

**Estimation of Serum Igg in Periodontitis
Patients and Healthy Controls – A Comparative Study**

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Certificate

THIS IS TO CERTIFY THAT DR. R. AROKIARAJ, POST GRADUATE STUDENT (2002-2005) IN THE DEPARTMENT OF PERIODONTICS, TAMIL NADU GOVT. DENTAL COLLEGE & HOSPITAL, CHENNAI-03 HAS DONE THIS DISSERTATION TITLED “ ESTIMATION OF SERUM IGG IN PERIODONTITIS PATIENTS AND HEALTHY CONTROLS – A comparative study” UNDER OUR DIRECT GUIDANCE AND SUPERVISION IN PARTIAL FULFILLMENT OF THE REGULATIONS LAID DOWN BY THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI, FOR M.D.S., BRANCH – II PERIODONTICS DEGREE EXAMINATION.

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CONTENTS

TITLE

1. INTRODUCTION
2. AIMS AND OBJECTIVES
3. REVIEW OF LITERATURE
4. MATERIALS AND METHOD
5. RESULTS
6. DISCUSSION
7. SUMMARY AND CONCLUSION
8. BIBLIOGRAPHY

INTRODUCTION

Recent trend in the etiopathogenesis of periodontal disease is mainly along the bacterial and immunological reactions involved. Since periodontitis is considered as a chronic inflammatory oral infection caused by specific pathogenic subgingival microflora. Periodontal bacteria like *P. gingivalis* and *A.actinomycetemcomitans* are regarded as specific pathogens in various types of periodontal diseases. Several studies^{7,14,34,35,73} have demonstrated that the antibody titers to these two organisms are increased in patients with periodontitis compared with subjects without periodontal disease. Further Aukil⁴ et al have demonstrated that serum antibody titer to *P.gingivalis* was reduced in subjects with advanced periodontitis following treatment. This shows a cause and effect relationship between periodontitis and serum antibody levels.

Also patients who originally had high levels of IgG to *P.gingivalis* had better treatment outcome in terms of a reduced number of deep pockets than patients with lower titers, indicating the protective role of IgG in the pathogenesis of periodontal disease.

Hence the present study was undertaken to compare the levels of serum IgG in healthy subjects to that of periodontitis patients.

REVIEW OF LITERATURE

The immunoglobulins are proteins with antibody activity, i.e., they combine specifically with the substance that elicited their formation. They constitute the humoral arm of the immune response where majority of them belong to gammaglobulins on serum electrophoresis. The two-hallmark features of immunoglobulins are “specificity” and “diversity” in addition to its secondary biologic activities.

Historic background of immunoglobulins:

George H.F. Nuttall, 1888 demonstrated the first antibodies which he called as serum bactericidins in the preantibiotic era.

Kraus, 1897 demonstrated the biofunctional property of antibodies which prompted him to call them as precipitins.

Sir Almoth Wright, 1903 reported that antibodies could aid in the process of phagocytosis. These features of antibodies made him name them as opsonins.

Arne Wilhelm Tiselum, 1937 identified that antibodies were serum proteins belonging to the group of globulins which are in the gamma region on electrophoresis.

Susumu Tonegawa, 1978 put forth the genetic principle for generation of antibody diversity and the intricacies in immunoglobulin gene rearrangement.

Baruj Benaceraff, Jean Decusset, 1980 discovered genetically determined structure on the cell surface that regulates immunologic reactions.

Basic structure of immunoglobulin:

Immunoglobulins are glycoproteins composed of 82-96 %of polypeptide and 4-18 % of carbohydrate. The polypeptide component possesses almost all of the biological properties associated with antibody molecules.

Immunoglobulin G:

In normal human adults,IgG constitute approximately 75% of the total serum immunoglobulin,within the IgG class,the relative concentration of the 4 subclasses are approximately.

IgG1 - 60 – 70 %

IgG2 - 14 – 20 %

IgG3 - 4 – 8 %

IgG4 - 2 - 6 %

These figures vary from individual to individual.thus the capacity of a given individual to produce antibodies of one or another IgG subclass may be under genetic control.

IgG is the only class of immunoglobulin that can cross placenta in human and it is responsible for protection of the newborn during the first month of life. IgG2 being transferred slowly than others.IgG is also capable of fixing serum complement.

