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Correlation Of Viral And Bacterial Concentrations In Plaque And Glycemic Status In Patients With Chronic Periodontitis With And Without Type 2 Diabetes Following Phase 1 Therapy - A Clinical, Microbiological And Biochemical Assessment

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Introduction

Background & Objectives: With increasing evidences implicating the role of viruses in periodontal disease and diabetes, the present study aimed to assess, compare and correlate the change in clinical periodontal parameters; viral and bacterial pathogen concentrations in plaque and Glycated hemoglobin levels (HbA1c) in patients with chronic periodontitis with and without type 2 diabetes.

Method :120 patients with chronic periodontitis (mild to moderate) were divided into two main groups as Group 1 (control n=30 non-diabetic patients) and Group 2(test n= 90 patients with type 2 diabetes) which were further subdivided based on glycemic control. Clinical parameters viz gingival index, plaque index, probing pocket depth & relative attachment level; and HbA1c levels were evaluated at baseline,1 and 4 months following phase -1 therapy, along with microbiological parameters viz bacteria (Pg,Pi,Tf,A.a) and viruses (HSV,EBV,HCMV) in the plaque samples by PCR analysis.

Results: Significant improvements in clinical and microbiologic parameters and HbA1c levels ($p<0.05$) were observed within all groups with more significant clinical changes in group 1(control) compared to the (test) groups following phase 1 therapy. Viruses were not detected in most of the samples tested. Significant positive correlation of clinical parameters with bacterial counts($p<0.05$) but not with the viral counts, and specifically probing depths with HbA1c levels in the diabetic group ($p<0.05$), were observed.

Conclusion: Although phase-1 therapy resulted in enhancement of clinical parameters, glycemic status and reduction in bacterial counts, the role of viruses in periodontal disease seems questionable as the proportion detected was negligible.

Keywords: Chronic periodontitis, Type-2 diabetes, Microorganisms, Phase-1 therapy, Glycemic control.

Clinical Relevance:

Scientific rationale for study: Our study intended to explore the impact of viral-bacterial associations on periodontitis patients with diabetes and glycemic control, and in turn, oral health; as not much has been reported in this regard.

Principal findings: Although phase 1 therapy led to improved periodontal and glycemic status, with reduced microbial counts, viruses could be detected in very few samples, thus being inconclusive with regard to their role.

Practical implications: Although the study could not specifically implicate viruses in periodontitis patients with diabetes, phase 1 therapy results in improvement in periodontal health as well as glycemic control.

Introduction

Periodontal disease is a microbial infection involving a variety of microbes that trigger inflammation, loss of connective tissue attachment and alveolar bone around the teeth. The primary etiologic factor of periodontitis is bacterial plaque involving largely gram negative species that express pathogenic factors eliciting host defense responses resulting in inflammation and tissue destruction. In fact the propensity of periodontitis to proceed with periods of exacerbation and remission could suggest that the presence of other organisms contribute to the disease[1] viz nonbacterial microorganisms such as viruses, mycoplasma, yeasts and protozoa[2]. The development of human periodontitis may depend upon cooperative interactions among herpes viruses, specific pathogenic bacteria and tissue destructive inflammatory mediators. Recent studies have shown that human viruses, especially Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and Herpes simplex virus (HSV-1) may play a role in the pathogenesis of periodontal disease[3-5]. The subgingival presence of both EBV and HCMV was reported to be associated with the major periodontopathic bacteria and the severity of periodontal disease.[6,7]

At present, a bidirectional pathway has been suggested between DM and periodontitis, since patients with DM are more predisposed to periodontal diseases, the established periodontitis may simultaneously impair adequate glycemic control[8]. Moreover, poorly controlled diabetic subjects, presenting elevated levels of glycosylated hemoglobin (HbA1c), show higher attachment and alveolar bone loss and local inflammatory cytokines than well controlled patients[8,9]. Exacerbated local inflammation in diabetic subjects with inadequate glycemic control could modify the subgingival environment, and consequently, the subgingival microbial profile.

Evidence has shown variations in the micro flora in non-diabetic and diabetic individuals; and also within the diabetic individuals as well, based on their glycemic control[10-12]. However, the effect of periodontal intervention on these elements is yet to be ascertained. A recent study[13] also reported the presence of viruses in diabetes with periodontal disease.

