IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 2 Ver. 9 February. (2018), PP 63-72
www.iosrjournals.org

"Comparative evaluation of Serum Total IgG in Aggressive and Chronic Periodontitis patients – An Immunoanalytical study"

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Date of Submission: 08-02-2018 Date of acceptance: 23-02-2018

I. Introduction

Periodontal disease is an irreversible infectious disease which causes progressive destruction of the supporting tissues of the tooth as a result of plaque accumulation and is manifested as local inflammation of the periodontium. Currently, two main forms of destructive periodontal disease are recognized, chronic and aggressive periodontitis **(Armitage 1999)[1]**.

Mounting a significant level of serum immunoglobulin G (IgG) antibodies has been considered important in preventing periodontal destruction in patients with aggressive periodontitis and chronic periodontitis (formerly adult periodontitis).

Most research has focused on the immune response to specific periodontal pathogens, thus there is little information about the general serum levels of IgG in patients with periodontitis, and studies that have been done report diverse results. Although no differences in the total IgG levels were found between patients and controls with chronic periodontitis and generalized juvenile periodontitis **(Lu et al.1994, Reinhardt et al 1989)[2, 3]**, **Wilton et al. (1992)[4]** reported elevated IgG2 levels in patients with chronic periodontitis.

Hence, in the present study an attempt has been made to study the impact of aggressive and chronic periodontitis on systemic inflammatory marker serumIgG.

II. Aims And Objectives

Our aim was to analyze whether serum total immunoglobulin G (IgG) can be used as marker to differentiate aggressive & chronic periodontitis patients and healthy controls by estimating & comparing serum total IgG levels using radial immunodiffusion method. Also an attempt has been made to observe whether serum total IgG levels are correlated with extent of periodontal destruction.

III. Material And Methods

Sixty patients comprising of 33 males and 27 females were part of the present study. The subjects included in the test groups were selected from the patients in Periodontology clinics in Government Dental College & Hospital, Aurangabad. Patients' with age between 17-35 years, minimum complement of 20 teeth, Periodontal probing depth ≥4mm and clinical attachment loss >2mm, Radiological evidence of bone loss were included in the study as Periodontitis patients and later segregated in to Aggressive Periodontitis (AgP), & Chronic Periodontitis (CP) according to the classification of American Academy of Periodontology **(Armitage, 1999)**[1]

The test subjects were divided in to following groups:

1. **Group I - Aggressive Periodontitis (AgP):** 20 patients

Further Group I was divided in to two subgroups on extent based division

Group IA - Generalized Aggressive Periodontitis (GAP): 10 patients

Group IB - Localized Aggressive Periodontitis (LAP): 10 patients

2. **Group II - Chronic Periodontitis (CP):** 20 patients

Further Group II was divided in to two subgroups on extent based division

Group IIA – Generalized Chronic Periodontitis (GCP): 10 patients

Group IIB - Localized Chronic Periodontitis (LCP): 10 patients

The control group was derived from those subjects who attended the restorative dental clinics and graduate students of Government Dental College & Hospital, Aurangabad with similar demographic characteristics as that of test group and showing no clinical evidence of gingival inflammation and loss of attachment.

Group III - Healthy Periodontal Status (HPS): 20 subjects

Patient with any systemic disease, Chronic ingestion of drugs or any type of medication / any medical condition (flu, allergy, sinusitis, etc) / Physical trauma in last two weeks, any history of dental treatment since

last six months including oral prophylaxis or any type of periodontal surgery**,** tobacco smokers or chewers**,** Periodontal abscess or any necrotizing form of gingival disease&Gingivitis and mild periodontitiscases were excluded from the study.

Informed consent was obtained from all patient participants and protocol was in accordance with the Helsinki Declaration of 1975 that was revised in 2000 and approved by Institutional ethical committee and Maharashtra University of Health Sciences, Nashik, Maharashtra (India). Procedure was fully explained to all patients before the study. A special case history proforma was designed so as to have systematic and methodical recording of all the observations and information requisite for the study. The proforma included a detailed case history including clinical examination, radiographic interpretation, laboratory investigations (which included blood sugar at fasting and postprandial and also complete hemogram).The probeable pocket depth (PPD) and clinical attachment level (CAL) measurements were also obtained.

After the diagnosis,five ml of venous blood sample was collected from each of the 60 patients by venipuncture in the antecubital fossa without excessive venous stasis. The blood samples were collected in a plain test tube containing no anticoagulant and allowed to clot to separate the serum. The serum samples were then centrifuged at 3000 rpm for 10 min. These samples were then stored at -20^{o}C till its estimation.

