"TO COMPARE THE LEVELS OF TNF-ALPHA IN SERUM OF GESTATIONAL AND NON GESTATIONAL DIABETES PATIENTS WITH CHRONIC PERIODONTITIS – A CASE CONTROL STUDY"

BY

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In

PERIODONTICS

(BRANCH – II)

Under the guidance of

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LIST OFABBREVATIONS

A	
AAP	American Academy of Periodontology
AGE	Advanced Glycation End Products
AL	Attachment Loss
AMI	Acute Myocardial Infarction
ANOVA	
AS	Ankylosing Spondylitis
B	
BI	Bleeding Index
BMI	Body Mass Index
BOP	Bleeding On Probing
C	
CAL	Clinical Attachment Loss
CD	Crohn’s Disease
CIs	Confidence Intervals
CRP	C-Reactive Protein
D	
DD	Death Domain
DM	Diabetes Mellitus
E	
ELISA	Enzyme-Linked Immunosorbent Assay
F	
FPG	Fasting Plasma Glucose

G	
gm	gram
GCF	Gingival Crevicular Fluid
GCT	Glucose Challenge Test
GDM	Gestational Diabetes Mellitus
GM	Gingival Margin Location
GIGT	Gestational Impaired Glucose Tolerance
GLUT-4	Glucose Transporter Type 4
H	
HbA1c	Hemoglobin A1c Or *Glycated Hemoglobin*
HPL	Human Placental Lactogen
I	
IL-10	Interleukin-10
IL-33	Interleukin-33
IR	Insulin Resistance
IUD	Intrauterine Device
K	
kDa	kilodaltons
M	
mg	milligram
MI	Tooth Mobility Index
N	
NHANES	National Health & Nutrition Examination Survey
NON-GDM	Non - Gestational Diabetes Mellitus

O	
OGTT	Oral Glucose Tolerance Test
ORs	Odds Ratio
P	
PD	Probing Depth
PI	Plaque Index
PIH	Pregnancy-Induced Hypertension
Pre-BMI	Pre - Body Mass Index
PMA	Papillary Marginal Attachment
PsA	Prostate-specific Antigen
R	
RA	Rheumatoid Arthritis
RAGE	Receptor For Ages
RCT	Randomized Controlled Trials
S	
SBI	Sulcus Bleeding Index
SD	Standard Deviation
SE	Standard Error
SNPs	Single Nucleotide Polymorphisms
T	
TNFR 1	Tumor Necrosis Factor RECEPTOR 1
TNFR 2	Tumor Necrosis Factor RECEPTOR 2
TNF α	Tumor Necrosis Factor A
T1dm	Type 1 Diabetes Mellitus

ABSTRACT

Background: The bidirectional association of periodontitis and diabetes are commonly reported as complex and chronic diseases. Gestational Diabetes Mellitus is believed to develop in the third trimester where progressive insulin resistance occurs with dysfunction of pancreatic ß-cell mass and insulin secretion. The maternal gingival inflammation including TNF - α is hypothesized to be the cause of insulin resistance. TNF – α, an insulin antagonist, is often used as an inflammatory marker to evaluate inflammatory status in patients with Gestational Diabetes Mellitus and periodontitis.

Objective: To compare the levels of serum TNF-alpha in Non-Gestational Diabetes and Gestational Diabetes patients with Chronic Periodontitis.

Material and Methods: The present case control study was conducted on subjects between 18 - 30 years of age. 72 subjects were selected from the Out Patient Department of Obstetrics and Gynecology, KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi during the period of January 2019 – January 2020. Subjects of Gestational Diabetes Mellitus and Non Gestational Diabetes were selected on the basis of inclusion and exclusion criteria. Maternal statistics were collected from their patient records from the Department of the Obstetrics and Gynaecology and a full-mouth periodontal examination was performed. The clinical parameters recorded were Plaque index (PI), Bleeding index (BI), Probing depth (PD) and Clinical attachment loss (CAL). Measurements were done by single calibrated examiner. After selection of the patients, Women diagnosed with GDM and chronic periodontitis were the case group for the current study and the Control group included women with chronic periodontitis who were not diagnosed with GDM or any other

types of diabetes. For TNF-α, blood samples were drawn to obtain the serum samples for analysis. Levels of TNF-α were determined using a human ELISA kit in accordance with the manufacturer's protocol. The following methods were implemented in the statistical evaluation: Mann Whitney U test, Independent T test, Paired T test, Wilcoxon signed rank test & Karl Pearson's / Spearman's rank correlation for clinical and biochemical parameters.

Results: GDM patients showed higher values in BMI, knowledge regarding dental oral hygiene, family history of diabetes or GDM, in comparison to non-GDM patient. The Non-GDM patients showed higher values in terms of single time gestation period (72%) compared to GDM patients (28%). Plaque index (PI) and Bleeding index (BI) showed non-significant correlation, whereas, PPD & CAL showed significant correlation in GDM group (pValue = 0.0001). TNF – α levels when compared with clinical parameters of both GDM & Non-GDM, were statistically significant in GDM group (pValue = 0.0001).

Conclusion: Within the limitations of the study, it can be concluded that BMI, PPD, CAL has significant correlation with TNF – α in GDM patients.

Keywords: Diabetes, Gestational; Tumour necrosis factor-alpha, Periodontal disease, Serum.

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INTRODUCTION

Periodontal disease is an inflammatory disease of bacterial origin. This leads to attachment and bone loss, which could lead to tooth loss if left untreated. The bacteria present in the plaque affect the periodontal tissues and provokes the destruction of the supporting tissues of the teeth. This destructive inflammatory process results because of an inadequate interaction between the oral microflora present in plaque biofilm and the host defense mechanisms. Chiefly, the microorganisms that are colonized in the sub-gingival region might cause a rise in local and systemic pro-inflammatory cytokines. This elevation in cytokines like, Interleukin -33 (IL-33), Tumor Necrosis Factor-α (TNF-α), Interleukin -1 Receptor Antagonist (IL-1ra), adiponectin have direct influence on periodontal tissue destruction. The periodontal disease is associated with systemic diseases which include cardiovascular disease, metabolic syndrome, and diabetes.

The bidirectional association of periodontitis and diabetes is commonly reported as complex and chronic diseases. This is observed when poor glycemic control is reported with increased severity of the periodontal disease. And likewise, the severe periodontal conditions are observed with glycemic controls that are compromised. Along with glycemic control, the improvement is observed in HbA1c levels as well. The management periodontal condition in diabetics, thus, becomes important. The role of the dentist is very important in the management of the diabeticpatient.[1]

“Gestational diabetes mellitus (GDM) is defined as glucose intolerance with first onset or presentation in pregnancy.” GDM is believed to develop in the third trimester where progressive insulin resistance occurs with dysfunction of pancreatic ß-

cell mass and insulin secretion. Amongpregnant women, there is a prevalence of GDM up to 10%. There are various associated risks leading to conditions like pre-term birth, changes in diabetes status at later stages and pre-eclampsia.[2] Globally, type 2 DM is a common chronic diseaseamong individuals. Hence, GDM has a key role in recognizing risk factors to prevent DM in later stages. During pregnancy, maternal carbohydrate metabolism increases with time. Development of GDM occurs when placental hormones entail a indemnifying increase in insulin secretion. This takes place as pregnancy advances.

There are immune system modulations during pregnancy. TNF- α appears to be elemental in promoting regular outcomes of pregnancy. The cytokines coincidently are seen associated with periodontal destruction by attachment and resorption of bone. TNF- α is also reported to be an insulin antagonist. It is a predictor of pregnancy-induced insulin resistance which is highly synthesized and released from the placenta. Hence it is often used as an inflammatory marker to evaluate inflammatory status in patients with GDM and periodontitis.[4]

The maternal gingival inflammation is hypothesized to be the cause of insulin resistance by the induction of systemic inflammatory response. Pre-existent pregnancy persuades “insulin resistance and impaired glucose tolerance”. The insulin resistance exerted by maternal gingival inflammation could be an aggravating factor and in due course lead to GDM. The aim of the present study is to evaluate the clinical periodontal status as well as serum levels of TNF-α in women with or withoutGDM.

AIM OF THE STUDY

To compare the levels of serum TNF-alpha in Non-Gestational Diabetes and Gestational Diabetes patients with Chronic Periodontitis.

OBJECTIVES OF THE STUDY

1. To assess the Probing Depth (PD), Clinical Attachment Loss (CAL), Plaque Index (PI) and Bleeding Index (BI) of non-gestational diabetes patients.
2. To assess the probing depth (PD), Clinical attachment loss (CAL), Plaque Index (PI) and Bleeding Index (BI) of gestational diabetes patients.
3. To evaluate the TNF-alpha levels in serum of non-gestational diabetes patients with chronic periodontitis
4. To evaluate the TNF-alpha levels in serum of gestational diabetes patients with chronic periodontitis.
5. To compare TNF – alpha levels in serum of gestational and non-gestational diabetes patients with chronic periodontitis.

REVIEW OF LITERATURE

History[5]

Hippocrates mentioned Diabetes mellitus as “making the water too often”. In 1500 BC, the “Greek Father of Medicine mentioned” that women with uncontrolled DM at the time of pregnancy and soon after it were at utmost danger. In 1961,O’ Sullivan coined the term “Gestational Diabetes Mellitus”.

Gestational Diabetes Mellitus is defined as “the carbohydrate intolerance of varying severity with onset or first recognized during pregnancy.” In the year 1954, Boston witnessed its first prospective study on pregnancy carbohydrate metabolism where a 1-hour screening test with 50gm was experimented. During pregnancy, diabetogenic stress and insulin resistance both are accelerated. GDM develops when the compensatory placental hormones, responsible for this resistance and stress, are imbalanced.

Classification of diabetes complicating pregnancy[5]

	Onset	FBG	2 hrs PPG	Therapy
A1	**Gestational**	**< 95 mg/dl**	**< 120 mg/dl**	**Diet**
A2	**Gestational**	**> 95 mg/dl**	**> 120 mg/dl**	**Insulin**
Class	**Age of onset**	**Duration**	**Vascular disease**	
B	**Over 20 years**	**<10 years**	**None**	**Insulin**
C	**10-19 years**	**10-19 years**	**None**	**Insulin**
D	**< 10 years**	**> 20 years**	**B. Retinopathy**	**Insulin**
F	**Any**	**Any**	**Nephropathy**	**Insulin**
R	**Any**	**Any**	**Pro. Retinopathy**	**Insulin**
H	**Any**	**Any**	**Heart**	**Insulin**

Gestational Diabetes[3, 6]

"GDM is carbohydrate intolerance of variable severity with onset or first recognition during present pregnancy. GDM comprises intolerance of glucose which might antedate the current pregnancy. It is caused by sluggish first phase insulin release and because of the effect of anti-insulin hormones for glucose utilization and insulin secretion in pregnancy".