However, till date, no study has evaluated the relationship between the glycemic status and the occurrence of a viral infection in periodontal pockets of type 2 diabetic subjects with chronic periodontitis. Since previous studies were almost exclusively centered on a bacterial contribution to periodontal disease, the possible involvement of viruses in the etiology of periodontitis in diabetic subjects may be relevant.

The present study was therefore attempted to assess, compare and correlate the presence of viral and bacterial pathogen concentrations and glycemic status in type 2 diabetic and non-diabetic patients with chronic periodontitis following phase 1 therapy.

Materials and Methods

Method of collection of data

After obtaining required permissions and clearance from the Ethical Committee of the institute; 120 patients of both sexes aged between 23 to 45 years, with chronic periodontitis(mild to moderate); visiting the Department of Periodontics at a Dental Institute and various diabetic centers in the city; were divided into 2 main groups based on their glycemic status following initial screening.

Glycated hemoglobin levels were assessed using standardized assay - High Pressure Liquid Chromatography (HPLC) to ascertain the glycemic status.

Group-1-Control (n=30) consisted of 30 non-diabetic patients with chronic periodontitis.

Group-2-Test: (n=90) consisted of 90 diabetic patients who were further divided into 3 subgroups [14]

2(A)- 30 Patients with diabetic patients with chronic periodontitis. (Good control (HbA1c<5%))

2(B)- 30 Patients with diabetic patients with chronic periodontitis. (Moderate control (HbA1c<7%))

2(C)- 30 Patients with diabetic patients with chronic periodontitis. (Poor control (HbA1c>8%))

Inclusion Criteria

- Patients with chronic periodontitis (mild to moderate with up to 4mm RAL) and with the presence of true periodontal pocket (up to 6mm) in ≥30% of sites in the mouth.
- Diabetic patients having chronic periodontitis with varied levels of glycemic control (group 2)

Exclusion Criteria

• Patients with any systemic diseases (group 1)

• Presence of any systemic or debilitating diseases apart from diabetes in group 2.

• Pregnant or Lactating women.

• A recent history or presence of any acute or chronic infections.

• Patients with history of any drug intake including antibiotics, analgesics or any other drugs (apart from anti diabetic drugs for group 2 patients) three months prior to study.

• Patients who have undergone periodontal therapy in the last six months.

• Patients who are smokers/paan/tobacco/betelnut users.

• Patients who are physically or mentally challenged.

The following parameters were assessed in all the patients at baseline (0), 1 months and 4 months

1) Clinical Parameters

- **Gingival index (GI) Loe H and Silness J (1963)[15]**
- **Plaque index- (PI) SILLNESS P and LOE H. (1964)[16]**
- **Probing pocket depth (PPD):** was recorded by noting the distance from gingival margin to base of the pocket (only true periodontal pockets were considered) using Williams periodontal probe.
- **Clinical (relative) attachment level** was calculated as the distance between the fixed reference point (base of the customize acrylic stent) and the base of the pocket.

All the 120 patients were subjected to phase 1 therapy which consisted of ultrasonic scaling followed by thorough root planing with the help of Gracey curettes after assessing the clinical parameters at baseline. Patients were advised Oral hygiene instructions without use of any chemical agents.

Patients were then recalled at the above time intervals during which time, plaque samples were collected and clinical parameters were recorded.

2) Microbiological Parameters

Subgingival plaque samples were collected from the deepest part of the pocket by using a sterile curette in 2 different eppendorf tubes containing tris-EDTA buffer.

The plaque samples were then subjected to multiplex Polymerase Chain Reaction(RT - PCR) using Hemoklen Taq enzyme for detecting viruses (HSV, CMV, EBV) and for detecting bacteria (P. gingivalis, T. forsythia, P. intermedia, A.actinomycetemcomitans)

3) Biochemical Analysis

Glycated hemoglobin levels by using standardized assay - High Pressure Liquid Chromatography (HPLC) were

recorded along with the clinical and microbiological parameters.