Table-I**SALIENT FEATURES OF IgG**

Heavy chain class	Gamma
Heavy chain subclasses	$\gamma_1, \gamma_2, \gamma_3, \gamma_4$
Light chain class	κ & λ
Molecular formula	$\gamma_2 L_2$
Sedimentation co-efficient	6 – 7
Molecular weight	150,000
Serum concentration (mg/dl)	1000
Serum half life	23 days
Complement fixation	+
Placental transfer	+
Anti bacterial lysis	+

Table –II- Properties of human IgG subclasses

IgG subclass	IgG1	IgG2	IgG3	IgG4
% of total IgG	70	20	6	4
Half life in serum(days)	23	23	7	23
Placental passage	+++	+	+++	+++
Complement fixation	++	+	+++	-
Binding to Fc receptor	+++	+	+++	-

Tests available for detecting antigen – antibody reaction :

- 1.Immunoelectrophoresis
- 2.Immunofixation electrophoresis
- 3.Electro immuno diffusion
- 4.Nephelometry
- 5.Radioimmuno assay
- 6.ELISA
- 7.Fluorescence polarization immunoassay
- 8.Enzymatic radio immunoassay
- 9.Immunofluorescence

Immunodiffusion:

In 1946, Oudin described a system of single diffusion of antigen and antibody in agar filled tubes. This important advance was soon followed by Ouchterlony's⁵¹ classic description of double diffusion on agar layered on slides. This method is used in the detection and analysis of precipitating antigen antibody system.

Immunodiffusion may be classified as single or double. In single immunodiffusion, either antigen or antibody remains fixed and other reactant is allowed to move and complex with it. In double immunodiffusion both reactants are free to move toward each other and precipitate.

Methodology & interpretation:

Mancini⁴³, 1965 introduced a novel technique for accurate quantitative determination of antigen & antibody.

The formation of antigen, antibody complexes in a semisolid medium such as agar is dependent on buffer electrolytes, pH and temperature while the most important determinants are the relative concentration of antigen and antibody.

Serum immunoglobulin levels in health and disease:

Serum immunoglobulin levels is dependent on a variety of developmental genetic and environmental factors. These included ethnic

background like age, sex, history of allergies or recurrent infections and geographic factors. (E.g., increased IgE levels in endemic parasite infection).

Normal human infants are born with very low serum immunoglobulin levels, the entire immunoglobulin portion of cord serum has been transferred transplacentally from the mother. After birth the maternal IgG decays and increase in autologous IgG synthesis.

Table – III
Serum IgG of normal subjects at different ages

Age	Level of IgG (mg/dl) range	Level of IgG % Of adult level
New born	1031 ± 200	89 ± 17
1 – 3 months	430 ± 119	37 ± 10
4 – 6 months	427 ± 186	37 ± 16
25 – 36 months	892 ± 183	77 ± 16
3 – 2 years	929 ± 228	80 ± 20
6 – 8 years	923 ± 256	80 ± 22
12 –16 years	946 ± 124	82 ± 11
Adults	1158 ± 305	100 ± 26

TABLE – IV

Serum IgG in disease

Disease	Changes in IgG level
Immunodeficiency	Moderate – marked decrease
Monoclonal gammopathies	Marked increase
Infections	Moderate increase
Liver diseases	Slight to moderate increase
Collagen disorder	Slight to moderate increase

IgG in periodontitis:

Richard L.Reiff⁵⁸ 1983 undertook a study to determine and compare IgG and IgA levels in both serum and saliva before to and after therapy. He found that the less severe the periodontal involvement more consistent was the reduction in IgG and IgA following initial therapy.

Ebersole¹⁷ et al,1985, evaluated the relation between scaling and root-planing treatment on systemic antibody response especially IgG. Peak levels of IgG response were noted at 2 – 4 months post treatment to microorganism detected in the subgingival plaque.

Ebersole¹⁶ et al ,1986, studied the serum IgG level to members of bacteroides genus and found that there was a significant elevation in serum IgG titer to adult and advanced destructive periodontitis.

Lai CH³⁴ etal, 1986 compared the serum IgA, IgG antibodies to Treponema vincentii and Treponema denticola in adult periodontitis and healthy subjects. They concluded that there was no significant correlation between serum antibody titre to any of the oral spirochaete tested.

Mandell RL⁴² etal 1987 assessed the clinical, immunological and microbiological features of active disease sites in juvenile periodontitis. They found that only A. actinomycetemcomitans was related to a marked increase in attachment loss.

Schenck, K. Michaelsen⁶¹ TE 1987 conducted a study to find the IgG subclass distribution in serum against lipo poly saccharide from B. gingivalis in health and disease. They concluded that periodontitis patients had significantly higher anti lipo poly saccharide IgG levels compared to healthy subjects where IgG2, was the dominating subclass.

Peng⁵³ 1988 evaluated serum antibodies to native and denatured type I and type III collagen in patients with periodontal disease. He found that serum IgG level to antibodies to native and denatured type I and type III collagen in patients with juvenile periodontitis were significantly higher than those of normal subjects.

Zambon⁷³ et al 1988 examined the subgingival microflora and serum IgG level in periodontitis patient with non insulin dependent diabetes mellitus. They demonstrated that there was a increase in response to *B. gingivalis* in non insulin dependent diabetes mellitus group than other groups.