Measures for screening:

Low and high risk

1) Age above or below 25years
2) Intra Uterine Device / anomaly - Low ethnic group
3) Repeated pregnancy loss – absence of DM family history
4) Family history of DM – normal BM

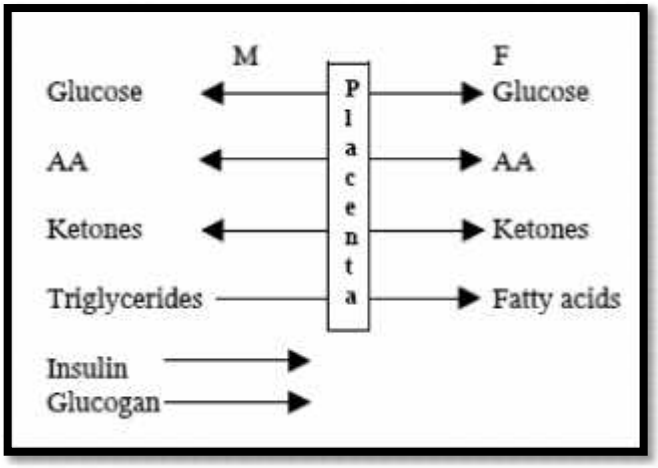
5) Macrosomia - Good obstetric output

6) Obese women

7) PIH

8) Polyhydramnios

9) Twin pregnancy



Screening:

Urine glucose:

The renal threshold for glucose is lowered at the time of pregnancy. It renders "glycosuria" less precise for detection of GDM. Hence it is not advised for diagnostic test.

Blood glucose:

The 24 and 28 weeks of gestation is the usual recommendation for screening. The current recommendation suggests glucose tolerance test for all the pregnant women in the 1st trimester itself. Repetition test is advisable 24 to 28 weeks followed by 32 to 34 weeks if negative.

ADA Recommendation:

1. **One Step Procedure:**

Diagnostic 100 gm oral glucose tolerance test without prior serum glucose screening.

2. **Two Step Approach:**

Initial screening by 50 gm oral glucose and if Blood Glucose > 140 mg % patient is asked for diagnostic OGTT, this method identifies up to 80% of GDM.

3. **Carpenter and Coustan diagnostic criteria:**

The American Diabetes Association (ADA) has adopted two diagnostic Carpenter criteria. The carpenter and Coustan's criteria are used for screening and diagnostic purpose.

Time 100 g OGTT 75 g OGTT (sacht'scriteria)

Fasting	95 mg/dl
1 hour	180 mg/dl
2 hour	155 mg/dl
3 hour	140 mg/dl

Two or more of the venous plasma concentrations must be met or exceeded for a positive diagnosis.

Gogeneni H et. al.,[7]a case control study, concluded that the likelihood of poor pregnancy outcomes is increased by GDMand gingivitis.PCR determined oral infections with 3 main periodontal pathogens. By quantification of CRP by EIA, systemic inflammation was determined. At the time of pregnancy,gingivitis was associated with oral infections and combinations of PG, Ff and Td. Gingivitis during pregnancy resulted in 325% spike in systemic CRP.

Chokwiriyachit A *et al*. (2013)[8] performed a case-control study in KhonKaen, Thailand.For the measurement of C-reactive protein (CRP), tumor necrosis factor-alpha, and interleukin-6 levels, serum samples were collected. In contrast to 26% of the controls, 50 % of cases of females had periodontitis. Females with GDM had higher PD and CAL and increased CRP. GDM was substantially related with periodontitis. With additional adjustment for family history of diabetes, pregnancy body mass index, and weight gain during pregnancy, the correlation remained significant.

Esteves Lima RP et. al., (2013)[6] explained, "the possible interaction and the impact of risk variables.360 females, 90 with GDM and 270 controls were included in this case control analysis. Participants received a periodontal full mouth examination with a record of probing bleeding (BOP), probing depth (PD), and extent of clinical attachment (CAL). The prevalence of periodontitis in the case group (GDM) was 40 percent and 46.3 percent in the control group. There was a lack of interaction (OR = 0.74; 95 % CI = 0.40 to 1.38) between periodontitis and GDM. The multivariate final logistic regression model retained as important GDM-related variables the following Maternal age (OR = 2.65; 95 percent CI = 1.97 to 3.56), chronic hypertension (OR = 3.16; 95 percent CI = 1.97 to 3.56), chronic hypertension (OR = 3.16) 1.35 to 7.42),

and the index of body mass (OR = 1.99; 95 % CI = 1.41 to 2.81). Between cases and controls, a high prevalence of periodontitis was observed, with no association between periodontitis and GDM".

Chapple ILC, Genco R, and on behalf of working group 2 of the joint EFP/AAP workshop (2013)[9] reported Epidemiological evidence of the effect of periodontal disease on the incidence, control and complications of diabetes was documented from cross-sectional, prospective and intervention research and identified possible underpinning mechanisms.

Epidemiological evidence of the effect of periodontal disease on the incidence, control and complications of diabetes was documented from cross-sectional, prospective and intervention research and identified possible underpinning mechanisms.There is no current evidence to support adjunctive use of antimicrobials for periodontal management of diabetes patients.

Ruiz DR, Romito GA, Dib SA (2011)[10] checked the relationship between periodontal disease and its clinical variables in Brazilian pregnant women who are not diabetic (C), gestational diabetes mellitus (GDM), or type 1 diabetes mellitus (T1DM). A single-blinded calibrated examiner recording plaque index (PI), gingival index (GI), bleeding index (BI), gingival margin position (GM), probing depth (PD), clinical attachment level (CAL), bleeding on test (BOP), and tooth mobility index (MI) carried out a periodontal review in one hundred and sixty-one pregnant women (GDM:80; T1DM:31; C:50). GI, GM, PD, CAL, BOP, and MI were far higher. Between GDM and T1DM, as for C. The PI between C and T1DM was higher in GDM and similar. In contrast with non-diabetic pregnant women (C), the presence of periodontal disease was substantially higher in Brazilian diabetic pregnancies (GDM

and T1DM). Between the GDM and T1DM classes, the level of periodontal disease was comparable.

Dasanayake AP, Chhun N, Tanner AC, Craig RG, Lee MJ, Moore AF, *et al*. (2008)[11] Compared to women who do not develop gestational diabetes mellitus, the hypothesis tested, those who do develop it will have a higher exposure to clinical and other periodontal parameters, clinical, bacteriological (in plaque and cervico-vaginal samples), immunological, and inflammatory mediator parameters measured. In 265 mainly Hispanic women in New York, 7 weeks prior to the diagnosis of gestational diabetes mellitus. Twenty-two gestational diabetes mellitus cases emerged from the cohort. Higher pre-pregnancy body mass index vaginal levels of Tannerella forsythia serum C-reactive protein and previous gestational diabetes mellitus emerged as risk factors as contrasted with safe control individuals. There was no statistical significance for clinical periodontal disease. They concluded that gestational diabetes mellitus may be associated with clinical periodontal disease.

Novak KF et. al.,[12]concluded that, patients with gestational diabetes mellitus will have more serious periodontal disease than normal patients.

Xiong X et. al.,[13]a case-control study in which 53 pregnant women with GDM and 106 pregnant women without GDM were analysed. PD and CAL were used to assess the seriousness of periodontal disease.The percentage of periodontitis was 77.4% in women with GDM and 57.5% in women without GDM, and the theory of an association between periodontal disease and GDM is confirmed by the outcome of this research.

TNF-α[14]"Tumor necrosis factor (TNF),a 17kDa protein consisting of 157 amino acids, is a homotrimer in solution, and its bioactivity is mainly regulated by soluble TNFα- binding receptors. In humans, the gene maps to chromosome 6".

The major sources of TNF alpha are

- activated macrophages
- T lymphocytes
- And natural killer (NK) cells
- fibroblasts
- smooth muscle cells
- tumor cells

TNF is involved in the following pathways

- Circadian rhythm.
- pathogenesis rheumatoid arthritis (RA)
- Crohn's disease
- psoriatic arthritis
- ankylosing spondylitis(AS).
- Bone resorption.

TNF - α in GDM

Gao XL, Yang HX, Yi ZH (2008)[17] conductedcase-control study. 22 women with GDM,GIGT and healthy pregnant women were chosen. The levels of tumor necrosis factor-α (TNF-α), leptin and adiponectin were determined. Women with GDM have the highest values of TNF-α and leptin and the lowest value of adiponectin compared with those with GIGT and the healthy controls. Positive correlation was

shown between the levels of TNF-α and leptin with the BMI, while adiponectin was negatively correlated.

Kirwan JP et. al.,[20]correlated, "changes in insulin sensitivity during pregnancy have been associated with changes in placental hormones, cortisol, leptin, and tumour necrosis factor (TNF) alpha. Insulin resistance was tested in women using euglycemic-hyper-insulinemic clamping,in pre-pregnancy (pregravid) and early and early pregnancy (pregravid). Of all the hormonal changes measured in this analysis, the only important indicator of the shift in insulin sensitivity was the improvement in TNF-alpha from pre-gravid to late pregnancy. Placental reproductive hormones and cortisol did not correlate with late pregnancy insulin sensitivity".

Periodontitis, GDM and TNF - α,Özçaka Ö, Ceyhan-Öztürk B, Gümüş P, Akcalı A, Nalbantsoy A, Buduneli N (2016)[2]

Evaluated for Clinical periodontal and gingival crevicular fluid (GCF) findings and serum tumor necrosis factor-alpha (TNF-alpha), interleukin-10 (IL-10) and IL-33 levels among women with and without gestational diabetes mellitus (GDM). Samples of serum and GCF were obtained, with full-mouth recordings.

Clinical periodontal and gingival crevicular fluid (GCF) findings and serum tumour necrosis factor-alpha (TNF-alpha), interleukin-10 (IL-10) and IL-33 levels were compared among women with and without gas indicated by higher PI and BOP in the GDM community, elevated GCF IL-10 levels may be a consequence of higher levels of inflammation. The clinical parameters examined may not have a major impact on levels of TNF-alpha and IL-33.estational diabetes mellitus (GDM). Samples of serum and GCF were obtained.

MATERIALS AND METHODS

SAMPLE SIZE & SOURCE OF DATA

The present case control study was conducted on subjects between 18 - 30 years of age. 72 subjects were selected from the Out Patient Department of Obstetrics and Gynecology, KLES, Dr.PrabhakarKore Hospital and Medical Research Centre, Belagavi" during the period of January 2019 – January 2020. Subjects of Gestational Diabetes Mellitus and Non-Gestational Diabetes were selected on the basis of inclusion and exclusion criteria.

Inclusion criteria:

1. Patients above the age of 18 years to 30years
2. Gestational age between 10 to 28weeks
3. Patients diagnosed with the Gestational Diabetes Mellitus
4. Chronic Periodontitis (AAP 1999classification.)

Exclusion criteria:

1. Known type 1 and type 2diabetes
2. Patient with any known systemic diseases.
3. Patients with < 20teeth
4. Patients with previous history of periodontal treatment in past 3months
5. Patients with a history of antibiotics prescriptions for 6months
6. Consumption of alcohol or smoking during pregnancy

STUDY DESIGN

Screening and enrollment of subjects

The patients reportingto the Out Patient Department of Obstetrics and Gynecology at the KLES Dr.PrabhakarKore Hospital and Medical Research center" who satisfied the inclusion criteria were selected for the study. After obtaining informed consent from the patient willing to participate, Maternal statistics were collected from their patient records from the "Department of the Obstetrics and Gynaecology and a full-mouth periodontal examination was performed (Annexure- III).