Statistical Analysis

All the clinical, microbiological and biochemical parameters recorded were statistically analyzed using SPSS software version 13. Descriptive analysis that included mean, standard deviation & percentages were found for each parameter in the four groups and used for analysis.

Within each group, paired't' test was performed to compare post treatment changes at various time intervals from baseline. For assessment between groups post hock test was performed.

In order to determine the correlation between clinical, microbiological and biochemical parameters Pearson's correlation test was performed.

A 'P' value of 0.05 or less was considered statistically significant.

Assessment of Clinical Parameters

Intra group assessment showed that all 4 clinical parameters viz, GI, PI, PPD and RAL showed significant improvement in values (P< 0.05) at the various time intervals within the test and control groups.[TABLE 1]

Intergroup comparisons revealed significant reduction in the clinical parameters viz GI, PI, PPD AND RAL in group 1 (control) compared to the test groups (2A, 2B & 2 C) at 1 month(P< 0.05) and additionally with regard to PPD & RAL , also at 4 months.(P<0.05).

Among the test groups, there was more significant reduction in group 2A compared to group 2B & 2C with GI& PI & RAL and also in group 2B over group 2C with PPD, at the end of 1 month only.

With regard to correlation assessment,(TABLE 4,5,6&7); significant correlation was found between GI scores and A.a counts at the end of the 1 month only, in group 1(control) (P<0.05); and also between the mean PI scores at baseline and Hba1c levels, (P>0.05)and between plaque index scores and Pi counts at the end of the 4th month, (p<0.05) in test group 2B. However, a negative correlation was found between GI scores and Pg counts in test group 2B at 4 months.

In addition, significant correlation was also observed in group 2A(test) between the mean PPD scores and Tf counts at baseline (P<0.05); and in **Group-2C** (**Test**) between PPD scores and Hba1c levels at the end of the 1month and 4 months (p<0.05). However, a negative correlation was observed in **Group-2B** (**Test**) between PPD scores and Pi and Tf counts at the end of the 1month (p<0.05)

Furthermore, significant correlation in group 1(control) was found between RAL scores and Tf counts at the end of the 4months (p<0.05) and in group 2C(test) between RAL scores and Tf counts at baseline and RAL scores and Aa counts at the end of the 4months .(p<0.05)

Glycated Hemoglobin Levels (Hba1c): (Table 1)

Intra group assessment showed that the mean differences in glycated hemoglobin levels were found to be statistically significant (P<0.05) at baseline to 1 month, baseline to 4 months & from 1 month to 4 months within all 3 test groups(2A, 2B & 2C).

Intergroup comparisons of Hba1c levels at 1 month and 4 months revealed significant reduction in 2A over 2B and 2C and 2B over 2C (P<0.05).

Assessment of Microbiological Parameters

Bacterial counts: (TABLE 2)

Intragroup comparisons of bacterial counts show significant reductions in the counts of P. Gingivalis, P. intermedia, T. Forsythia and A. comitans within all the groups ie. Group 1 (control) and group 2A, 2B & 2C(test) at the various time intervals.(p<0.05) However , with regard to T. Forsythia counts, although there was significant reduction in the test groups 2A, 2B & 2C, no

significant changes were observed within group 1 (control).($p>0.05$).

Intergroup comparisons revealed no significant differences between the groups with regard to the bacterial counts of P. gingivalis, P. intermedia, T. forsythia and A. comitans at the various time intervals with the exception of P. gingivalis counts in test group 2A & 2B which showed significant improvement over test group 2C.($P<0.05$)

Viral counts: (TABLE 3)

With regard to viral counts, Herpes simplex virus(HSV) was detected in group 2A & 2B test groups at baseline only and **Epstein-barr virus(EBV)** in group 2B (test) at baseline only. **Human Cytomegalo virus(HCMV)** could not be detected in any of the time intervals in any of the groups.

Due to inherent discrepancies in these numbers, tests of statistical analysis could not be applied.