Serum total IgG assay was performed by single radial immunodiffusion (RID).RID plates containing mono-specific antibody to IgG in agarose gel were used **[Fahey &McKelvey (1965)**[5]**, Mancini et al. (1965)** [6]]. **(BINDARID® KITS, The Binding Site Ltd, Birmingham, UK).** The calibrator, control and test samples were gently mixed immediately before use. Two wells in each plate were filled with 5µl of the high calibrator and control serum using a micropipette. The remaining wells were then be filled with 5µl of test samples. The lid was tightly closed and the plates were stored flat at room temperature 25^{o}-30^{o} C and were incubated for 48 hours. After the required diffusion time, ring diameters were measured to the nearest 0.1 mm, using digital vernier caliper. The concentration of immunoglobulin G in each test sample can be read directly from the RID Reference Table (based on linear calibration curve) provided by the manufacturer.

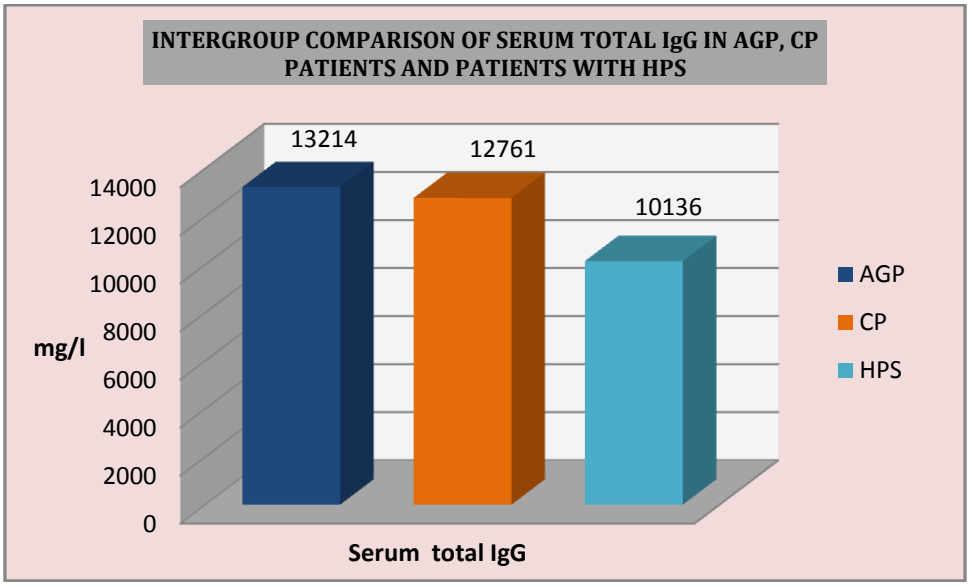
Test of statistical significance such as unpaired student 't' test, ANOVA and correlation co-efficient were applied to the test results. For all analyses, only p values <0.05 were considered statistically significant.

IV. Results

Table 1: Intergroup comparison between mean levels of serum total IgG in AgP and CP patients & patients with HPS

	AgP	CP	HPS	p
S. IgG (mg/l)	13214 ± 2140	12761 ± 2455	10136 ± 2166	0.01**
**= statistically highly significant				