Definition of Cases and Controls

Based on the recommendation of the ACOG, all pregnant women undergo a recommended laboratory screening test for GDM between 24 to ≥ 30 weeks of gestation.[21] During this test, they are first screened for carbohydrate intolerance by performing a standard 1-hour, 50-g oral glucose challenge test (GCT). If the glucose level was > 135 mg/dl (GCT positive), the women underwent a 3-hour, 100-g oral glucose tolerance test (OGTT) after a 10 to 12 hour overnight fast".[22] "Women with glucose levels two times higher than the OGTT values (fasting, 1, 2, and 3 hours) were diagnosed as having GDM. Women diagnosed with GDM were the case group for the current study.[22] The Control group included women who were not diagnosed with GDM or any other types of diabetes. The case and control groups were selected from the O.P.D of Department of Obstetricsand Gynecology at the KLES Dr.PrabhakarKore Hospital and Medical Research center.

Periodontal Disease Measurements and Definitions

After the selection of the patients, a full-mouth periodontal examination was performed and measurements were made using William's periodontal probe and a mouth mirror. To eliminate inter-examiner variability, all periodontal measurements were performed by single calibrated examiner.

The Clinical parameters recorded were:

1. Plaque index (PI)(Silness J and Loe H, 1964)
2. Bleeding index (BI) (Muhlemann H.R and Son S, 1971)
3. Probing depth (PD)
4. Clinical attachment loss (CAL)

Armamentarium for Recording Clinical Parameters:

1. Mouth mask
2. Diagnostic and gloves
3. Mouth mirrors
4. Tweezer
5. William's graduated periodontal probe
6. Tissue forceps
7. Kidney tray
8. Cotton swabs

Plaque Index (Silness J and Loe H,1964)[23]

In this index, plaque thickness is given consideration. The evaluation or scoring is done on the entire dentition or on selected teeth (16, 12, 24, 36, 32, 44). Only plaque of the cervical third of the tooth is evaluated. The surfaces examined are four gingival areas of tooth i.e. disto - facial, facial, mesio- facial and lingual surfaces. The lingual surface is considered as one unit. This index is one of the most widely used and recognized amongst the plaque indices and has demonstrated good validity and reliability. It can be used on all surfaces of all or selected teeth or for selected surfaces of all or selected teeth. However, one criticism is the subjectivity in estimating plaque. Therefore, it was recommended that a single examiner be trained and used with each group of patients throughout a clinical trial.

Procedure

The tooth was dried and examined visually with the help of mouth mirror and explorer. When no plaque was visible, an explorer was used to test the surface. The explorer was passed across the tooth surface in the cervical third and near the entrance to the sulcus. The evaluation or scoring was done on the entire dentition. Only plaque of the cervical third of the tooth was evaluated. The surfaces examined were four gingival areas of tooth i.e. disto - facial, facial, mesio - facial and lingual surfaces. The lingual surface was considered as one unit.

Scoring:

Score	Inference
0	This score is given when the gingival area of the tooth surface is literally free of plaque. The surface is tested by running a pointed probe across the tooth surface at the entrance of the gingival crevice after the tooth has been properly dried and if no soft matter adheres to the point of the probe, the area is considered clean.
1	This score is given when no plaque can be observed in situ by the unarmed eye, but when plaque is made visible on the point of the probe after this has been moved across the tooth surface at the entrance of the gingival crevice. Disclosing solution has not been used.
2	This score is given when the gingival area is covered with a thin to moderately thick layer of plaque. The deposit is visible to the naked eye.
3	Heavy accumulation of soft matter, the thickness of which fills out the niche produced by the gingival margin and the tooth surface. The interdental area is stuffed with soft debris.

Calculation

Plaque score per person = Total Plaque scores / Total number of sites examined x 4

Interpretation

Rating	Scores
Excellent	0
Good	0.1- 0.9
Fair	1.0- 1.9
Poor	2.0- 3.0

Sulcus Bleeding Index (Muhlemann H. R. and Son. S,1971)[24]

As early as 1958, Sulcus Bleeding Index was introduced and termed PM index in which bleeding after gentle probing was the leading symptom. To avoid confusion from PMA index the initials were changed to Sulcus Bleeding Index. The index was developed to locate areas of gingival sulcus bleeding upon gentle probing and thus recognize and record the presence of early inflammatory gingival disease.

The tissue surrounding each tooth is divided into four gingival scoring units-

1. Munits
    a) Labial marginalgingiva
    b) Lingual marginalgingiva

1. Punits
    a) Mesial papillarygingiva
    b) Distal papillarygingiva

A standardized light was used while probing each of four areas. The probe was held parallel with the long axis of the tooth for M units, and the probe was directed towards the col area for P unit. Scoring was done 30 seconds after probing the apparently healthy gingival units. The gingiva was gently dried if necessary to observe colorchanges.

Score	Inference
0	Healthy P and M, no bleeding on probing.
1	Bleeding on probing, no color change, no swelling of P and M.
2	Bleeding on probing, change in color, no swelling of P and M
3	Bleeding on probing, change in color, slight swelling of P and M
4	Bleeding on probing, change in color, obvious swelling of P and M
5	Bleeding on probing, spontaneous bleeding, change in color, marked swelling with or without ulceration.

Calculation

Gingival score per person = Total gingival scores / Total number of sites examined x 4

Interpretation

Gingival Scores	Degree of Gingivitis
0.1 – 1.0	Mild
1.1 – 2.0	Moderate
2.1 – 3.0	Severe

Measurement of Clinical Parameter

Probing pocket depth[25] and clinical attachment level[26] were recorded by using William's graduated periodontal probe. The probe was inserted parallel to the vertical axis of the tooth and 'walked' circumferentially around each surface of each tooth to detect the areas of deepest penetration. Probing pocket depth was measured from the crest of the marginal gingiva to base of the pocket and clinical attachment level was measured from the cement-enamel junction of the tooth to the base of the pocket.

Grouping

After recording the clinical parameters for periodontitis, the 72 subjects were equally divided into two groups:

Group 1 (n=32): GDM patients with Chronic Periodontitis Group 2 (n=32): Non-GDM patients with Chronic Periodontitis

Following the grouping of the patients, the procedure for analysis of TNF-α was initiated.

Collection of Serum & TNF-a analysis

1. The analysis of TNF-alpha levels for both the groups was done at the department of biochemistry at KLEs Dr.PrabhakarKorehospital.
2. After recording the clinical parameters, blood samples were drawn to obtain the serumsamples.
3. Blood sample of patients was collected from the ante-cubital fossa in a 5 ml plastic test tube and stored at room temperature (20C to 25C) for 30min.

4. It was then transferred to a refrigerator (2C to 8C) and centrifuged within 2 hours at the speed of 3,150 rpm for 20minutes.
5. Each specimen was equally divided into four (0.5-mL) aliquots of serum and stored in a -20C freezer for laboratoryanalysis.
6. The samples were then analyzed for TNF alpha inflammatorymarker.
7. GDM status was masked to the laboratory to avoid bias. Levels of TNF-α were determined using a human ELISA kit (Diaclone, Besancon Cedex, France) in accordance with the manufacturer'sprotocol.

Stepwise procedure for determination of TNF-alpha: (Specimen collection, processing & storage)

1. Removal of serum from the clot or red cells, respectively, as soon as possible after clotting and separation was done.
2. Cell culture supernatants: After removal of the particulates, aggregation by spinning at approximately 1000 x g for 10 min wasperformed.
3. Serumpyrogen/endotoxin free collecting tubes were used. Serum wasremoved rapidly and carefully from the red cells after clotting. Following clotting, centrifugation at approximately 1000 x g for 10 min was done and serum wasremoved.
4. Plasma: EDTA, citrate and heparin plasma were assayed. Spin samples at 1000 x g for 30 min to remove particulates was done. Plasma washarvested.
5. Storage: If not analysed shortly after collection, samples had to be aliquoted (250-500µl) to avoid repeated freeze-thaw cycles and stored frozen at –70°C. Multiple freeze-thaw cycles of frozen specimens wereavoided.

6. Recommendation: It was recommended not thaw by heating at 37°C or 56°C. Thawing was done at room temperature and the sample was completely thawed and homogeneous in nature beforeuse.
7. According to standard concentrations and corresponding Optical density (OD) values, the linear regression equation of the standard curvewascalculated. Then according to the OD value of samples, the concentration of the corresponding sample was calculated.
8. The values were obtained inpg/µL

Armamentarium for Serum Preparation –

1. Spirit
2. Cotton
3. Tourniquet
4. 5 mlsyringe
5. 10 ml plastic testtube
6. Centrifugationmachine

Armamentarium for Serum Analysis –

1. Elisa kit (Diaclone, Besancon Cedex, France)

STATISTICAL ANALYSIS:

All the descriptive data that include mean and standard deviation were determined. The following methods were implemented in the statistical evaluation: Mann Whitney-U test, Independent T-test, Paired T-test, Wilcoxon signed rank test & Karl Pearson's / Spearman's rank correlation

The data derived for each group was analyzed by paired 't' test. For all tests, a p value of <0.05 was considered significant and p value of <0.001 was considered highly significant. The following formulae were employed:

1. Mean: $X = \Sigma X i / n$

   Where, $X i = 1,2 \ldots\ldots n$

2. Standard deviation: $SD = \sqrt{\Sigma (X i - X)^2 / n - 1}$

3. Standard error: $SE = SD/\sqrt{n}$

4. Paired 't' test: $t = \bar{d} / SE$ ofdifference

   Where, d = mean of differences

Fig 1: Armamentarium

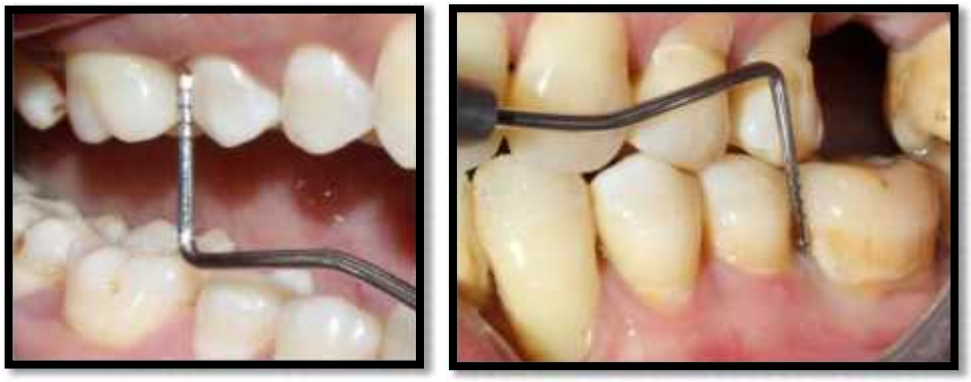


Fig 2: Measurement of clinical parameters using Williams periodontal probe:

Pocket probing depth and clinical attachment loss (PPD AND CAL)

Fig 3: Blood sample of patients was collected from the ante-cubital fossa in a 5 ml plastic test tube.