Discussion

Periodontitis usually results from the complex interactions of the microbial communities with the host elements in the gingival sulcus that leads to inflammation, loss of connective tissue attachment and alveolar bone around the teeth.[17] Recent evidences suggest that several forms of aggressive periodontitis and periodontal destruction may be associated with the coexistence of periodontal herpes viruses, especially HCMV, EBV and periodontopathic bacteria.[5,18,19,20]

Diabetes mellitus (DM) is well recognized as a risk factor for periodontal disease, whilst periodontitis is thought to influence the systemic inflammatory condition, lipid and glucose metabolism and insulin resistance.[21] Diabetes mellitus, especially when uncontrolled, appears to be an important risk factor for periodontal destruction, since an alteration in host-response and microbiological aspects occurs in diabetic subjects.[22]

Studies have shown that the microbiota associated with diabetes does not appear different from the microbiota of non-diabetic patients.[23-27] Although the microbiology of periodontal disease in DM is fairly well understood, recent reports have pointed to a strong viral influence. It is not still clear whether the presence of viruses in the periodontal microflora of diabetic patients could possibly influence the severity and progression of disease as well as the glycemic status. It was therefore attempted to firstly identify whether the viruses HSV-1,HCMV,EBV-1 were associated with periodontal bacteria in the subgingival plaque samples of diabetic patients with chronic periodontitis and if present, whether phase-1 therapy alone was sufficient to significantly alter their proportions and influence glycemic control.

The sample size of 120 patients evaluated in this study was arrived at, following consultation with the statistician which was in accordance with the vast majority of clinical periodontal treatment studies in humans [28,29].In order to understand this phenomenon with better sensitivity and specificity, it was decided to carry out the study by dividing the 120 patients into 4 groups based on the level of glycemic control.

Glycated (Glycosylated) Hemoglobin is a form of hemoglobin used primarily to identify the average plasma glucose concentration over a prolonged period of time. Literature suggests that periodontal treatment in T2DM patients with chronic periodontitis(CPD) may lead to improved diabetic control as measured by serum glycosylated hemoglobin (HbA1C) levels.[30-32] Glycated hemoglobin is formed when glucose molecules react with hemoglobin, during the normal 120-day life span of the red blood cell (RBC). Once a hemoglobin molecule is glycated, it remains in this form. Therefore, glycated hemoglobin levels were evaluated in keeping with the life cycle of an average erythrocyte ie 120 days (4 months)[33]

and accordingly the other parameters were also assessed in the same time frame.

The clinical parameters(GI,PI,PPD,RAL) were assessed to evaluate the disease severity and progression at regular intervals and also to correlate the changes on the bacterial-viral concentrations in patients with chronic periodontitis with and without diabetes following phase 1 therapy. The gold standard for recording changes in periodontal status is the longitudinal measurements with clinical attachment level (CAL) from CEJ to the base of the pocket .Due to the relative inconsistencies in determining CEJ accurately at the selected sites, it was decided to use a customized acrylic stent and use the base of the stent as the fixed reference point and evaluate relative attachment level (RAL).

In addition, microbiological analysis with the help of multiplex PCR technique was done to detect the bacteria ; A. actinomycetemcomitans, P. gingivalis, P. intermedia, and T. forsythia; and viruses HSV, EBV, and HCMV. PCR is a rapid, accurate and sensitive technique for the detection of bacterial and viral DNA sequences. The sensitivity of PCR allows detection of periodontal pathogens below the normal level of detection by culture methods, immunofluorescence, enzyme based tests and DNA probes.[18]

Within the limitations of the study, following the statistical analysis of the results obtained, intragroup comparison showed a significant improvement in the clinical parameters assessed viz, GI, PI, PPD and attacment levels at the various time intervals within both the test (diabetic) group and the control group(non diabetic). This is in accordance with evidence from studies with similar findings. [34- 36]

Interestingly, inter group comparisons showed significant improvements in the clinical parameters in the non-diabetic group as compared to the diabetic groups at the various time intervals. Additionally, significant reduction was also found in moderately controlled group compared to the poorly controlled group. This is in accordance with evidence with similar findings thereby suggesting that the level of glycemic control does have an impact on clinical outcomes in patients with chronic periodontitis.[34,37,38]

All the clinical parameters showed positive correlation with the microbial counts at the various time intervals in the non-diabetic and diabetic groups with various levels of control, with the exception of gingival index and probing depths in the moderately controlled diabetic groups which saw a negative correlation thereby indicating that reduced gingival inflammation and pocket depths did not significantly affect microbial counts.