GRAPH 1

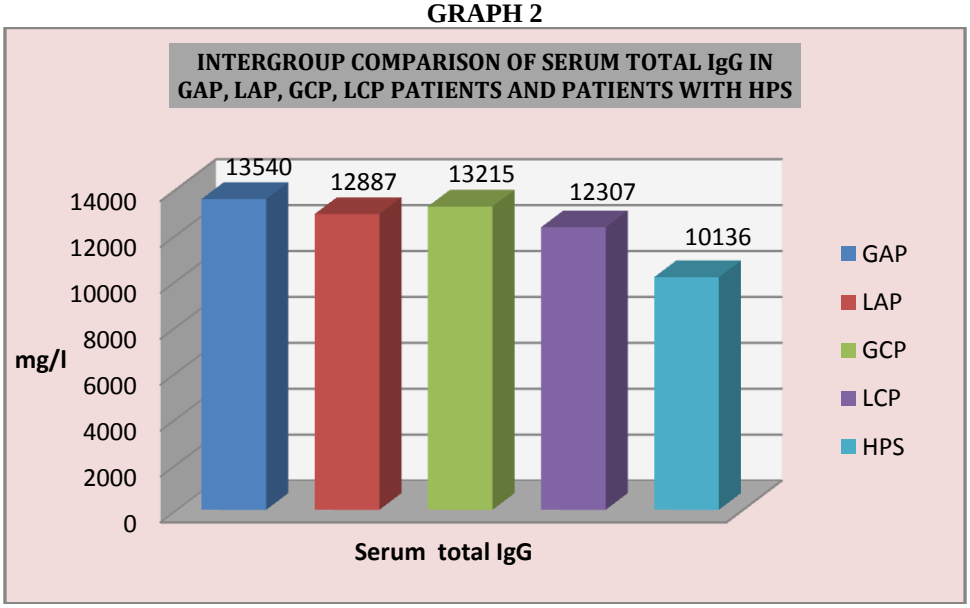


There was statistically highly significant difference for serum total IgG levels in AgP and CP patients when compared to patient with HPS. However AgP and CP did not differ significantly for serum total IgG. (TABLE 1& GRAPH 1)

Table 2: Intergroup comparison between mean levels of serum total IgG in GAP, LAP, GCP, LCP patients and patients with HPS

	AgP		CP		HPS	p
	GAP	LAP	GCP	LCP		
S. IgG (mg/l)	13540 ± 2161	12887 ± 2182	13215 ± 2014	12307 ± 1880	10136 ± 2166	0.02*
*= statistically significant						

GRAPH 2



When extent based sub divisions were compared with HPS, there was statistically significant difference for serum total IgG levels in GAP, LAP and GCP patients. LCP patients did not differ significantly for serum total IgG from periodontally healthy patients.(TABLE 2& GRAPH 2)

Table 3: Intragroup comparison between mean levels of serum total IgG in GAP and LAP patients

	GAP	LAP	P
S. IgG (mg/l)	13540 ± 2161	12887 ± 2182	0.51

Table 4: Intragroup comparison between mean levels of serum total IgG in GCP and LCP patients

	GCP	LCP	p
S. IgG (mg/l)	13215 ± 2014	12307 ± 1880	0.31

There was no statistical significant difference between GAP & LAP and GCP & LCP for serum total IgG (TABLE 3 & 4)

Table 5: Correlation Coefficients of blood leucocytes and serum proteins with clinical parameters (PPD & CAL) in patients with GAP & LAP

	GAP		LAP	
	PPD	CAL	PPD	CAL
S. IgG	0.85**	0.87**	0.84**	0.80**
*= statistically significant		**= statistically highly significant		

Table 6: Correlation Coefficients of blood leucocytes and serum proteins with clinical parameters (PPD & CAL) in patients with GCP & LCP

	GCP		LCP	
	PPD	CAL	PPD	CAL
S. IgG	0.72*	0.74*	0.80**	0.84**
= statistically highly significant= statistically highly significant				

Serum IgG levels were statistically significantly correlated with extent of periodontal infection .i.e PPD & CAL(TABLE 5 & 6)

V. Discussion

Traditional thinking / paradigm have maintained that periodontitis is an oral disease and that the tissue destructive response remains localized within the periodontium, limiting its effects of the disease to oral tissues supporting the teeth. Thus in the present study we focused on differences in the immune response viz. serum total IgG in Aggressive periodontitis (AgP), Chronic periodontitis (CP) and patients with healthy periodontal status (HPS). Differences in the extent based subdivisions i.e. generalized aggressive periodontitis (GAP), localized aggressive periodontitis (LAP), generalized chronic periodontitis (GCP) and localized chronic periodontitis (LCP) was also determined. Also there is a gap in the knowledge regarding serum IgG levels and periodontal destruction. Thus the correlation of serum IgG levels was assessed with the extent of periodontal destruction as determined by probeable pocket depth (PPD) & clinical attachment level (CAL).

Humoral immune response i.e antibodies are produced against foreign antigens in order to detoxify and neutralize them.**Ebersole et al. (1982a)**[7]**, Ranney et al. (1982)**[8], **Susan Doty et al. (1982)**[9]**, Gunsolley et al. (1987)**[10] in their studies in the early 80's and **Kinane et al. (1993)**[11] investigated serum antibody responses to various periodontal bacteria. Using the accepted nomenclature of that time, these studies primarily compared subjects with LJP or GJP and, to a lesser extent, subjects with adult periodontitis, and demonstrated an inverse relationship between titre levels and extent of disease. A protective nature of antibody responses was inferred for investigated titres to periodontal species.