Fig 4: Vacutainer containing blood sample

Fig 5: storage done at 2C to 8C with clot activator

Fig 6 : Reagents of theELISAkit

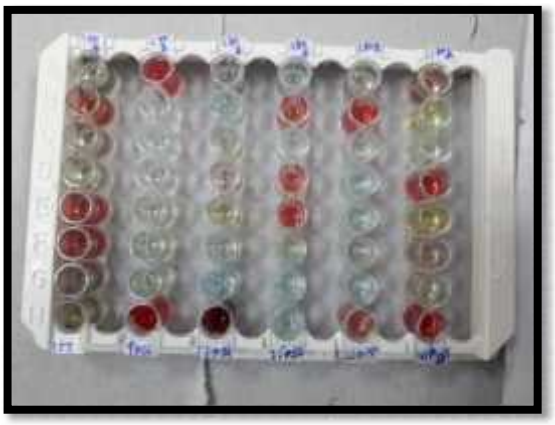
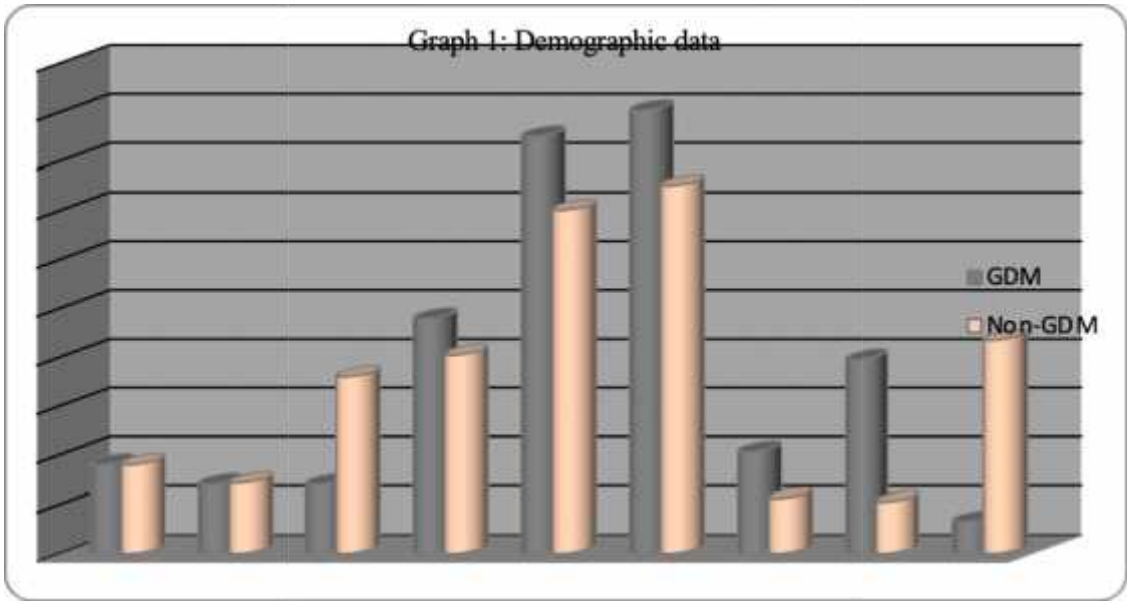


Fig 7: ELISAmicroplate

RESULTS AND OBSERVATIONS

Table 1: Demographic Data			
No.	**Parameters**	**GDM**	**Non-GDM**
1	**Number of subjects** (n)	36	36
2	**Age** (years)	28.0 ± 2.7	28.6 ± 3.5
3	**Single time gestation** (%)	28%	72%
4	**Fasting levels** (mg/dl)	95.03 ± 8.6	80.56 ± 3.25
5	**1 hour**	169.25 ± 7.5	140.54 ± 5.6
6	**2 hour**	180.05 ± 9.5	150.87 ± 5.6
7	**BMI** (kg/m2)	33.4 ± 6.4	22.4 ± 2.4
8	**Family history of diabetes or GDM** (%)	79%	21%
9	**Awareness about dental hygiene maintenance** (%)	13%	87%



Observation of Table 1 (Graph 1):

Table 1 demonstrates demographic data distribution and its clinical, anthropometric and laboratory findings. The total number of participants in the study was 72. The mean age of participants in GDM group was 28.0 ± 2.7 and in Non-GDM group was 28.6 ± 3.5. Percentage of 72% single time gestation was observed in Non-GDM group and 28% in GDM. The fasting levels in GDM was found to be 95.03 ± 8.6 which was higher in comparison to Non-GDM group 80.56 ± 3.25.

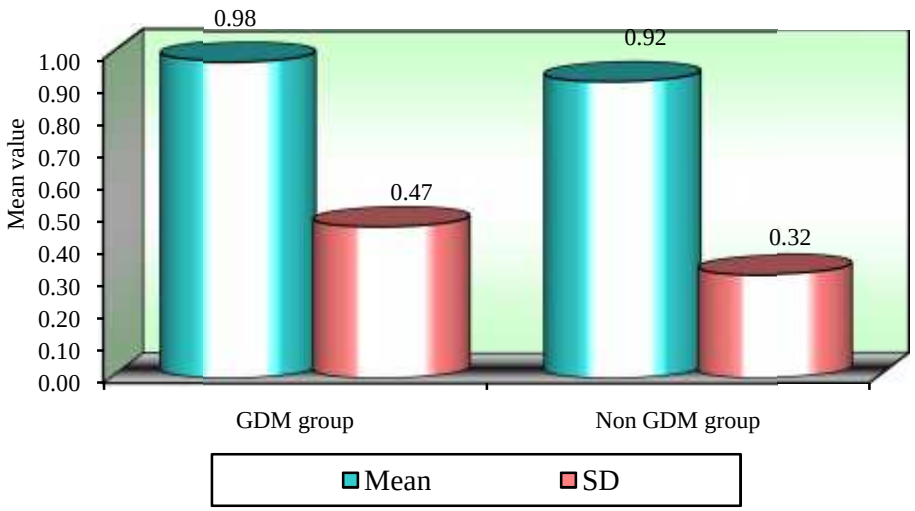
The Body Mass Index recorded was observed to be higher in GDM group (33.4 ± 6.4) compared to Non-GDM (22.4 ± 2.4). The history of diabetes or GDM in family members recorded was higher in GDM group (79%) than the Non-GDM group (21%). The awareness about dental hygiene maintenance was highest in Non-GDM group by 87% than in GDM group (13%).

Table 2: Comparison of GDM and Non GDM groups with plaque index scores					
Groups	**Mean**	**SD**	**U-value**	**Z-value**	**P-value**
GDM group	0.98	0.47	631.50	-0.1858	0.8526
Non GDM group	0.92	0.32			

*Test applied: Man-whitney –U,

*p value<0.05 was statistically significant

Graph 2: Comparison of GDM and Non GDM groups with plaque index scores



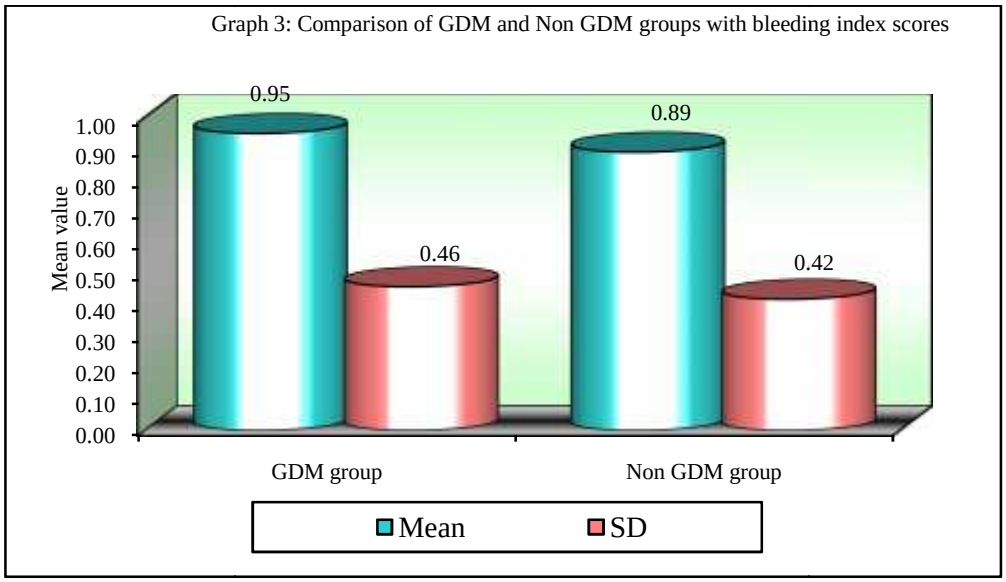
Observation of Table 2 (Graph 2):

The Plaque Index (PI) score by Mann-Whitney U test in GDM Group was 0.98 ± 0.47, whereas, in Non-GDM group was 0.92 ± 0.32. There was no significant difference of Plaque Index when compared with both GDM and Non-GDM group. The Plaque index was statistically non-significant with the (p Value = 0.8526).

Table 3: Comparison of GDM and Non GDM groups with bleeding index scores					
Groups	**Mean**	**SD**	**U-value**	**Z-value**	**P-value**
GDM group	0.95	0.46	613.50	-0.3885	0.6976
Non GDM group	0.89	0.42			

*Test applied: Man-whitney –U,

*p value<0.05 was statistically significant



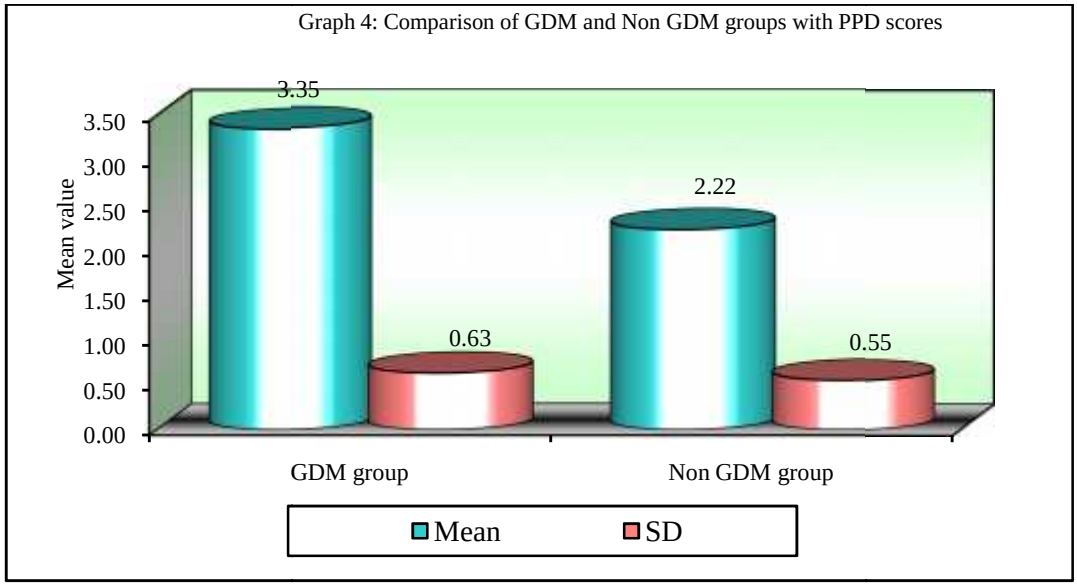
Observation of Table 3 (Graph 3):

The Bleeding Index (BI) score by Mann-Whitney U test in GDM Group was 0.95 ± 0.46, whereas, in Non-GDM group was 0.89 ± 0.42. There was no significant difference of Bleeding Index when compared with both GDM and Non-GDM group. The Bleeding Index was statistically non-significant with the (p Value = 0.6976).

