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### RESEARCH ARTICLE

#### BETA GLUCURONIDASE ACTIVITY IN DIABETES ASSOCIATED PERIODONTITIS

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#### Abstract

Diabetes mellitus is a risk factor for development of periodontal disease with progressive periodontal destruction seen in diabetic patients. Advances in oral and periodontal disease diagnostic research are moving towards methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers. The aim of this study was to identify the presence of beta glucuronidase activity as an enzymatic biomarker of periodontal tissue destruction in patients of Diabetes mellitus associated with Periodontitis.  $\beta$  Glucuronidase activity was estimated in saliva, GCF (Gingival Crevicular Fluid) and serum in healthy subjects and patients with Diabetes mellitus, Periodontitis and Diabetes associated periodontitis. Enzyme activity was compared with clinical parameters like Probing pocket depth, Plaque Index and Gingival Index. It was maximally raised in diabetic cases with periodontitis. Enzyme activity was found to be highest in crevicular fluid compared to saliva and serum. However, serum Glucuronidase correlated significantly with clinical indices.  $\beta$ -glucuronidase may be employed on routine basis as a chair side test for screening and diagnosis of patients with periodontitis in diabetics.

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#### Introduction:-

The oral cavity provides a continuous source of infectious agents, and its condition often reflects progression of systemic pathologies. Historically, oral infections were thought to be localized to the oral cavity except in the case of some associated syndromes and untreated odontogenic abscesses. A change in paradigm has dispelled this notion, and a whole new concept of the status of oral cavity and its impact on systemic health and disease has been evolved. [1]

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting from the defects in insulin secretion, insulin action or both. Type 1 diabetes results from autoimmune destruction of insulin-producing  $\beta$ -cells in the pancreas, leading to total loss of insulin secretion. Type 2 diabetes results from insulin resistance rather than from total absence of insulin production. Patients with type 2 diabetes can remain undiagnosed for years because hyperglycemia appears gradually and often without symptoms. Insulin resistance results in a decreased capacity to transfer glucose into target cells; thus, hyperglycemia develops. [2]

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Long-term diabetic oral complications include microvascular diseases like Xerostomia, greater susceptibility of oral tissues to trauma, more opportunistic infections (e.g., candidiasis), greater accumulation of plaque, greater risk of caries, greater susceptibility to periodontal disease, greater risk of developing periodontal abscesses when periodontitis is present, delayed healing. [3]

Gingival and periodontal diseases, in their various forms, have afflicted mankind since the dawn of history. [4] Periodontal disease is an infection that affects periodontium, the tissue that supports the teeth. It's a chronic inflammatory disease of bacterial ideology. [5], [6] Periodontitis is much more than a localized oral infection. [7] Since periodontitis is asymptomatic, the affected subjects are largely unaware and refrain from periodontal treatment. [8]

The association between periodontal disease and diabetes has been explored in several studies over the years, and it is generally accepted that periodontal disease is more prevalent and more severe in persons with diabetes than in nondiabetic persons. Indeed, the periodontal signs and symptoms are now recognized as the "sixth complication" of diabetes. [6]

Although, the high prevalence of periodontitis in diabetics is cognizant by the dental professionals, it is not a well-known fact in the medical community. Periodontitis should receive due attention as a "pandemic" by the respective national and world health governance. Conventional methods for diagnosing periodontal disease measure the damage from the past episodes of destruction as having experienced a significant anatomic event. [9] Advances in oral and periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers. [10] Biochemical analysis may include detection of specific inflammatory markers, proteolytic and hydrolytic enzymes, cell death and connective tissue degradation products and bone-related biomarkers.

The purpose of the study was to identify the presence of beta glucuronidase activity as an enzymatic biomarker of periodontal tissue destruction and to correlate levels in the three oral fluids saliva, GCF and serum with the severity of periodontal disease in diabetic patients.

#### **Literature Survey:**

Diabetes is the commonest metabolic disorder and its incidence is on the increase all over the world. Periodontal disease has been labeled as the "Sixth Complication" of diabetes. The oral cavity frequently undergoes changes that are related to the diabetic condition, and oral infections may adversely affect metabolic control of the diabetic state. [2] Diabetes is a risk factor for development of periodontal disease and there is a significant progressive periodontal destruction in diabetic patients. Periodontitis is a "low grade infection" capable of developing a "low grade systemic inflammation" with an ability to influence the general systemic health. Very often, course of periodontal disease is modified by the systemic disorder of patients. Diabetes mellitus plays an important role in the etiology of periodontal disease. Uncontrolled diabetic patients frequently show a combination of inflammatory and degenerative changes ranging from a mild gingivitis to advanced chronic periodontitis with a widened periodontal ligament, exudation from periodontal pockets, and/or multiple lateral periodontal abscesses. Diabetic patients with periodontal disease may have increased risk of diabetic complications. [7] Studies have proved that control of periodontal infection has an impact on improvement of glycemic control evidenced by a decrease in demand for insulin and decreased Hemoglobin A<sub>1C</sub> levels. [1] The increased susceptibility of diabetic patients to periodontal breakdown may be due to an abnormal neutrophil functioning. Within the gingival crevice, Neutrophils play an important role in the innate cellular immune response, ingesting bacteria and secreting proteolytic enzymes. [11] During inflammation seen in gingivitis and periodontitis, the extracellular release of Polymorphonuclear leukocytes in the host gingiva may contribute significantly to the degradation of the gingival tissues and the pathogenesis of the periodontal disease. [12]

