Oral mucosal Cryoglobulin [ICD-10-CM Diagnostic Code D89.9 Cryoglobulin], Oral Mucosal Herd Immunity and the Immune Herd Plots among Periodontal Disease Patients

Ibrahim M. S. Shnawa a*, Baha H. H. AL-Amedie b and Zainab H. AL-Fatlawi b

a Department of Biotechnology, College of Biotechnology, University of Qasim and Hilla University College, Babylon, Iraq.

b College of Dentistry, Babylon University, Babylon, Iraq.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2022/v34i18A35773

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/82708

Received 20 December 2021
Accepted 26 February 2022
Published 02 March 2022

ABSTRACT

Forty five patients were diagnosed by specialist dentist of the team as periodontal disease patients. Basically they were found to be chronic periodontitis and chronic gingivitis. The study male and female patient groups [25 males and 20 females] were subjected to the detection of oral mucosal cryoglobulin and tempts to use cryoglobulin as a marker for oral mucosal herd immunity among these patients. Oral mucosal cyoprotein solutions were separated and characterized as oral mucosal cryoglobulin [ICD-10-CM Diagnostic code, D89.9 Cryoglobulin]. Mucosal cryocrit percentages were ranged from one to seven percent. Oral mucosal cryoglobulin concentrations were ranged between 0.13 up to 2 mg/dl. In chronic periodontitis patients and 0.15 up to1.96 mg/dl.in chronic gingivitis patients. The individuals Mucosal herd immunity constitutes three basic immune fractions; low, moderate and high cryoglobulin responders. The immune herd plot types were matched as Gaussian distribution plot types. The findings of oral mucosal cryoglobulin among these patients in clinically indicative concentrations for the potential role may be played in the pathogenesis of chronic gingivitis and chronic periodontitis as it may cause precipitates in the local blood vessels of gum and periodontium.
Keywords: Cryoglobulin; oral; mucosal; gingivitis; periodontitis.

1. INTRODUCTION

In a series of papers concerning the oral immunology that have been published by Shnawa and Raghunathan 2015 various aspects of this topics were tackled [1]. The nature of the oral immune responses have been reviewed by Shnawa [2]. Human immunoglobulin are classified in accordance with heat sensitivity into; normoglobulin (Normopotent), cryoglobulin (Cryopotent) and pyroglobulin (Pyroapotent) [3,4]. Cryoglobulin is an immunoglobulin that precipitate at 4°C and dissolved in either 37°C or 45°C and associated with chronic intracellular microbial infections, autoimmune and lymphoproliferative as well as some neoplastic human diseases [5,6,7,8,9,10]. Human body fluids rather than that of blood contains cryoglobulins [6]. Local low grade B cell lymphoma secreting cryoglobulin or normoglobulin reported in association with membranoproliferative glomerulonephritis [7]. Acute kidney injury in patients with cryoglobulinemia secondary to hepatic mucosa associated lymphoid tissue lymphoma were reported [8]. Shnawa and Algebori reviewed the mucosal cryoglobulin [10] and they have been reported the presence of urinary mucosal cryoglobulin among pulmonary tuberculosis patients at Babylon province Iraq [10]. AL-Ameidi et al. have been reported the association of serum cryoglobulin with periodontitis patients [11]. The objective of the present work was to report on the presence of cryoglobulin responses in the oral mucosal materials of chronic periodontitis and chronic gingivitis patients together with tempts to use cryoglobulin responses as probes for mapping of oral mucosal herd immunity.

2. MATERIALS AND METHODS

Forty-five dental ill subjects from both sex, Table 1, were the dental clinic attendance of the college of dentistry university of Babylon to the year 2020-2021. The specialized dentist of the research team interviewed them and putdown the clinical diagnoses as chronic periodontitis and chronic gingivitis [12]. Patients consent were obtained before clinical sampling. Gum and periodontium affected materials were collected by the team dentist using standard standardized technique for collection and temporary maintenance of their contents [13]. Direct Giemsa and Gram stained films were done on the spot from the materials and examined [14]. Then both of the clinical materials were cleared off from their cellular contents as pellets by centrifugation at 5000rpm for five minutes. Supernatant fluids were kept for protein separation using PEG 6000 6% solution as protein precipitant. Equal volumes of PEG and the supernatant fluids were gently mixed and incubated at 4°C overnight. The precipitated protein collected as pellets and dissolved in two mls. amounts of sterile normal saline, loaded in cryoglobulin tubes in an upright positions. The loaded tubes were incubated at 4°C for 1 to 5 days to record the cryocrit percentages [5,15,16]. The cryoprecipitate were characterized [5] and cryoglobulin concentration were determined by Biurt method [16]. Immunofixation was done on the recovered cryoglobulin using low leveled immunoglobulin G,A,M [Manufacturar instructions]. Biometery for the findings were performed. In tables minimum, maximum, mean and ranges were calculated for cryoglobulin concentrations, cryocrit values, herd plots were done using concentration class interval of cryoglobulin concentrations as in [17].