Table 4: Comparison of GDM and Non GDM groups with PPD scores				
Groups	**Mean**	**SD**	**t-value**	**P-value**
GDM group	3.35	0.63	8.1280	0.0001*
Non GDM group	2.22	0.55		

*Test applied: Independent t test,

*p value<0.05 was statistically significant



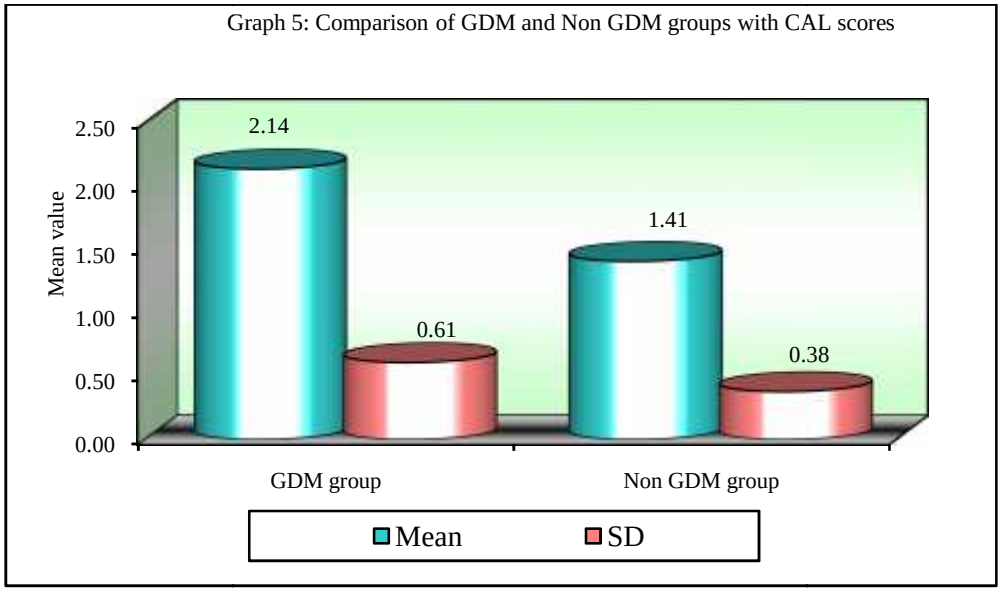
Observation of Table 4 (Graph 4):

The Periodontal Probing Depth (PPD) score by Independent test in GDM Group was 3.35 ± 0.63, whereas, in Non-GDM group was 2.22 ± 0.55. Significant difference was observed between GDM & non-GDM with mean PPD scores. PPD score was higher in GDM compared to non-GDM. The PPD was statistically significant with the (p Value = 0.0001).

Table 5: Comparison of GDM and Non GDM groups with CAL scores				
Groups	**Mean**	**SD**	**t-value**	**P-value**
GDM group	2.14	0.61	6.1183	0.0001*
Non GDM group	1.41	0.38		

*Test applied: Independent t test,

*p value<0.05 was statistically significant



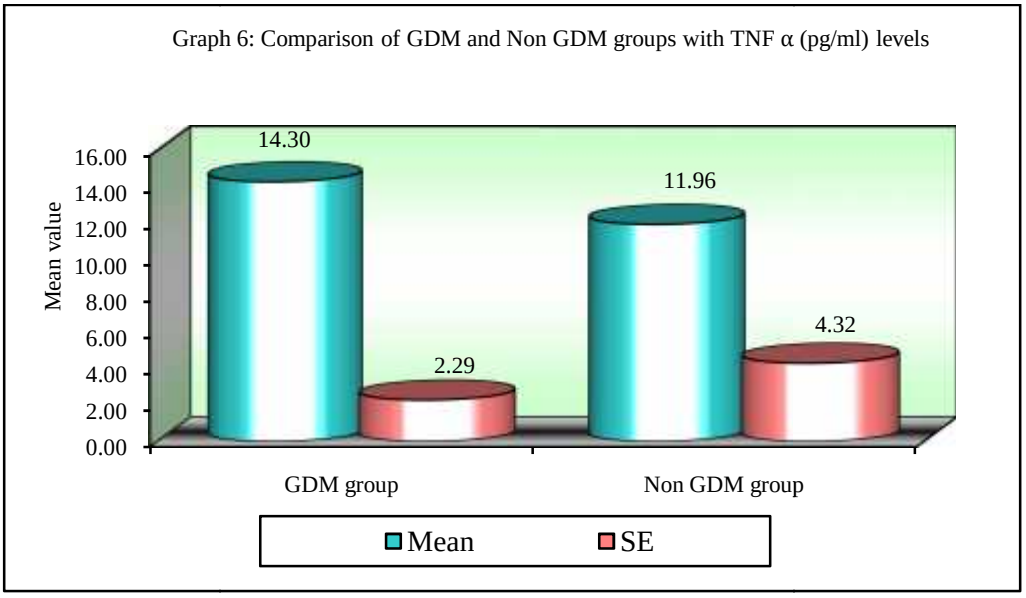
Observation of Table 5 (Graph 5):

The Clinical Attachment Level (CAL) score by Independent test in GDM Group was 2.14 ± 0.61, whereas, in Non-GDM group was 1.41 ± 0.38. Significant difference was observed between GDM & non-GDM with mean CAL scores. CAL score was higher in GDM than in non-GDM. The CAL was statistically significant with the (p Value = 0.0001).

Table 6: Comparison of GDM and Non GDM groups with TNF - α (pg/ml) levels					
Groups	**Mean**	**SD**	**U-value**	**Z-value**	**P-value**
GDM group	14.30	13.75	452.00	-2.2074	0.0273*
Non GDM group	11.96	25.90			

*Test applied: Man-whitney - U ,

*p value<0.05 was statistically significant.



Observation of Table 6 (Graph 6):

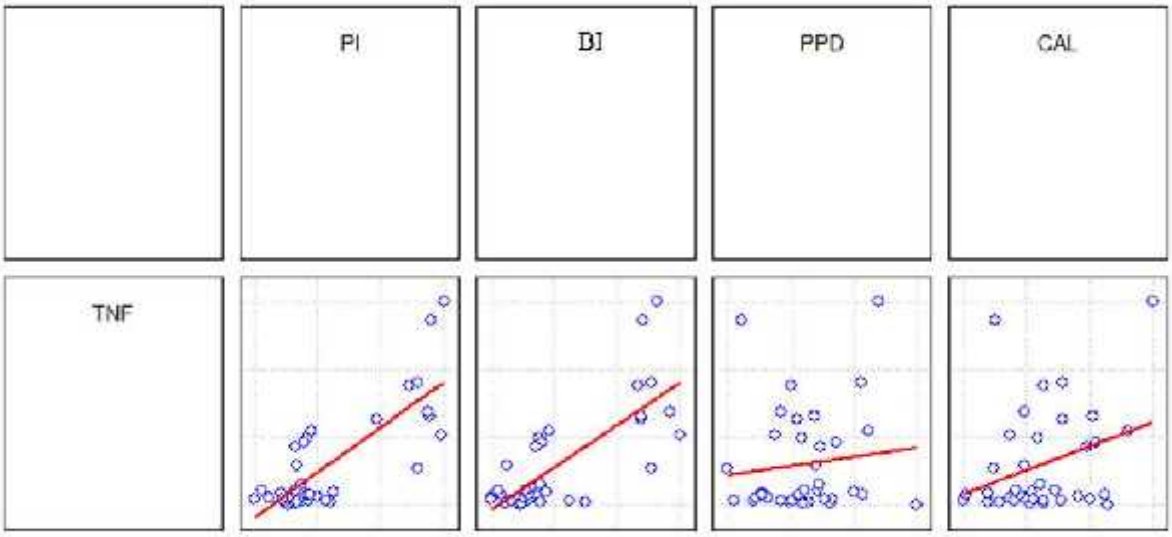
The TNF- α level inGDM group recorded was 14.30 ± 13.75 and in the Non-GDM group was significantly lower with 11.96 ± 25.90. The TNF- α value in GDM group was statistically significant (p value = 0.0273). TNF- α levels are significantly higher in GDM group when compared to non-GDM. TNF- α levels have a significant difference with mean TNF- α level at 5% level of significance.

Table 7: Correlation between TNF - α (pg/ml) levels with PI, BI, PPD and CAL in GDM group				
Parameters	**N**	**Spearman R**	**t-value**	**p-level**
Plaque index	36	0.6057	4.4387	0.0001*
Bleeding index	36	0.6461	4.9361	0.0001*
PPD	36	0.0810	0.4741	0.6384
CAL	36	0.2117	1.2632	0.2151

***Test applied: Spearman's rank correlation coefficient,**

***p value<0.05 was statistically significant**

Graph 7: Scatter diagram showing correlation between TNF - α (pg/ml) levels with Plaque index, Bleeding index, PPD and CAL in GDM group



Observation of Table 7 (Graph 7):

In GDM, statistical significant & positive correlation was observed among Plaque Index & Bleeding Index with TNF - α. Statistically non-significant but positive correlation was observed among Pocket Probing Depth and Clinical Attachment Loss with TNF - α.

Scatter graph 7 explains the TNF - α correlation among the clinical parameters. Only in regards to Plaque & Bleeding index the bar was observed to be inclined suggesting its clinical significance in GDM group.