Both diseases are thought to share a common pathogenesis that involves an enhanced inflammatory response which can be observed at the local and systemic level. The inflammatory response is mainly caused by the chronic effects of hyperglycemia and specifically the formation of biologically active glycated proteins and lipids that promote inflammatory responses. [1]

Periodontitis is not only the result of adverse microbial activity but as an interaction among various other factors like genetics, systemic health, immunity, environmental factors like tobacco and stress. The above mentioned factors

play an important role in the modification of host response to the disease process. [2] In addition to the local destruction, this inflammation involves increased permeability of the capillaries leading to potential portals to the systemic circulation for the inflammatory mediators as well as the products of the bacterial infection. [5] The balance between the protective host factors and microbial challenge is greatly influenced by environmental and genetic factors that have an impact on the immuno-inflammatory response of the host. [13]

Traditional diagnostic procedures are inherently limited, in that only disease history, not current disease status, can be assessed. Advances in diagnostic research in oral and periodontal disease are moving toward methods whereby periodontal risk can be identified and quantified by objective measures (e.g., biomarkers). [14] Extending the diagnostic procedures and treatment monitoring by methods based on determination of the level of inflammatory mediators in gingival crevicular fluid or in saliva may facilitate diagnosis, increase treatment effectiveness, and provide data on the patho-mechanism of periodontal diseases.

Gingival fluid has been extensively investigated for the release of host response factors. [10] There are more than 50 indicators of inflammatory and immune response identified in this fluid. Sampling of GCF is relatively easy, non-invasive, convenient, and efficient means to sample biomarkers of inflammation and bone resorption in the oral cavity. [14]

The response of an organism to periodontal infection includes production of several enzymes released from stromal, epithelial, inflammatory, or bacterial cells. These intracellular enzymes are released increasingly from the damaged cells of periodontal tissues into the gingival crevicular fluid (GCF) and saliva.

Interest in Saliva as a diagnostic medium has advanced exponentially in the last 10 years. Saliva has been used as a diagnostic fluid in medicine and dentistry. [15] Saliva composed of water, electrolyte and organic molecules like amino acids, peptides, proteins, glycoprotein and glycolipid is derived from local vasculature originating from carotid arteries. Saliva contains biomarkers derived from serum, gingival crevicular fluid and mucosal transudate. Salivary components for periodontal diagnosis include enzymes and immunoglobulins, hormones of host origin, bacteria and bacterial products, ions, and volatile compounds. [16] Recent studies have shown that these enzymes and components can be quantified easily in saliva sample. [9] Salivary diagnostics is an emerging field in Proteomics.

Despite of increased understanding of etiology and pathogenesis of periodontal infections the diagnosis and classification of this disease is still based almost entirely on traditional clinical assessment. [17] A problem central to periodontology is inability to detect actively deteriorating sites and highly susceptible patients. [18] Conventional disease diagnosis techniques lack the capacity to identify highly susceptible patients who are at risk for future breakdown. Both automated periodontal probes and subtraction radiography techniques are most often seen in the research setting and seldom in clinical practice. Researchers are confronted then with the need for an innovative diagnostic test that focuses on the early recognition of the microbial challenge to the host. [19]

Both saliva and GCF being non-invasive and having simple collection procedures, their analysis may be especially beneficial in the determination of current periodontal status and may act as a means of monitoring response to treatment. [10]

In the present study, we have attempted to study the association of diabetes mellitus with periodontal disease using  $\beta$  Glucuronidase activity as means of diagnostic test.

## **Methodology/Approach: -**

### **Aim:**

To detect the efficacy of Beta Glucuronidase activity as an enzymatic biomarker of periodontal tissue destruction, aiding in diagnosis of periodontitis in patients with Diabetes mellitus.

### **Objectives:-**

1. To estimate and compare the levels of Beta Glucuronidase in saliva, GCF and serum in healthy subjects and patients with Diabetes mellitus, Periodontitis and Diabetes associated periodontitis.
2. To determine the most appropriate biological fluid as an index of periodontitis.

3. To assess whether this biomarker is a suitable indicator of clinical indices of periodontitis
4. To study the association of diabetes mellitus with periodontal disease using  $\beta$  Glucuronidase activity as means of diagnostic tests.