3. RESULTS

3.1 Demography

The demography of periodontal patients was depicted in Table 1.

3.2 Cryoprotein Identification

The physical texture of the cryoproteins were of colloidal natures precipitated by PEG 6000 6%. at 4°C within 24 hrs. Cryoglobulins were precipitated at 4C within 1-5 days, dissolved at 37°C and re-precipitated at 4°C. Cryoglobulin may and may not be in association with rheumatoid factor Table 2.

3.3 Cryocrit

The mean cryocrit percentage values among periodontal patients were higher than that of controls. Chronic gingivalitis patients have shown cryocrit percent as 1-7% while the cryocrit percent for the chronic periodontitis patients were ranging 1-6% Table 3.

3.4 Cryoglobulin

The cryoglobulin concentration means for the periodontal patients were found approaching five
to six folds than that of control subjects. The cryoglobulin concentration means were; 0.23, 1.82 and 1.37 mg/dl for the control, chronic gingivitis and chronic periodontitis patients respectively Table 4.

3.5 Oral Mucosal Cryoglobulin Herd Immunity

Herd oral mucosal immune responses were found to be of three response types as low, moderate and high responders Table 5. When cryoglobulin concentration were tempted to plot herd immune response, it was normal Gaussian distribution both in Periodontitis and In chronic gingivitis patients Figs. 1 & 2.

3.6 Cryo-Globulin Immuno-Fixation

The cryoglobulin preparations were showing no precipitation zones in the low leveled IgG, IgA, & IgM partigens.

Table 1. The individual and herd demography of the periodontal test patients

<table>
<thead>
<tr>
<th>Features</th>
<th>Chronic Gingivitis</th>
<th>Chronic Periodontitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Herd level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd size</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Male-Female ratio</td>
<td>1.3: 1</td>
<td>1.2: 1</td>
</tr>
<tr>
<td><strong>Age range</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22-46 Y</td>
<td>30-52 Y</td>
</tr>
<tr>
<td>Female</td>
<td>30-47 Y</td>
<td>30-42 Y</td>
</tr>
<tr>
<td>Overall</td>
<td>22-47 Y</td>
<td>30-52 Y</td>
</tr>
</tbody>
</table>

Table 2. Identification of oral mucosal Cryoprotein and cryoglobulin in periodontal patients

**Cryoprotein**

Initial precipitation with PEG 6000 6% at 4 C for 24 hrs
Physical texture Initial precipitation: Colloidal appearance

**Cryoglobulin**

Initial duration of precipitation at 4C was 1-5 days
Dissolution at 37C
Re-precipitation at 4 C
Nature of Physical Texture Crystalline Gelatinous
Chronic gingivitis 5:23(21.73%) 18:23(78.26%)
Chronic periodontitis 6:22 (27.27%) 16:22(72.72%)
Positive Blurt Reaction
Rheumatoid factor positive cases
Chronic Gingivitis 4:23(17.39%)
Chronic Periodontitis 4:22(18.19%)

Table 3. Mucosal cryocrit percentages of periodontal patients

<table>
<thead>
<tr>
<th>Biometric features</th>
<th>Control</th>
<th>Chronic gingivitis</th>
<th>Chronic periodontitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
<td>1.83</td>
<td>3.1</td>
<td>3.55</td>
</tr>
<tr>
<td>Maximum</td>
<td>3</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Range</td>
<td>1-3</td>
<td>1-7</td>
<td>1-6</td>
</tr>
</tbody>
</table>
Table 4. Oral Mucosal cryoglobulin concentrations in mg/dl

<table>
<thead>
<tr>
<th>Biometric Features</th>
<th>Control</th>
<th>Chronic gingivitis</th>
<th>Chronic Periodontits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0.12</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>Median</td>
<td>0.18</td>
<td>1.84</td>
<td>1.96</td>
</tr>
<tr>
<td>Mean</td>
<td>0.23</td>
<td>1.82</td>
<td>1.37</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.4</td>
<td>1.96</td>
<td>2</td>
</tr>
</tbody>
</table>

**Range**

- Male: 0.13-0.4, 0.16-1.96, 0.14-2
- Female: 0.12-0.4, 0.15-1.96, 0.13-1.96
- Overall: 0.12-0.4, 0.15-1.96, 0.13-2

**Reference Normal Values for**

- Mixed I: 0.5 mg/dl.
- Mixed II: 0.1 mg/dl.
- Mixed III: 2.5 mg/dl.