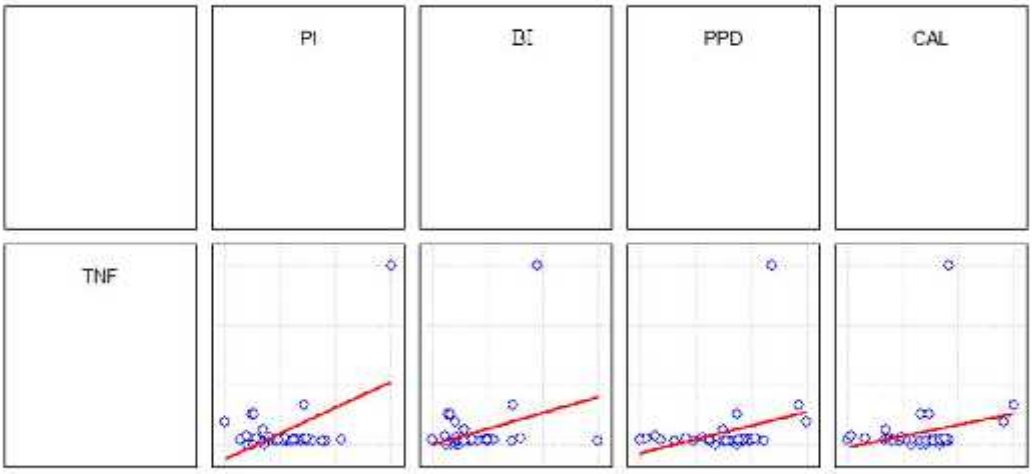
Table 8: Correlation between TNF - α (pg/ml) levels with PI, BI, PPD and CAL in Non-GDM group

Parameters	N	Spearman R	t-value	p-level
Plaque index	36	-0.0066	-0.0383	0.9697
Bleeding index	36	0.0989	0.5796	0.5660
PPD	36	0.1222	0.7179	0.4777
CAL	36	-0.0609	-0.3556	0.7243

***Test applied: Spearman's rank correlation coefficient,**

***p value<0.05 was statistically significant**

Graph 8: Scatter diagram showing correlation between TNF - α (pg/ml) levels with Plaque index, Bleeding index, PPD and CAL in non-GDM group



Observation of Table 8 (Graph 8):

Statistically non-significant correlation was observed among all the clinical parameters - Plaque Index, Bleeding Index, Pocket Probing Depth, Clinical Attachment Loss, and TNF- α. When compared with GDM group, the Non-GDM group showed lesser values for all the clinical parameters.

Scatter graph 8 explains the TNF - α correlation with the clinical parameters. The bar was not inclined towards any parameter suggesting the clinical non-significance between the TNF - α and clinical parameters.

Table 9: Correlation between TNF α (pg/ml) levels with PI, BI, PPD and CAL in both GDM and Non-GDM group				
Parameters	**N**	**Spearman R**	**t-value**	**p-level**
Plaque index	72	0.2819	2.4581	0.0164*
Bleeding index	72	0.3417	3.0415	0.0033*
PPD	72	0.2832	2.4702	0.0159*
CAL	72	0.2654	2.3033	0.0242*

***Test applied: Spearman's rank correlation coefficient,**

***p value<0.05 was statistically significant**

Observation of Table 9:

A positive correlation was observed among all the clinical parameters - Plaque Index, Bleeding Index, Pocket Probing Depth, Clinical Attachment Loss, and TNF- α in GDM group. GDM group comparatively showed significant correlation with TNF- α than in non-GDM group.

DISCUSSION

Periodontal diseases are bacterial infections chronic in nature. This progressive infection results in inflammation causing destruction of tooth supporting tissues. Pathogenesis of periodontitis is complex involving bacteria and host factors. Immune response of the host has a major impaction disease progression and severity of periodontitis.[20]

"Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine. They are released by macrophages. TNF-α mediate bone loss in periodontal disease playing significant role in destruction. Increased concentration observed in periodontitis correlate closely with the tissue destruction and immune response. TNF-α also inhibits insulin transduction and contributes to insulin resistance in diabetes mellitus".[20]

Gestational Diabetes Mellitus (GDM) is defined as "carbohydrate intolerance first recognized during pregnancy."[27] GDM is a unique physiologic state that is distinguishable from the normal metabolic changes induced by pregnancy. "Glucose metabolism disorder is a common complication during pregnancy and its pathogenesis is associated with Insulin Resistance (IR) and deficiency of insulin secretion.[28, 29] It has been presumed for a long time that glucocorticoid and placenta secreted hormones (Human Placental Lactogen (HPL), progesterone, estrogen and prolactin) mediate the Insulin resistance. However, recent studies have focused on several new factors including TNF-α,[20]leptin[30] and adiponectin,[31] which could lead to Insulin resistance.[32] Kirwanetal[20]stated that among all the markers being tested including TNF-α, leptin, glucocorticoid, estrogen, progesterone and HPL, TNF-α was an independent predictive factor for insulin sensitivity, but no statistical significance

was noted among insulin sensitivity, placental hormones and glucocorticoid in the late trimester."

Our study was done to evaluate and compare the levels of serum TNF- alpha in Non-Gestational Diabetes Mellitus (Non GDM) and Gestational Diabetes Mellitus (GDM) patients having Chronic Periodontitis wherein a total of 72 female patients, satisfying the inclusion criteria were selected for this case control study. The case group included the patient diagnosed with GDM and Chronic Periodontitis, while the control group had non GDM patients with periodontitis.

The age range of 18-30 years are considered most fertile years for a woman to conceive.[33] It is during these years that a woman conceives without much complication within their biologic clock.[34] The current study adapted similar range as inclusion criteria and the mean average age observed was 28 years for both GDM & Non-GDM group as seen in Table 1. Along with the strong family history of GDM, the second time gestation increases the risk of recurrence of GDM in subsequent pregnancies.[35] However, 79% history of GDM and diabetes was observed among family members in GDM group and 21% was observed in the Non GDM group.

The increase of Body Mass Index (BMI) during pregnancy increases the risk of GDM during pregnancy.[36] The Body Mass Index was found to be high in GDM patients (33.4+6.4) which in accordance to the study conducted by Winkler et. al.[7]Several potential mechanisms of TNF- αin relation to BMI have been explained. In adipose cells, TNF-αcan inhibit*GLUT-4* resulting in a decreasing glucose transport. TNF-αpromotesSerphosphorylationofIRS-1, leading to a reduction in insulin receptor-mediated signaling.[37] This data explains of how BMI could be associated with

increased chances of GDM, where the BMI values obtained in Non-GDM patients were below the GDM values. Maternal obesity is strongly seen associated with GBM. This can be attributed to insulin signaling dysfunction. In a study by Friedman and colleagues[39] they have found that defective phosphorylation of tyrosine kinase receptors in patients with GDM. Due to this there was lesser transportation of glucose to the mother as well as the fetus.[40]

The Plaque Index (PI) is one of the most widely used and recognized indices with good validity and reliability. It is used in clinical trials for an insight to individual's level of oral hygiene maintenance.[41] The Plaque Index was found to bestatistically non-significant in our study when compared between the GDM group and the non GDM group (p value 0.8526 - Table 2)

The Sulcus Bleeding Index (SBI) provides an objective and easily reproducible measurement of gingival status. Since patients can easily appreciate it, the SBI can be used to enhance the patient's motivation for plaque control.[42] The Bleeding Index (BI) was found to be statistically non- significant in our study when compared between the GDM group and the non GDM group (p value 0.6976 - Table3)

During pregnancy, gingivitis is characterized by erythema, oedema, hyperplasia and increased bleeding. This could progress to increase in pocket depth. The alterations that occur in subgingivalmicroflora may be attributed to elevated levels of progesterone or estriol.[43] The loss of attachment, considered as progression of gingivitis to periodontitis, is assessed by PPD & CAL. The presence of periodontal pockets can lead to more chances of harbouring pathogenic microorganisms which may evoke a host response leading to a systemic effect.[44] GDM is associated with

increased incidence of periodontal diseases.[45] In our study, we have found higher PPD and CAL in patients with GDM($p<0.001$ - Table 4 and 5). The elevated PPD & CAL in our study have influenced TNF-a levels in the current study. Increased TNF-$\alpha$is believed to be found during periodontal breakdown of the tissues.[46]

Oral hygiene maintenance and its awareness both play a pivotal role in any individual, especially during pregnancy. Due to improper oral hygiene maintenance, the clinical parameters like PI & BI were found to be statistically significant and had a positive correlation with TNF-alpha in GDM group ($p<0.001$) (Table 7). Higher TNF–A levels in GDM group than the non-GDM group was found in the current study which is in accordance to the study by Xiang and colleagues.[47]

A local and chronic subclinical inflammation results in periodontal infections provoking local and host immune response. the destruction of Pancreatic beta cells can result from increased levels of IL-1 and TNF- α. This further leads to insulin resistance.[48]

TNF-α levelsis a predictor of insulin resistance during pregnancy.[29] During third term of pregnancy, TNF-$\alpha$is correlated inversely with insulin sensitivity. In order to improve insulin sensitivity, neutralization of the TNF-αsignaling is important.[48] Sphingomyelinase and ceramides are increased by TNF-αactivation which leads to interference with auto-phosphorylation of insulin receptor. The raised TNF-$\alpha$is associated with insulin resistance imaging, obesity, septicemia and also seen after damage of muscle.[20]

There are various studies showing TNF- α association with GDM. However, the study carried out by Özçaka Ö et al showed negative correlation of TNF – α with GDM and a positive correlation of TNF- α with Non GDM group.[2]

Inter study variation in TNF-*α* concentration may be due to several factors including periodontal disease severity, subject age, sample type, population type, and technique details such as storage temperature and pre-test storage time.

Thus, within its limits, our study suggested significant correlation between periodontitis (PPD, CAL) BMI and TNF – α with GDM. We recommend the further studies with a larger sample size. There was a family history of GDM and diabetes among the pregnant women with GDM. To assess whether periodontal disease is an independent risk factor for GDM, further research is required. Further prospective studies are essential whether periodontal disease is a contributing factor for GDM.

Periodontitis is a preventable and curable disease. Treating any form of periodontal disease and rectifying oral health before or during pregnancy can prevent the occurrence of type 2 diabetes mellitus. Hence, GDM can provide opportunities which might prevent development of type 2 diabetes. Once sufficient literature data is obtained regarding the positive correlation of GDM with periodontal disease, there will be room for further studies.

SUMMARY & CONCLUSION

"The aim of the study was to compare the levels of serum TNF-α in NonGestational Diabetes Mellitus and Gestational Diabetes Mellitus patients with Chronic Periodontitis. A total of 72 female subjects with an age range of 18 to 30 years from the period of January 2019 – January 2020 were included for this case control study. The patients satisfying the inclusion criteria were selected for the study, followed by which the recording of maternal statistics and periodontal findings was done. The case group included the patient diagnosed with GDM with chronic periodontitis, whereas, the control group included non- GDM patients with periodontitis".

Clinical parameters like Plaque index (PI), Bleeding index (BI), probing pocket depth(PPD), clinical attachmentloss (CAL) were recorded and their maternal statistics were taken from their OPD records (Annexure – III). All the data were subjected to statisticalanalysis.

The following conclusions were drawn from the current study:

- GDM patients showed higher values in BMI (33.4+ 6.4), family history of diabetes or GDM (79%), in comparison to non-GDM patients.
- The Non-GDM patients showed higher values in terms of single time gestation period (72%), knowledge regarding dental oral hygiene (87%) in comparison to GDM patients.
- Plaque index (PI) and Bleeding index (BI) showed non-significant correlation when compared with GDM and Non –GDM group.
- PPD &CAL showed significant correlation in GDM group, whereas the Non-

GDM group showed non-significant correlation.