Our study indicated that high antibody levels do not necessarily confer protection from widespread disease. This is in accordance with **Ebersole et al. (1984)**[12]**, Gunsolley et al. (1990)**[13]**,Lamster et al. (1998)**[14]**, Albandar et al. (2001)**[15]**, Craig et al. (2002)**[16]**, Craig et al. (2003)**[17]**, Furuichi et al. (2003)**[18]**, Graswinkel et al. (2004)**[19]**, Shi et al. (2008)**[20]. This change in paradigm raised questions about the protective function of antibodies. The lymphokine and enzyme secretion induced by IgG–Antigen–complement complex can cause local tissue destruction in periodontitis and the disseminating lipopolysaccharide-specific antibody response provides a source of potentially inflammatory mediators, including PGE_2, TNF-α and IL-1β, which also play significant role in local tissue destruction and in systemic complications.

. The serum total IgG levels in our study group was positively correlated with the extent of destruction i.e. with increasing PPD & CAL, serum IgG levels concomitantly increased. High levels of antibody in patients with periodontitis may be either indicative of an effective host response, which can contain the infection, or a sign of a chronic failure to control the disease **(Page 1998)**. Our study supports the notion that high antibody levels do not necessarily confer protection.

Limitations of our study

1. Further studies with larger sample sizes are required to evaluate other genetic or environmental factors such as the time point of blood sampling and the composition of microbial species, which may also affect the serum IgG concentrations.
2. This was a cross sectional study examining the patient at only one point of time. Substantial longitudinal studies that monitors antibody levels prior to disease onset, during progression to severe disease, and following stabilization due to clinical intervention or spontaneous remission are necessary to fully understand the role of antibodies in tissue destruction and their potential for use in risk assessment of periodontal disease management and its associated systemic complications.

Conclusions &Inferences

1. Serum total IgG can be used as systemic inflammatory markers to distinguish between disease and health.
2. Different forms of periodontitis i.e. AgP& CP, GAP & LAP and GCP & LCP cannot be distinguished using serum total IgG levels.
3. High antibody levels do not necessarily confer protection from widespread periodontal disease.

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Figure legends:

1. Fig# 1 : Armamentarium for clinical diagnosis
2. Fig# 2 : Armamentarium for blood collection procedure
3. Fig# 3 : Procedure of blood collection
4. Fig# 4 : Armamentarium for estimation of serum total IgG by Radial Immunodiffusion method
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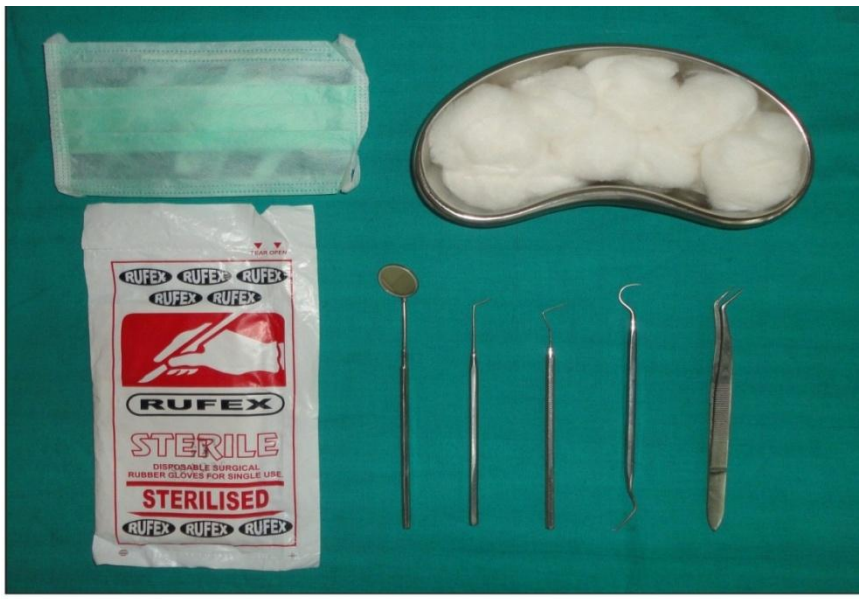
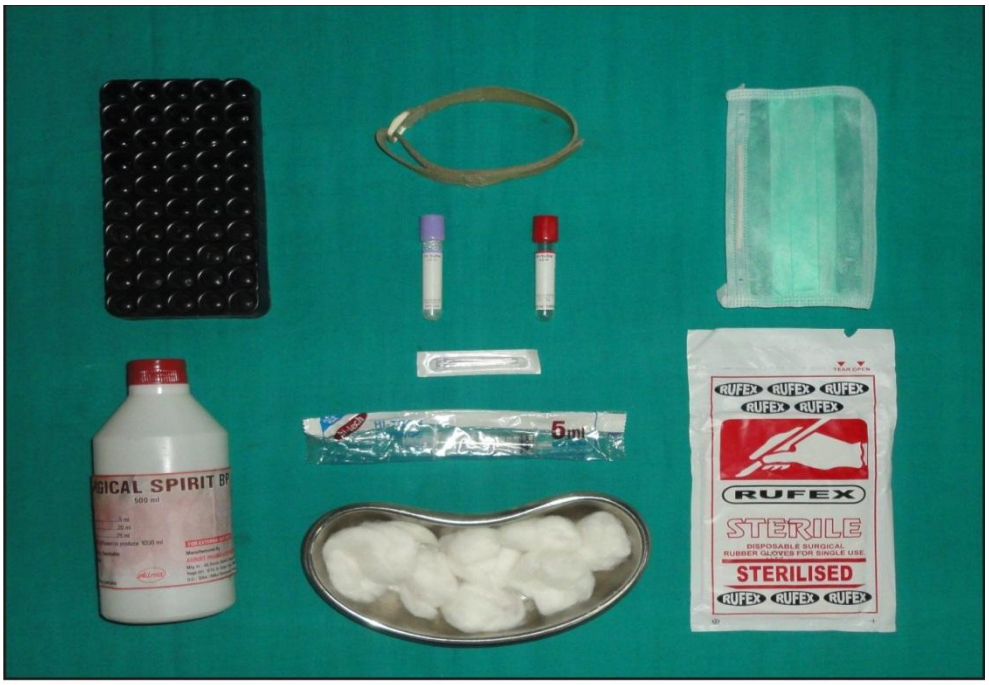
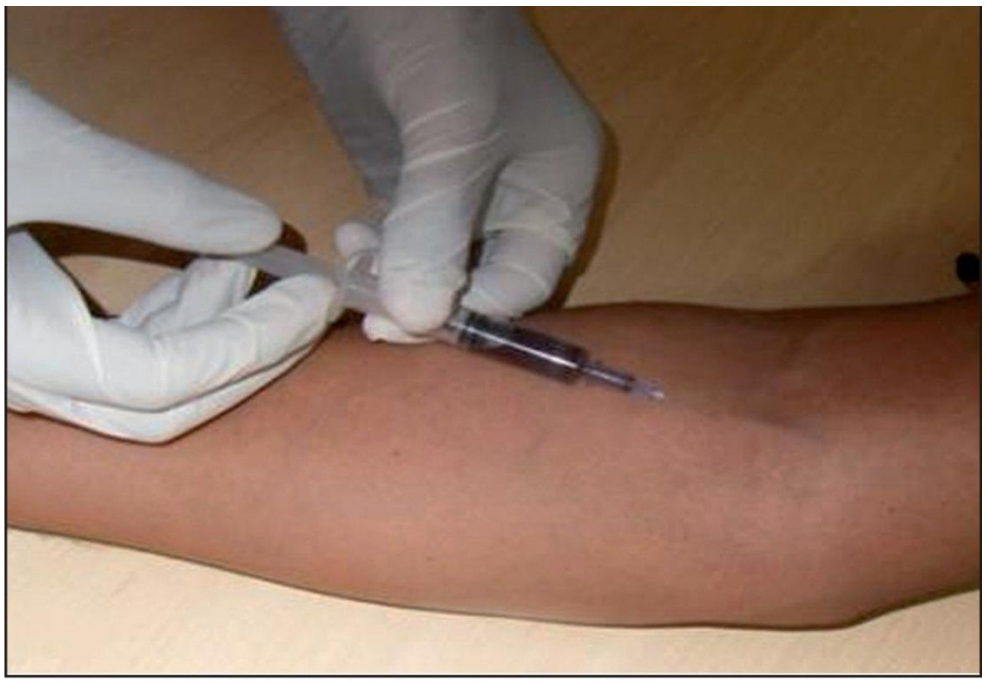
6. Fig# 6 : Immunoprecipitation rings after incubation period of 48 hrs
7. Fig# 7& 8 : Clinical picture of Generalized Aggressive Periodontitis (GAP) and Localized Aggressive Periodontitis (LAP) patients
8. Fig# 9 & 10 : Clinical picture of Generalized Chronic Periodontitis (GCP) and Localized Chronic Periodontitis (LCP) patients
9. Fig# 11: Clinical picture of patients with Healthy Periodontal Status (HPS)