Table 5. Herd Immune cryoglobulin Responders among periodontal patients

<table>
<thead>
<tr>
<th>Entity</th>
<th>Low responder concentration mg/dl.</th>
<th>Moderate responder concentration mg/dl.</th>
<th>High responder concentrations mg/dl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic gingivitis</td>
<td>0.13-0.9</td>
<td>1-1.8</td>
<td>1.9-2.9</td>
</tr>
<tr>
<td></td>
<td>5*:23 (21.7%)</td>
<td>12:23(52.17%)</td>
<td>6:23(26.1%)</td>
</tr>
<tr>
<td>Chronic Periodontitis</td>
<td>0.1-0.8</td>
<td>0.9-1.9</td>
<td>2-2.9</td>
</tr>
<tr>
<td></td>
<td>4*:22(18.18%)</td>
<td>16:22(72.72%)</td>
<td>2:22(9.1%)</td>
</tr>
</tbody>
</table>

*Number of patients

Fig. 1. Oral mucosal cryoglobulin herd plot of chronic gingivitis patients

*Cryoglobulin concentration in Gingivitis in mg/dl. The x axis, the class interval concentrations were: 1 - 0.09 = 0 2 - 0.1 - 0.9 = 4, 3 - 1.9 = 16, 4 - 2 - 2.9 = 2, 5 - 3 - 3.9 mg/dl = 0. And the Y axis were; the number of patients

4. DISCUSSION

Local body fluid other than blood had been advocated to contain cryoglobulins [6]. Local low grade B cell lymphoma secreting monoclonal cryoglobulin or normoglobulin in association with membrano-proliferative glomerulonephritis (MALToma) have been reported in two rare cases [7], and acute kidney injury in patient with cryoglobulinemia secondary to hepatic mucosa associated lymphoid tissue lymphoma [8]. Mucosal cryoglobulin report and review [9]. Urinary mucosal cryoglobulin has been reported in pulmonary tuberculosis patients in Babylon
Fig. 2. Oral mucosal cryoglobulin herd plot of chronic periodontitis patients
Cryoglobulin concentration in periodontitis in mg/dl. The X axis concentration class intervals were: 1-0.0-0.09 = O, 2- 0.1- 0.9 = 5 , 3- 1-1.9 = 12 , 4- 2 – 2.9 = 5 , 5- 3 – 3.9 mg/dl. = 0. And the Y axis were; the number of patients

province Iraq [11]. Cryoproteins were recovered from periodontal and gum materials of chronic periodontitis and chronic gingivitis patients by the veritu of separation by protein precipitant PEG at 4 C and being of positive Biurt test. The recovered cryoproteins were either of crystalline or gelatinous natures, with revers-able precipitation at 4C and solvation at 37C with an evident cryocrit % values. Characteristics which are consistent with cryoglobulin [5,6,7,8]. The cryoglobulin concentration means were higher in patients than in controls., Table 4. The findings of negative precipitation zones in IgG, IgA and IgM partigens may be attributed to:i- the difference in mucosal cryoglobulin antigenic specificity than mucosal normo-globulin antigenic specificity and /or secretory peace cryoglobulin specificity [17,18] and ii-the concentration limits of these cryoglobulins were below the limits of sensitivity of the tempted partigens [Manufacturer instruction].The recovered oral mucosal cryoglobulin concentrations were within the clinically indicative for a potential role that may be played in the pathogenesis immunity of chronic gingivitis and chronic periodontitis, as it may precipitate in the local blood vessels of gum and periodontium causing local vasculitis [20,21,17,19,22]. Three immune herd plots were noted as low, moderate and high responders, Table 5. Local and systemic infectious diseases that induce immune responses such responses are subjected to the influences of human individual variation of the individuals forming the herd and herd immunity though it is of more profound effects in communicable infectious diseases [11,23]. The herd plots nature was of normal distribution type in contrast to serum cryoglobulin which showed skwed typ. Oral mucosal cryoglobulin may constitute valid marker of herd immunity in periodontal diseases [11,24,23].

5. CONCLUSION

Oral mucosal cryoglobulin were identified in association with materials recovered from gum and periodontium of the chronic gingivitis and chronic periodontitis patients. The recovered cryoglobulins were indicating the potential role played in the pathogenesis of periodontal diseases The oral mucosal cryoglobulin response of the herd were evident as low , moderate and high responders .The immune herd plots were normal Gaussian distribution types. Oral mucosal cryoglobulin was found as valid probe for herd immunity.

CONSENT

Patients written consent were obtained before clinical sampling.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
REFERENCES


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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/82708