- TNF alphalevels were statistically significant in GDMgroup.
- TNF alphalevels were statistically non-significant in the Non- GDMgroup.
- The parameters, Plaque index (PI), Bleeding Index (BI), Probing Pocket Depth (PPD) & Clinical Attachment Loss (CAL) showed significant correlation with TNF – α in GDM group. However, Non-GDM group showed no statistical significantco-relation.
- Within the limitations of the study, it can be suggested that Gestational Diabetes Mellitus (GDM) with BMI, PPD, CAL has significant correlation with TNF– α.

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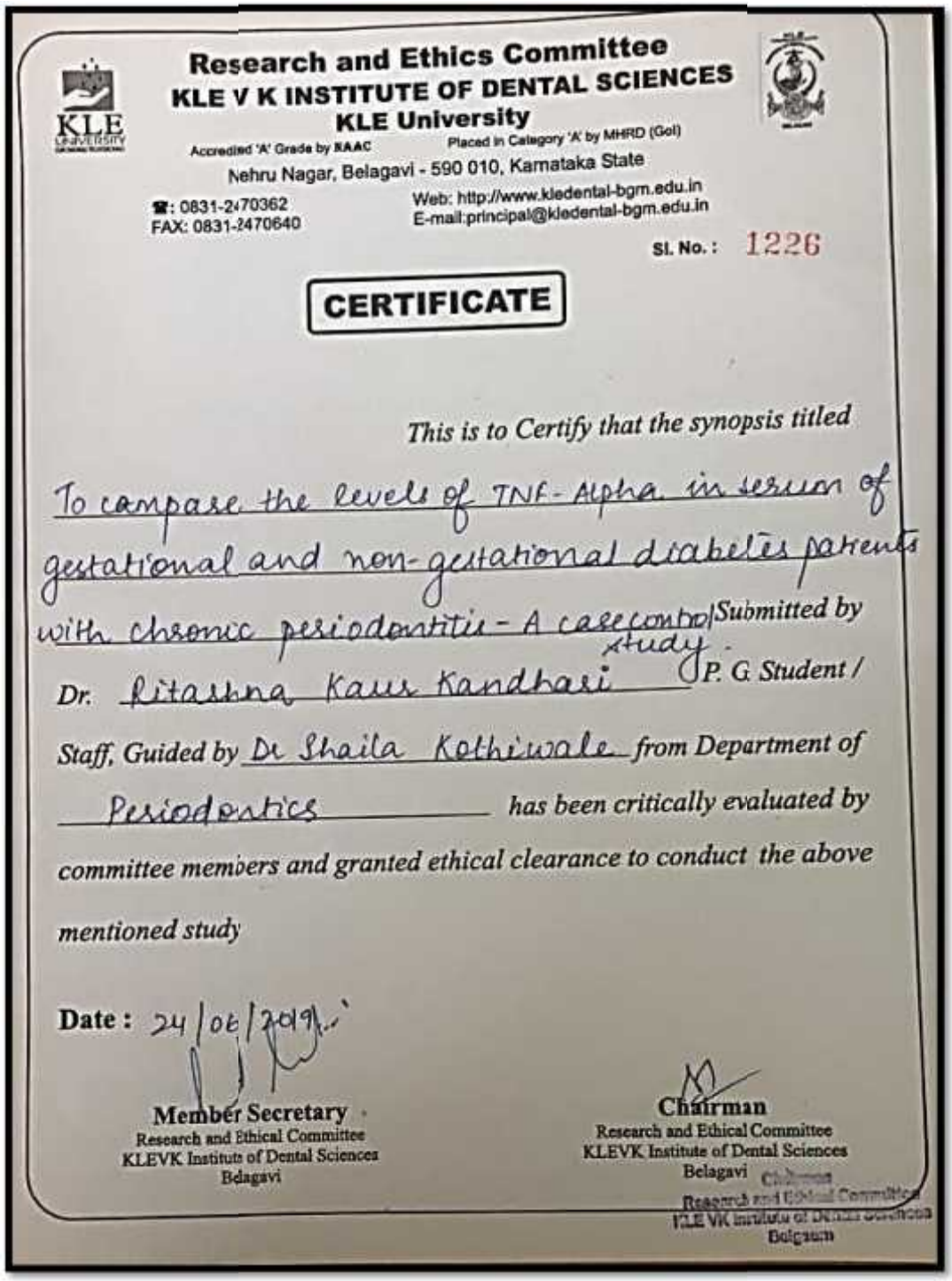
ANNEXURE 1

ETHICAL CERTIFICATE



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Sl. No.: 1226

CERTIFICATE

This is to Certify that the synopsis titled

To compare the levels of TNF-Alpha in serum of gestational and non-gestational diabetes parents with chronic periodontitis - A case control study

Submitted by Dr. Ritasha Kaur Kandhari *P. G Student / Staff. Guided by* Dr Shaila Kothiwale *from Department of* Periodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 24/06/2019

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE 2

KAHER'S KLE VK INSTITUTE OF DENTAL SCIENCES, BELAGAVI – 590010

TO COMPARE THE LEVELS OF TNF-ALPHA IN SERUM OF GESTATIONAL AND NON GESTATIONAL DIABETES PATIENTS WITH CHRONIC PERIODONTITIS – A CASE CONTROL STUDY.

OPERATOR: DR. RITASHNA KAUR

I, _____ aged _____ have been informed about my involvement in the study:

1) I agree to give my personal details like name, age, sex, address and the details required for the study to the best of my knowledge.
2) I agree for the periodontal examination to be done by the dentist
3) I permit the dentist to collect the blood samples for the study.
4) I permit the dentist to utilize the information given by me and results obtained from this study for presentation and publication purpose.
5) I will not claim any returns for my cooperation in the study, even if it is being sponsored by any agency. I am participating with my own will and wish.
6) I will follow the instructions given by the doctor.
7) During the study, if I wish to resign from the study, I am free to do so and my treatment will still be completed in the department.

In my full consciousness and presence of mind, after understanding all the procedure in my vernacular language, I am willing and give my consent to participate in this study.

Date:

Place:

Subject's Signature Signature of witness

KAHER'S KLE VK INSTITUTE OF DENTAL SCIENCES, BELAGAVI – 590010

TO COMPARE THE LEVELS OF TNF-ALPHA IN SERUM OF GESTATIONAL AND NON GESTATIONAL DIABETES PATIENTS WITH CHRONIC PERIODONTITIS – A CASE CONTROL STUDY.

ऑपरेटर: डॉ. रितेशनाकुर

मी, _____ वयाच्या_____

चाअभ्यासमाझ्यागुंतवणूकीबद्दलकळवलाआहे

1) मीमाझ्यावैयक्तिकमाहितीजसेकीनाव, वय, लिंग, पत्ताआणिअभ्यासक्रमासाठीआवश्यकअसलेल्यामाहितीसमाझेसर्वोत्तमज्ञानदेण्या साठीसहमतआहे.
2) मीदंतचिकित्सकानेकेलेल्यापीरियंटॉन्टलपरीक्षणासाठीसहमतआहे
1) 3). मीदंतचिकित्सकअभ्यासासाठीरक्तनमुनेगोळाकरण्यासपरवानगीदेतो.
3) मीदंतवैज्ञानिकांनामाझ्याद्वारेदिलेलीमाहितीआणिसादरीकरणआणिप्रकाशनउद्देशासा ठीयाअभ्यासातूनमिळालेल्यापरिणामांचावापरकरण्यासपरवानगीदेतो.
4) मीकोणत्याहीसंस्थेद्वारेप्रायोजितकेलेअसलेतरीही, अभ्यासातमाझ्यासहकार्यासाठीकोणत्याहीपरताव्याचादावाकरणारनाही. मीमाझ्याइच्छेनुसारआणिइच्छेनेभागघेतआहे
5) मीडॉक्टरांनीदिलेल्यानिर्देशांचेपालनकरू.
6) अभ्यासादरम्यान, जरमीअभ्यासातूनराजीनामादिलाअसेलतरमीतसेकरण्यासस्वतंत्रआहेआणिमाझेउप चारअद्यापविभागांतपूर्णकेलेजातील.

माझीसंपूर्णचेतनाआणिमनाचीउपस्थिती,

माझ्यास्थानिकभाषेतीलसर्वप्रक्रियासमजूनघेतल्यानंतर,

मीयाअभ्यासातसहभागीहोण्यासाठीमाझीइच्छाव्यक्तकरतोआणित्यासमान्यतादेतो.

तारीख:

ठिकाण:

साक्षीदाराचाविषयस्वाक्षरी

KAHER'S KLE VK INSTITUTE OF DENTAL SCIENCES, BELAGAVI – 590010

TO COMPARE THE LEVELS OF TNF-ALPHA IN SERUM OF GESTATIONAL AND NON GESTATIONAL DIABETES PATIENTS WITH CHRONIC PERIODONTITIS – A CASE CONTROL STUDY.

ಆಯೋಜಕ : DR. ರತ್ನಾನಾಕೂರ್

ನಾನು, _____ ವಯಸ್ಸಿನ__ _____

ಅಧ್ಯಯನದಲ್ಲಿನನ್ನಪಾಲ್ಗೊಳ್ಳುವಿಕ

ಬಗ್ಗೆತಿಳಿಸಲಾಗಿದ

"

1) ನನ್ನವ್ಯಯಕ್ತಿಕವಿವರಗಳನ್ನುಹಸರು, ವಯಸ್ಸು, ಲಿಂಗ, ವಿಳಾಸಮತ್ತುನನ್ನಜ್ಞಾನದಅತ್ಯುತ್ತಮಅಧ್ಯಯನಕ್ಕಅಗತ್ಯವಿರುವವಿವರಗಳನ್ನುನೀಡಲುಒಪ್ಪುತ್ತೇನ.
2) ದಂತವೈದ್ಯರುನಡಸಬೇಕಾದಅವಧಿಯಪರೀಕ್ಷಗನಾನುಒಪ್ಪುತ್ತೇನ
3) ಅಧ್ಯಯನಕ್ಕರಕ್ತಮಾದರಿಗಳನ್ನುಸಂಗ್ರಹಿಸಲುನಾನುದಂತವೈದ್ಯರಿಗಅನುಮತಿನೀಡುತ್ತೇನ.
4) ನಾನುನೀಡದಮಾಹಿತಿಮತ್ತುಪ್ರಸ್ತುತಿಮತ್ತುಪ್ರಕಟಣೆಯಉದ್ದೇಶಕ್ಕಾಗಿಈಅಧ್ಯಯನದಿಂದಪಡದ ಫಲಿತಾಂಶಗಳನ್ನುಬಳಸಿಕೊಳ್ಳಲುನಾನುದಂತವೈದ್ಯರಿಗಅನುಮತಿನೀಡುತ್ತೇನ.
5) ಯಾವುದೇಏಜನ್ಸಿಯಪ್ರಾಯೋಜಕತ್ವವನ್ನುಹೊಂದಿದ್ದರೂಸಹ, ಅಧ್ಯಯನದಲ್ಲಿನನ್ನಸಹಕಾರಕ್ಕಾಗಿಯಾವುದೇಲಾಭವನ್ನುನಾನುಪಡದುಕೊಳ್ಳುವುದಿಲ್ಲ. ನನ್ನಸ್ವಂತಇಚ್ಛಮತ್ತುಇಚ್ಛಯೊಂದಿಗನಾನುಭಾಗವಹಿಸುತ್ತಿದ್ದೇನ.