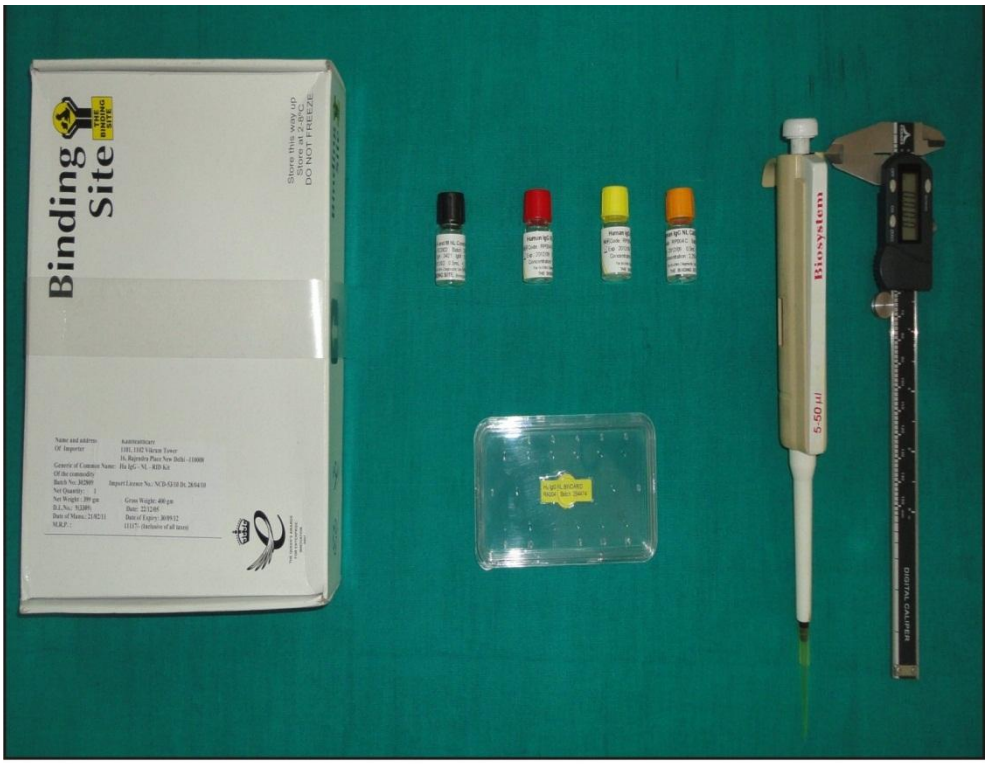
Photograph 1. Armamentarium for clinical diagnosis



Photograph 2. Armamentarium for blood collection procedure

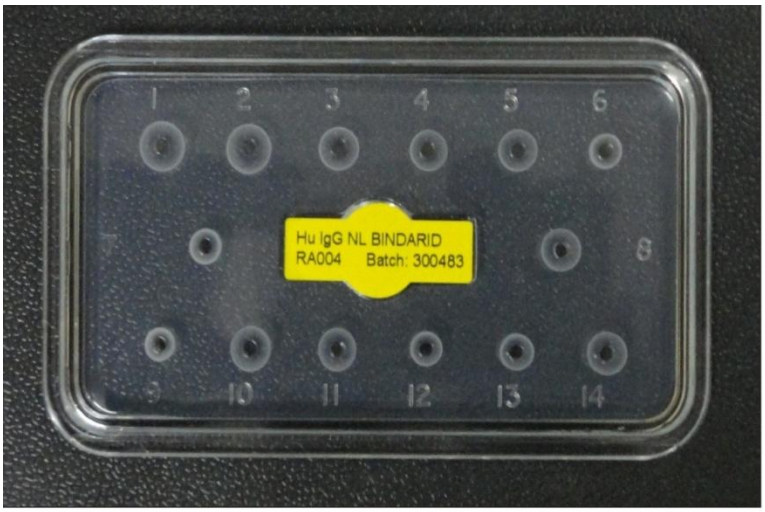


Photograph 3. Procedure of blood collection

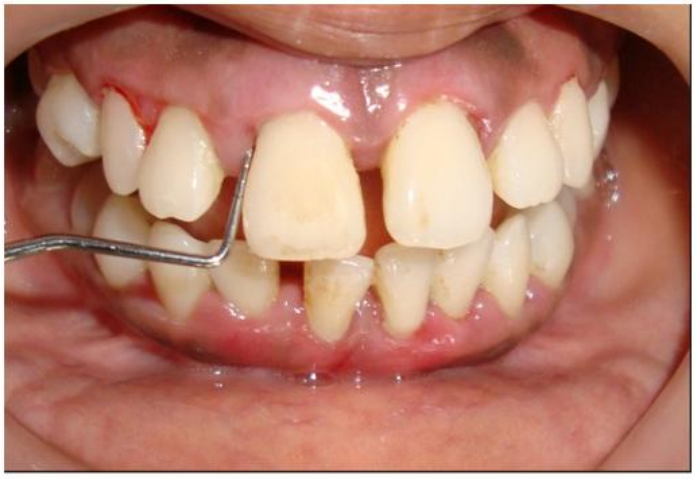


Photograph4. Armamentarium for estimation of Serum IgG by Radal immunodiffusion technique (RID Assay)