Kohyama³¹ A 1989 assessed clinical parameters like plaque index, probing depth, bleeding on probing and percent bone loss in addition to microbiological and serological investigations. He concluded that monitoring *B. gingivalis* in subgingival plaque serum IgG titre against it may aid in the adequate treatment of periodontal disease.

Cao⁷ et al 1990 showed that serum IgG to *B. gingivalis* tendered to be greater in accordance with the increase of disease severity and percentage of black pigmented bacteroides species.

Lamster³⁵ et al 1990 evaluated the relationship of GCF and serum antibody titer to periodontal pathogens. They found that the development of serum IgG antibody response to periodontal pathogen is consistent with a protective host response.

Chen¹⁰ et al 1991 studied the humoral immune response to porphyromonas gingivalis before and after therapy in RPP patients. They concluded that many RPP patients do not have protection levels of biologically

functional antibody during the course of their natural infection, but stimulated after treatment.

Ebersole¹⁴ et al,1991, assessed the serum antibody responses to *A.actinomycetemcomitans* infected patients with periodontitis . They demonstrated that the primary response to *A.actinomycetemcomitans* were of IgG1 and IgG3 subclasses ,whereas the primary subclass response in normal subjects was limited to the IgG2 subclasses.

Gregory RL²³ et al, 1992 observed that the IgG degrading enzymes in localized juvenile periodontitis due to extensive degradation of IgG and IgA in crevicular fluid samples from periodontal disease sites of localized juvenile periodontitis.

Lopatin DE³⁸ et al 1992, assessed the avidity and titre of IgG subclasses to *p. gingivalis* in adult periodontitis patients. They found that the predominant humoral response to *P.gingivalis* occurs in IgG2 subclass and showed significantly lower relative avidity.

Randal⁵⁷ et al 1993 studied the site-specific isolates of microbiota associated with NUG. It was concluded that relationship of serum IgG and spirochaetes was weak or non existent.

Kinane³⁰ et al, 1993 evaluated the local and systemic response in patients with chronic periodontitis They demonstrated that the chronic

periodontitis patients with greater pocket depth and more gingival inflammation had paradoxically lower antibody titre.

Lamster³⁶ et al 1994 examined the strategies for identifying individual risk profile for patients with periodontal disease based on host response .They found that β .glucuronidase levels in GCF were inversely correlated with the IgG serum antibody suggesting the essentially protective function of the systemic humoral response in periodontal disease.

Wilson⁷⁰ et al 1995 found that serum of patients with localized juvenile periodontitis which when employed in conjunction neutrophils that express receptors that are capable of recognizing this subclass and hence they are opsonic for A.actinomycetemcomitans.

Unsal⁶⁷ BT et al, 1996, assessed the effects of periodontal therapy on serum antibody levels to A.actinomycetemcomitans and P.gingivalis .He concluded that an increase of anti body level against specific bacteria is debatable .

Quinn SM⁵⁵ et al,1998 ,evaluated the influence of smoking on adult periodontitis and serum IgG2 .They demonstrated that there is a definite decrease in serum IgG2 in smokers than in non smokers .

Chung HY⁹ et al ,2003 ,studied the immunoglobulin G profiles in different forms of periodontitis and they concluded that microbial challenge

might not provoke significant changes in systemic IgG response in patients with chronic periodontitis.

Pussinen PJ⁵² et al, 2004, evaluated the association between periodontal pathogens and stroke using serum antibodies as a marker .The study concluded that there is serological evidence that an infection caused by major periodontal pathogens is associated with future stroke.

SUMMARY AND CONCLUSION

Twenty-four subjects were selected from among the outpatient attending the Department of Periodontics, TamilNadu Government Dental College and Hospital, Chennai-3. Twelve subject with probing depth ≤ 3 mm and no clinical attachment loss where taken as control group and remaining twelve patients with probing depth ≥ 4 mm and with clinical attachment loss ≥ 1 mm were taken as study group.

The periodontal status of both the groups were assessed by measuring the plaque index, probing depth and clinical attachment loss. Intra-oral radiographs were taken for radiographic assessment. Two ml of blood sample were obtained by venupuncture and transported to the clinical laboratory for serological investigations and quantitation of serum IgG levels was done using radial immunodiffusion assay.

Data collected were analysed statistically using student t-test and Pearson correlation analysis. Clinical periodontal parameters and serum IgG levels were compared with control and study groups. Plaque index, mean probing depth and mean clinical attachment loss were statistically significant, higher than the control group ($P < 0.001$). However serum IgG levels were not statistically significant between control and study groups ($P > 0.005$)

In conclusion, though both the groups were studied for comparable parameters. It was found that no statistically significant correlation exists between periodontitis and serum IgG levels. Further studies should be conducted with more number of samples to substantiate this finding.

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