On the contrary, probing depths positively correlated with glycated hemoglobin levels in the poorly controlled diabetic group in turn suggesting that improved glycemic status contribute to reduced pocket depths following phase 1 therapy.

The relationship between plaque accumulation and HbA1c levels remains controversial.[39-41] Some reports have observed an improved compliance with oral hygiene amongst diabetic subjects with better glycemic control, whilst others have not found this association.[42-44]

With regard to diabetic control, significant improvement in glycated hemoglobin levels was observed within moderately and poorly controlled diabetic groups following phase 1 therapy indicating that the well controlled group remained so throughout the duration of the study which was in accordance with similar evidence.[45]

When compared with each other, significant improvement was observed in the well-controlled and moderately controlled diabetic group as against the poorly controlled group which is in line with evidence that concluded that

phase-1 therapy improves the glycemic status of diabetic patients with chronic periodontitis.[37,38]

With regard to the microbiologic parameters, intra group comparisons revealed a significant reduction in the counts of Pg, Tf, Pi,& Aa counts within both the test (diabetic) and control (non-diabetic) group except with Tf counts within the control(non-diabetic) group at the various time intervals. This is in accordance with the findings of Contreras at al 1999[4] and Makiura et al 2008[13] who concluded that phase-1 therapy results in reduction of bacterial counts in patients with chronic periodontitis and diabetes.

Inter group comparisons revealed significant reduction in Pg& Tf counts in the non-diabetic group compared to the diabetic group at various time intervals. However, no such differences were observed in the Pi and Aa counts which suggests that glycemic control in diabetic patients may influence the periodontal microflora to a variable extent.

Evidence with regard to viruses in periodontal disease are diverse. In the present study, HCMV was not detected in any of the 4 groups. This is contradictory to the findings [45,46] where viruses were found in the plaque samples collected from the chronic periodontitis patients but they were detected in very less number of patients. More positive results may be observed in aggressive periodontitis patients than in chronic periodontitis patients.

HSV and EBV were detected only at baseline in 3 samples of the diabetic group and not in any of the groups, therefore based on the observation of our study, their role in periodontal disease with or without diabetes is questionable which is in accordance with Rotola et al [47] who found low prevalence of HSV and EBV in gingival biopsy samples but not in plaque samples.

Limitations

1. The inability of PCR to detect viruses, could be its inherent drawback in being able to detect only in a small population of plaque. Thus the plaque sample used may not be a true representative of the microbial population tested.

2. The study could involve a larger cross section of the population.

Within the limitations of the present study it can be safely concluded that phase 1 therapy showed better improvement in chronic periodontitis patients without diabetes than in diabetic patients and may contribute to reduction or elimination of microorganisms counts and improving glycaemic status . Since the viral content found in the samples in the study were inconsistent, the effect of phase 1 therapy on them could not be substantiated. Nevertheless, further long term cross sectional studies on larger populations could probably provide better insights into the credibility of the study.

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List of Table:

Table I - Clinical Parameters & Biochemical Parameters

PARAMETERS		GINGIVAL INDEX				PLAQUE INDEX				PROBING POCKET DEPTH				RAL				GLYCATED HB LEVELS			
Groups	Time intervals	Mean	SD	Sig.	Inf	Mean	SD	Sig.	Inf	Mean	SD	Sig.	Inf	Mean	SD	Sig.	Inf	Mean	SD	Sig.	Inf
Group1	Baseline-1month	0.58	0.33	.000	S	0.50	0.33	.000	S	.61	0.40	.000	S	0.94	1.06	.000	S				
	Baseline-4months	0.91	0.33	.000	S	0.81	0.39	.000	S	1.04	0.50	.000	S	1.85	0.94	.000	S				
	1month-4months	0.34	0.30	.000	S	0.31	0.30	.000	S	0.43	0.30	.000	S	0.90	0.64	.000	S				
Group-2A	Baseline-1month	0.70	0.43	.000	S	0.41	0.36	.000	S	0.62	0.50	.000	S	1.82	0.75	.000	S	0.11	0.23	.016	S
	Baseline-4months	1.20	0.42	.000	S	0.90	0.40	.000	S	1.60	0.33	.000	S	3.05	0.80	.000	S	0.14	0.32	.019	S
	1month-4months	0.50	0.30	.000	S	0.50	0.32	.000	S	0.99	0.41	.000	S	1.23	0.70	.000	S	0.37	0.22	.383	NS
Group-2B	Baseline-1month	0.43	0.34	.000	S	0.41	0.42	.000	S	0.89	0.41	.000	S	2.16	0.62	.000	S	0.50	0.57	.000	S
	Baseline-4months	0.77	0.48	.000	S	-1.58	14.08	.544	NS	1.47	0.35	.000	S	3.11	0.78	.000	S	0.87	0.73	.000	S
	1month-4months	0.33	0.30	.000	S	-1.99	14.09	0.446	NS	0.59	0.38	.000	S	0.96	0.69	.000	S	0.37	0.54	.001	S
Group-2C	Baseline-1month	0.61	0.50	.000	S	0.40	0.24	.000	S	1.06	0.52	.000	S	1.60	0.92	.000	S	0.82	0.85	.000	S
	Baseline-4months	1.24	0.50	.000	S	0.93	0.42	.000	S	1.99	0.54	.000	S	2.65	1.195	.000	S	1.50	1.17	.000	S
	1month-4months	0.63	0.40	.000	S	0.53	0.33	.000	S	0.93	0.48	.000	S	1.04	0.85	.000	S	0.68	0.74	.000	S

Table II - Micrbiological Parameters(Bacteria):

PARAMETERS		P. GINGIVALIS				T. FORSYTHIA				P. INTERMEDIA				A. COMITANS			
Groups	Time intervals	Mean	SD	Sig.	Inf	Mean	SD	Sig.	Inf	Mean	SD	Sig.	Inf	Mean	SD	Sig.	Inf
Group1	Baseline-1month	49260000000	5081385637	.000	S	70000000	2556533321	.144	NS	1755333333	3731564092	.015	S	41000000	1827066661	.229	NS
	Baseline-4months	50100000000	5069972454	.000	S	7633333333	2526751112	.109	NS	1775000000	3750355155	.015	S	3933333333	1811635544	.244	NS
	1month-4months	84000000	1790319879	.000	S	6333333	2428044 9	.164	NS	1966666666	3764473 9	.008	S	-1666666666	3790490	.023	S
Group-2A	Baseline-1month	428666666666	4992665885	.000	S	1995333333	4042906715	.011	S	1821333333	3716936756	.012	S	1736300000	3767043889	.017	S
	Baseline-4months	43120000000	5016730904	.000	S	1696551724	3768050154	.022	S	1799666666	3652283634	.011	S	1699200000	3696511798	.018	S
	1month-4months	25333333	4360744 4	.000	S	-23103448	1705893 86	.472	NS	-21666666	3104288 83	.705	NS	-37100000	1827765 45	.275	NS
Group-2B	Baseline-1month	46770000000	4902599697	.000	S	1626000000	3617409627	.020	S	2124666666	3927763014	.006	S	1366600000	3448798567	.038	S
	Baseline-4months	47636666666	4978032074	.000	S	1663333333	3699998446	.020	S	2169000000	3988301297	.006	S	1366233333	3448749385	.038	S
	1month-4months	866666666	2383467 96	.05	S	3733333333	1827365 41	.272	NS	4433333333	1842165 76	.198	NS	-366666666	1847334	.286	NS
Group-	Baseline-1month	68443333333	4733278140	.000	S	1099633333	3020280170	.056	S	2872666670	4219338784	.001	S	1436000000	3429714575	.029	S

2C	Baseline-4months	6731000000.	4727797001	.000	S	1044000000	3052032222	.071	NS	2926333336	5013420091	.003	S	1438666666	3428498951	.029	S
	1month-4months	-1333333.333333	5033634003.182888	.227	NS	-556333333	2433564833	.221	N.S	5366666	3707364404	.937	NS	2666666	1659524 9	.386	N.S

Table III - Microbiological Parameters (Viruses)