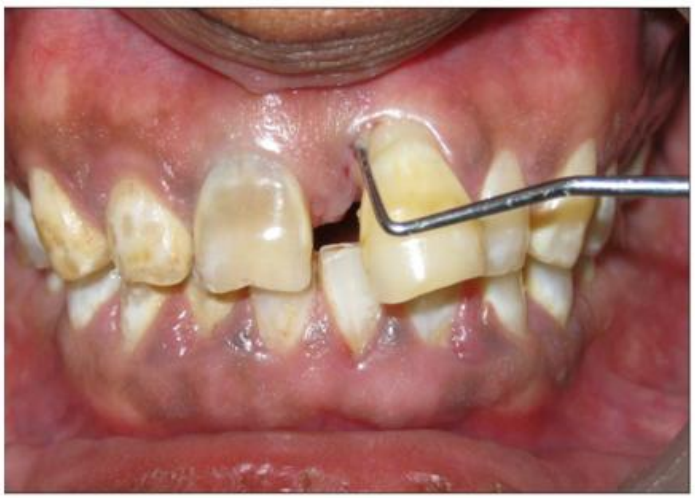
Photograph 5. Procedure of Radial immunodiffusion technique (RID Assay)



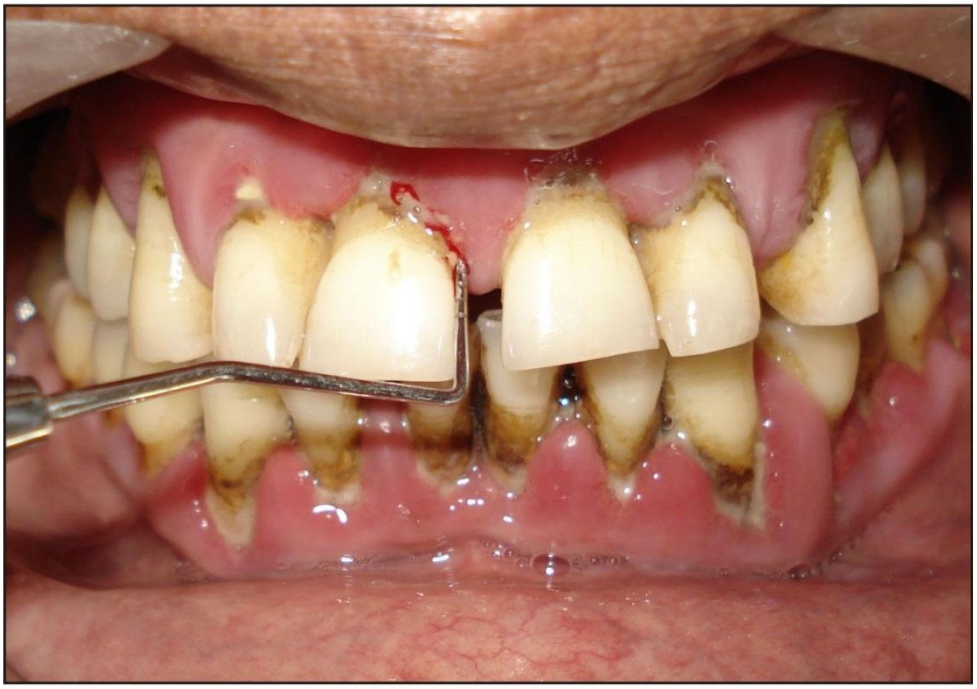
Photograph 6. Immunoprecipitation rings after incubation period of 48 hours