6) ವೈದ್ಯರುನೀಡಿದಸೂಚನೆಗಳನ್ನುನಾನುಅನುಸರಿಸುತ್ತೇನೆ.

7) ಅಧ್ಯಯನದಸಮಯದಲ್ಲಿ, ನಾನುಅಧ್ಯಯನದಿಂದರಾಜೀನಾಮೆನೀಡಲುಬಯಸಿದರೆ, ನಾನುಹಾಗೆಮಾಡಲುಮುಕ್ತನಾಗಿರುತ್ತೇನೆಮತ್ತುನನ್ನಚಿಕಿತ್ಸೆಯುಇನ್ನೂಇಲಾಖೆಯಲ್ಲಿಪೂರ್ಣಗೊಳ್ಳುತ್ತದೆ.

ನನ್ನಸಂಪೂರ್ಣಪ್ರಜ್ಞೆಮತ್ತುಮನಸ್ಸಿನಉಪಸ್ಥಿತಿಯಲ್ಲಿ,

ನನ್ನದೇಶೀಯಭಾಷೆಯಲ್ಲಿಎಲ್ಲವಿವರಣೆಗಳನ್ನುಅರ್ಥಮಾಡಿಕೊಂಡನಂತರ,

ನಾನುಈಅಧ್ಯಯನದಲ್ಲಿಪಾಲ್ಗೊಳ್ಳಲುನನ್ನಒಪ್ಪಿಗೆಯನ್ನುನೀಡುತ್ತೇನೆ.

ದಿನಾಂಕ:

ಸ್ಥಳ:

ವಿಷಯದಸಹಿಸಾಕ್ಷಿಸ

ANNEXURE III - PROFORMA

KAHER'S KLE VK INSTITUTE OF DENTAL SCIENCES, BELGAUM–590010

DEPARTMENT OF PERIODONTICS

TO COMPARE THE LEVELS OF TNF-ALPHA IN SERUM OF GESTATIONAL DIABETES PATIENTS WITH CHRONIC PERIODONTITIS – A CASE CONTROL STUDY

Case No: **OPD No:**

Name:

Age: **Sex:**

Occupation:

Address:

Chief Complaint:

Medical history:

Dental history:

Personal habits:

Oral hygiene habits:

Awareness of oral hygiene: YES ☐ **NO** ☐

Brushing habits:

Type of brush:

Dentifrice:

Frequency:

Direction / Technique:

Dietary habits:

Maintenance of oral hygiene during pregnancy:

GESTATIONAL DIABETES MELLITUS PARAMETERS:		
Single time gestation:		
Pre pregnancy	Weight	
	Height	
Body mass index:		

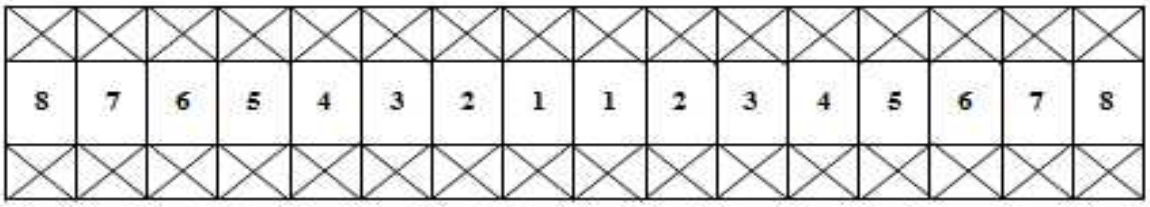
Gestational age at first prenatal care visit:	
Family history of diabetes or GDM:	YES / NO

OGTT – Oral Glucose Tolerance Test levels:	
Fasting levels(>95mg/dl):	mg/dl
1hour levels (>180mg/dl):	mg/dl
2 hour levels (>155mg/dl):	mg/dl

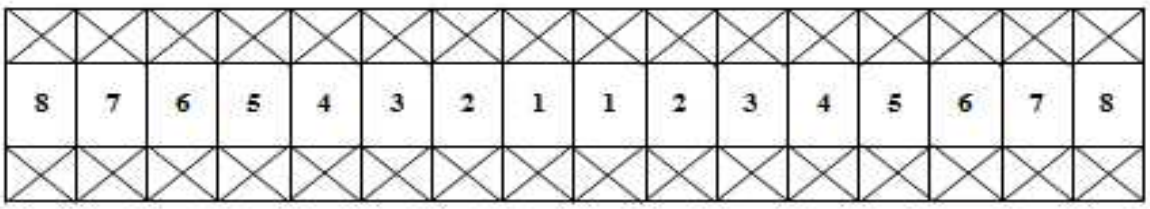
DENTAL PARAMETERS:

NO OF TEETH PRESENT:

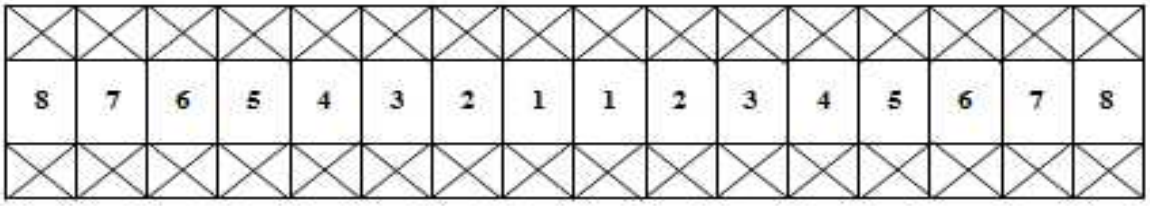
PLAQUE INDEX (PI) (Silness J and Loe H, 1964)



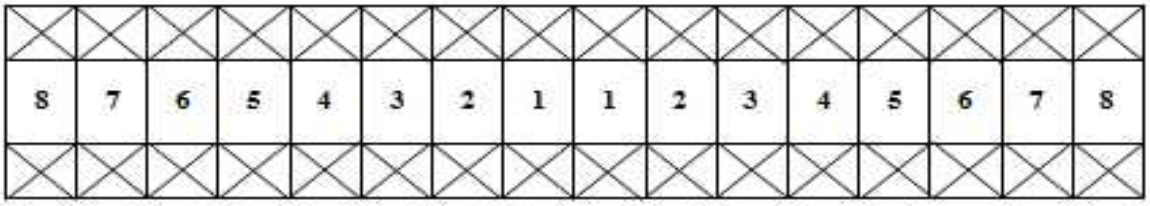
BLEEDING INDEX (BI) (Muhlemann H.R and Son S, 1971)



PERIODONTAL PROBING DEPTH (PPD)



CLINICAL ATTACHMENT LOSS (CAL)



LEVELS OF TNF-ALPHA IN SERUM :

ANNEXURE IV – MASTER CHART

Non-GDM group					
No	TNF - α	PI	GI	PPD	CAL
1	1.7	0.58	0.65	2.4	1.45
2	156	1.99	1.71	2.9	1.7
3	21	0.36	0.63	3.4	2.3
4	28	0.62	0.53	2.4	1.4
5	28	0.64	0.56	2.4	1.5
6	35.5	1.14	1.38	3.3	2.4
7	1.6	0.63	0.51	2.4	1.4
8	1.73	0.75	0.69	2.1	1.3
9	5.2	1.02	1.14	2.7	1.7
10	4.18	0.86	0.54	2.5	1.5
11	4.38	0.96	0.92	2.6	1.6

12	4.08	1.1	0.92	2	1.65
13	1.7	0.61	0.59	2.1	1.6
14	4.25	0.72	0.68	2.31	1.7
15	5.49	0.81	0.78	2.6	1.7
16	4.48	1.16	0.81	2.8	1.6
17	5.76	1.5	0.79	2	1.3
18	4.59	1.35	2.5	2.41	1.67
19	4.49	0.72	0.66	2.02	1.4
20	5.1	0.86	0.53	1.78	1.17
21	4.45	0.93	0.84	2.2	1.46
22	6.98	0.95	1.48	2.52	1.54
23	5.79	0.5	0.34	2.5	1.25
24	4.6	1.32	1.05	2.3	1.09
25	4	1.22	1.37	2.25	1.54
26	5.3	1.06	1.02	2.42	1.6
27	5.2	0.7	0.67	2	1.01
28	6.3	1.14	1.05	1.66	1.07
29	7.3	0.67	0.71	2.66	1.66
30	14.1	0.73	0.75	2.2	1.03
31	6.68	1.16	1.49	1.1	0.81
32	5.2	0.88	0.857	1.3	1.05
33	7.4	0.8	0.65	1.9	1.2
34	4.9	1.01	0.75	1.5	1.09
35	5.9	1.04	1.06	1.01	0.62
36	9.1	0.57	0.5	1.23	0.65
GDM group					

No	TNF - α	PI	GI	PPD	CAL
1	6.2	0.83	0.71	2.7	1.09
2	7.3	0.71	0.69	3.34	1.78
3	21.01	0.81	0.79	3.3	2.07
4	23	0.84	0.87	4.3	3.32
5	7.3	0.44	0.47	3.53	2.32
6	27	1.8	1.6	3.5	2.84
7	20.01	0.79	0.82	3.83	2.88
8	57.1	1.91	1.72	4.45	3.65
9	35	1.63	1.57	3.15	2.15
10	5	0.39	0.42	3.65	2.8
11	19.01	0.71	0.77	3.58	2.76
12	35.9	1.7	1.68	4.2	2.42
13	28.1	1.79	1.82	3	1.9
14	8.9	0.76	0.81	3.56	2.12
15	5.1	0.61	0.73	3.75	2.02
16	6.15	0.81	0.5	3.25	1.84
17	6.5	0.6	0.7	2.7	1.4
18	21.88	1.89	1.9	2.9	1.7
19	6.95	1.02	0.85	4.09	2
20	13.8	0.73	0.54	3.52	1.9
21	4.8	0.62	0.58	2.28	1.06
22	4.7	0.82	0.57	3	2.15
23	5.6	0.65	0.68	2.62	1.9
24	52.2	1.81	1.61	2.4	1.5
25	4.4	0.73	0.81	3.4	1.56

26	4.76	0.96	1.03	2.57	1.76
27	3.89	0.63	0.53	3.33	1.97
28	5.9	0.89	0.78	3.25	2.64
29	4.1	0.71	0.65	3.73	2.16
30	13	1.71	1.68	2.19	1.48
31	5.9	0.89	0.78	3.25	2.64
32	4.1	0.71	0.65	3.73	2.16
33	6.2	0.81	0.76	4.21	3
34	5.4	0.5	0.42	2.8	1.7
35	4.28	1.001	1.16	3.12	1.4
36	3.69	0.66	0.64	5.02	3.04