The human complement system is a major humoral effector mechanism that contributes to protective responses against foreign substances, and to damage to host tissues.9 Many of the biological effects of this enzyme system result from the release of activation peptides from the parent proteins consequent to limited proteolytic cleavage. Other biologically important phenomena result from the binding of these proteins or their cleavage products to specific receptors found on many cells including Neutrophils, Platelets, Mast cells, Macrophages and Erythrocytes. Complement activation may occur consequent to an antigen-antibody reaction or it may be brought about by several alternative mechanisms.10

Hemolytic complement activity depends on all the classical components, and altered in the concentration of any of the component influences the total activity.11 CH50 activity is a sensitive assay reflecting the overall integrity of the complement system in hemolytic (functional) terms. Increased hemolytic complement levels are associated with a wide variety of inflammatory and necrotic disorders.12,13 It has been reported that complement works synergistically with other systems especially with the immune system composed of immunoglobulins and ‘T’ and ‘B’ lymphocytes.14

Even though there is a central role for the complement in periodontal disease, which has not been well established, evidence has accumulated which indicates the potential role for the occurrence of complement mediated phenomena in periodontal disease. Norman et al.15 observed an elevated level of CH50, C3, C4 in patients with periodontitis. An elevation of hemolytic complement activity and its fractions C3 and C4 is observed in the present study. The activation of complement by micro-organisms associated with periodontal disease has been suggested by Synderman.16 He suggested that one mechanism for periodontal inflammation could be via the interaction of bacterial products and serum complements within the tissues.

Several investigators have demonstrated in vivo, complement consumption included by dental plaque and several species of oral bacteria. Activation of the alternative or classical pathways or both has been demonstrated.17-20 Thus, the variety and abundance of oral gram-positive and gram-negative bacteria capable of activating complement may be responsible for the inflammatory events associated with CGP.21

SUMMARY

The role of complement system in the pathogenesis of periodontal disease is not well established. The presence of complement proteins and their cleavage products, as well as activators of the complement pathways, in gingival environment has made it attractive to postulate that complement may contribute to the inflammatory process observed. Numerous and complex biological effects of complement proteins have been described, yet the important of these effects in human periodontal disease is not well established. Further studies may be required to support the present observation and to interpret the exact role of complement fractions in CGP.
ACKNOWLEDGEMENTS

We express our sincere thanks to Dr. M. Krishnan Nair, Director of Regional Cancer Centre, Trivandrum. The financial assistance given by the Kerala State Committee on Science, Technology and Environment is gratefully acknowledged.

REFERENCES


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