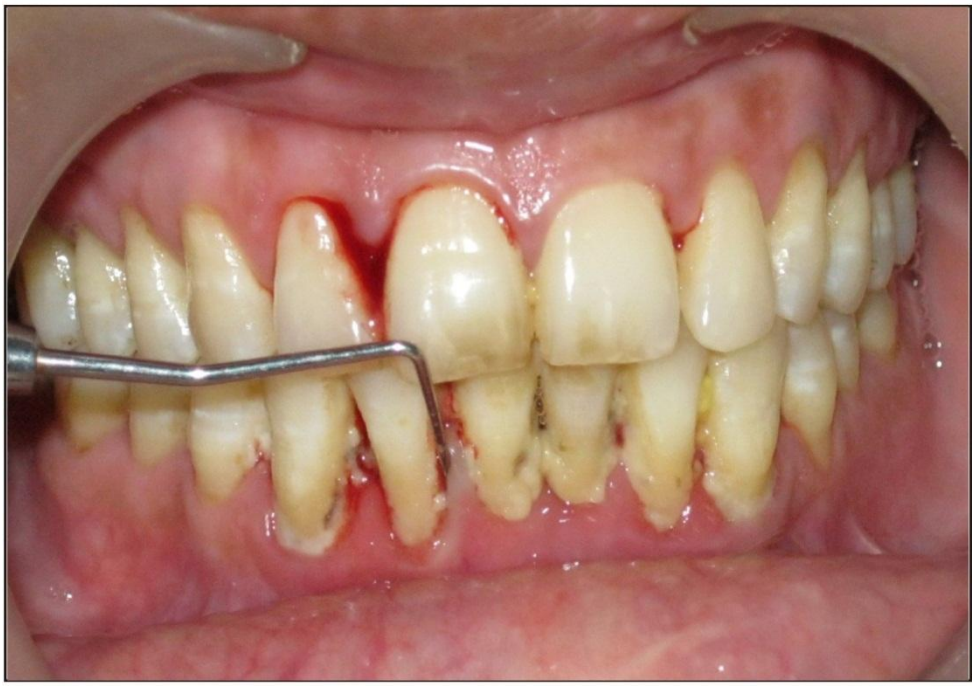
Photograph 7. Clinical picture of a generalized aggressive periodontitis (GAP) patient



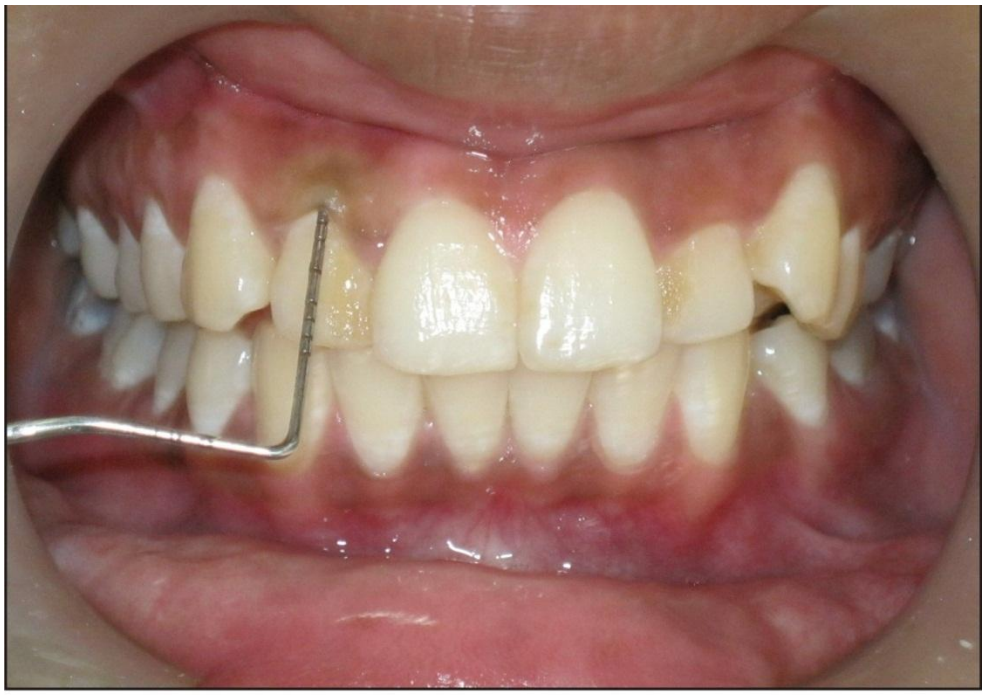
Photograph 8. Clinical picture of a localized aggressive periodontitis (LAP) patient



Photograph 9. Clinical picture of a generalized chronic periodontitis (GCP) patient



Photograph 10. Clinical picture of a localized chronic periodontitis (LCP) patient



Photograph 11. Clinical picture of a patient with healthy periodontal status (HPS)

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