PARAMETERS		HSV		EBV		HCMV	
Groups	Time intervals	Mean	SD	Mean	SD	Mean	SD
Group1	Baseline	.00	.000	0.50	0.33	.00	.000
	1 month	.00	.000	0.81	0.39	.00	.000
	4months	.00	.000	0.31	0.30	.00	.000
Group-2A	Baseline	333333	1825741	0.41	0.36	.00	.000
	1month	.00	.000	0.90	0.40	.00	.000
	4months	.00	.000	0.50	0.32	.00	.000
Group-2B	Baseline	1000000	3051285	0.41	0.42	.00	.000
	1 month	.00	.000	-1.58	14.08	.00	.000
	4months	.00	.000	-1.99	14.09	.00	.000
Group-2C	Baseline	.00	.000	0.40	0.24	.00	.000
	1 month	.00	.000	0.93	0.42	.00	.000
	4months	.00	.000	0.53	0.33	.00	.000

Table IV : Gi Correlated With Microbiological Parameters And Hba1c Levels

Groups	Time intervals		Pi	Pg	Tf	Aa	HSV	EBV	CMV	Hba1c
Group-1	Baseline	r	-.187	.305	.153	.106	0	0	0	0
		p	.324	.101	.421	.576	0	0	0	0
	1 month	r	.116	-.037	.016	.492**	0	0	0	0
		p	.543	.846	.934	.006	0	0	0	0
	4 months	r	-.149	.290	.084	.096	0	0	0	0
		p	.433	.120	.660	.615	0	0	0	0
Group-2A	Baseline	r	.130	-.120	-.021	.034	-.075	0	0	-.110
		p	.494	.527	.914	.857	.692	0	0	.561
	1 month	r	.015	-.097	-.227	-.240	0	0	0	.069
		p	.936	.610	.228	.201	0	0	0	.716
	4 months	r	-.119	-.189	.145	-.237	0	0	0	.090
		p	.533	.317	.453	.207	0	0	0	.637
Group-2B	Baseline	r	-.218	-.228	-.055	-.110	.013	.166	0	-.075
		p	.247	.225	.772	.564	.944	.380	0	.696
	1 month	r	-.317	-.035	-.041	.100	0	0	0	-.097
		p	.088	.854	.831	.600	0	0	0	.608
	4 months	r	.126	-.410*	.258	.223	0	0	0	.046

		p	.508	.024	.169	.237	0	0	0	.811
Group-2C	Baseline	r	.248	-.135	-.233	-.010	0	0	0	.160
		p	.186	.478	.215	.959	0	0	0	.398
	1 month	r	.252	-.199	.113	.259	0	0	0	.240
		p	.179	.292	.551	.167	0	0	0	.201
	4 months	r	-.044	.145	.091	.154	0	0	0	.203
		p	.819	.443	.631	.417	0	0	0	.282

Table V : Pi Correlated With Microbiological Parameters And Hba1c Levels

Groups	Time intervals		Pi	Pg	Tf	Aa	HSV	EBV	CMV	Hba1c
Group-1	Baseline	R	-.175	-.084	.054	-.040	0	0	0	0
		P	.356	.660	.776	.834	0	0	0	0
	1 month	R	-.027	.186	-.252	.176	0	0	0	0
		P	.888	.325	.180	.351	0	0	0	0
	4 months	R	-.203	.095	-.145	.055	0	0	0	0
		P	.283	.618	.444	.773	0	0	0	0
Group-2A	Baseline	R	-.107	.331	-.241	-.332	.090	0	0	.331
		P	.573	.074	.199	.073	.636	0	0	.074
	1 month	R	.194	-.048	-.230	.034	0	0	0	.291
		P	.305	.800	.222	.859	0	0	0	.119

	4 months	R	-.172	-.115	-.118	-.194	0	0	0	.188
		P	.364	.546	.541	.305	0	0	0	.319
Group-2B	Baseline	R	.043	-.175	.058	.052	.056	-.109	0	.389*
		P	.821	.356	.760	.787	.768	.567	0	.034
	1 month	R	.141	-.002	.286	-.091	0	0	0	-.064
		P	.458	.990	.126	.633	0	0	0	.738
	4 months	R	.681**	-.066	-.033	-.039	0	0	0	.052
		P	.000	.730	.864	.839	0	0	0	.783
Group-2C	Baseline	R	.089	-.168	-.063	-.257	0	0	0	-.089
		P	.641	.375	.741	.171	0	0	0	.638
	1 month	R	-.203	.069	.144	-.023	0	0	0	-.249
		P	.282	.719	.447	.902	0	0	0	.185
	4 months	R	.111	-.167	.073	.211	0	0	0	-.095
		P	.559	.379	.700	.263	0	0	0	.619

Table VI: Ppd Correlated With Microbiological Parameters and Hba1c Levels

Groups	Time intervals		Pi	Pg	Tf	Aa	HSV	EBV	CMV	Hba1c
Group-1	Baseline	r	.109	-.036	.177	.039	0	0	0	0
		p	.565	.848	.348	.836	0	0	0	0
	1 month	r	.081	-.042	.090	.313	0	0	0	0
		p	.672	.826	.636	.092	0	0	0	0
	4 months	r	-.231	.017	.087	-.111	0	0	0	0
		p	.220	.927	.648	.561	0	0	0	0
Group-2A	Baseline	r	.313	.302	.406*	.353	0	.174	0	-.273
		p	.092	.105	.026	.056	0	.357	0	.144
	1 month	r	.076	-.139	-.147	-.177	0	0	0	.030
		p	.692	.462	.439	.348	0	0	0	.877
	4 months	r	-.158	-.123	.153	-.215	0	0	0	.018
		p	.405	.517	.428	.254	0	0	0	.926
Group-2B	Baseline	r	.013	-.241	.082	.162	0	-.149	.247	-.157
		p	.948	.199	.668	.392	0	.432	.188	.408
	1 month	r	-.443*	-.293	-.472**	-.175	0	0	0	-.312
		p	.014	.117	.009	.356	0	0	0	.093
	4 months	r	-.072	-.063	-.115	-.122	0	0	0	-.009

		p	.705	.739	.543	.521	0	0	0	.964
Group-2C	Baseline	r	.134	.015	-.060	-.315	0	0	0	.344
		p	.482	.937	.755	.090	0	0	0	.063
	1 month	r	.211	.104	-.040	.303	0	0	0	.394*
		p	.263	.586	.833	.103	0	0	0	.031
	4 months	r	-.099	.037	.090	.123	0	0	0	.528**
		p	.605	.848	.636	.518	0	0	0	.003

Table VII : Ral Correlated With Microbiological Parameters And Hba1c Levels

Groups	Time intervals		Pi	Pg	Tf	Aa	HSV	EBV	CMV	Hba1c
Group-1	Baseline	r	.170	-.075	-.063	-.122	0	0	0	0
		p	.368	.692	.740	.519	0	0	0	0
	1 month	r	.064	.053	.208	.100	0	0	0	0
		p	.736	.781	.271	.600	0	0	0	0
	4 months	r	-.157	-.063	.389*	.001	0	0	0	0
		p	.408	.741	.034	.995	0	0	0	0
Group-2A	Baseline	r	.182	.155	.266	.240	.041	0	0	.256
		p	.337	.415	.155	.201	.829	0	0	.173
	1 month	r	-.044	-.027	.020	.061	0	0	0	.098
		p	.817	.887	.915	.749	0	0	0	.605

	4 months	r	-.218	.207	.257	-.158	0	0	0	.059
		p	.247	.273	.179	.404	0	0	0	.759
Group-2B	Baseline	r	.034	-.142	.084	.116	-.010	.153	0	-.319
		p	.858	.454	.658	.542	.957	.418	0	.085
	1 month	r	-.266	-.057	-.229	-.095	0	0	0	-.280
		p	.155	.765	.224	.617	0	0	0	.135
	4 months	r	.097	.113	.143	.164	0	0	0	-.081
		p	.609	.552	.450	.388	0	0	0	.670
Group-2C	Baseline	r	.003	.071	.380*	.237	0	0	0	.002
		p	.988	.711	.039	.207	0	0	0	.991
	1 month	r	-.052	.263	.048	.209	0	0	0	.131
		p	.785	.160	.801	.267	0	0	0	.490
	4 months	r	-.009	.060	-.189	.435*	0	0	0	.063
		p	.961	.753	.316	.016	0	0	0	.743