## **Materials and methods: -**

### **Subject selection:**

The study was conducted at the Department of Biochemistry, MGM Medical College Navi Mumbai and Periodontology OPD of MGM Dental College, Navi Mumbai. Ethical clearance was obtained from the Ethical committee of the institute, and a written informed consent was obtained from all the participants prior to the study.

A total of 40 subjects were equally categorized into four groups: Group-I (10 Healthy individuals), Group-II (10 Type-2 Diabetes without Periodontitis), Group-III (10 Periodontitis without Type-2 Diabetes) and Group-IV (10 Type-2 Diabetes with Periodontitis).

### **Inclusion criteria:**

1. Patients under diabetic treatment or had been diagnosed diabetes mellitus for at least last one year.
2. Patients diagnosed clinically as having periodontitis.
3. Not having any other systemic diseases.
4. Not having any history of diabetic complications like neuropathy, nephropathy, retinopathy etc.
5. Not undergone any periodontal treatment since last one year.
6. Willingness to participate in the study.

### **Exclusion criteria:**

1. Age less than 30yrs.
2. History of systemic disease with high liver enzyme activities
3. Medical therapy apart from antidiabetic treatment
4. Patients with diabetic complications such as neuropathy and retinopathy.
5. Smokers and ex-smokers.

The periodontal involvement was confirmed when presence of hard deposits or inflammation or bleeding on probing was observed.

### **Clinical parameters:**

The relevant clinical history was recorded for all the patients. A careful oral examination was carried out with the help of mouth mirror and graduated periodontal probe and following clinical parameters were recorded.

#### **a. Probing Pocket Depth (mm):**

It is the distance between the base of the pocket and the gingival margin. This distance is measured with a William's graduated periodontal probe held parallel to the vertical axis of the tooth and walking it circumferentially around each surface of each tooth. The area of deepest penetration is recorded for each individual tooth.

#### **b. Plaque Index:**

Plaque Index was assessed by Sillness and Loe, 1967 scoring criteria.

#### **Criteria for plaque index:**

1. No plaque in the gingival area
2. A film of plaque adhering to the gingival margin and adjacent area of the tooth. The plaque may be recognized only by running a probe across the tooth surface.
3. Moderate accumulation of soft deposits within the gingival pocket and on tooth, gingival margin and / or adjacent tooth surface which can be seen by the naked eye.
4. Abundance of soft deposits within the gingival pocket and / or on the gingiva and adjacent tooth surface

The plaque index score for a tooth was obtained by totaling the scores of all the four surfaces and dividing it by the number of surfaces examined. The plaque index score per person was obtained by adding the plaque index scores of each tooth and dividing by the number of teeth examined. Scores range from 0 to 3.

Rating	Scores
Excellent	0
Good	0.1 – 0.9
Fair	1.0 – 1.9
Poor	– 3.0 .1

**c. Gingival Index:**

Gingival Index was assessed by Sillness and Loe, 1963 scoring criteria.

**Criteria for gingival index:**

1. Normal gingiva
2. Mild inflammation, slight change in colour, slight oedema, no bleeding on probing
3. Moderate inflammation, redness, oedema, glazing and bleeding on probing
4. Severe inflammation, marked redness, oedema, ulceration and tendency to bleed spontaneously

Totaling the scores of each scoring unit and dividing it by 4 gives the gingival index score for a tooth. Totaling the scores of each tooth and dividing by the number of teeth examined provides gingival index score per person.

Gingival scores	Condition
0.1 - 1.0	Mild gingivitis
1.1 - 2.0	Moderate gingivitis
2.1 - 3.0	Severe gingivitis

**Sample collection and preparation:****Collection of Saliva:**

Approximately 1.0 ml of non-stimulated saliva samples were collected into sterile Eppendorf tubes which were homogenized by gentle mixing and centrifuged at 3000 rpm for 10 min. 5 µL of supernatant was then transferred to another sterilized Eppendorf tube containing 250 µL of phosphate buffer (pH-7) and stored at -20°C until it was analyzed for salivary enzymes.

**Collection of blood:**

Venous blood was collected under strict aseptic conditions in fluoride bulb for plasma and in plain bulb for serum. Plasma was collected after an overnight fast and two hours after meal for the fasting and postprandial blood glucose estimation. Serum was used for enzyme assays.

**Collection of Gingival Crevicular fluid:**

The subjects were seated comfortably in an upright position in the dental chair, with adequate lighting condition. The sites were chosen from maxillary teeth to avoid contamination with saliva. Supra gingival plaque and calculus were removed with hand instrumentation prior to the collection of GCF. The calibrated microcapillary tubes (0- 5- 10 µL range) were placed extracrevicular at the mesiofacial, distofacial, or midfacial surface of the tooth. A standardized volume of 5 µL of GCF was collected. Microcapillary tubes contaminated with blood were discarded.

The collected GCF was immediately transferred with a jet of air pressure from the capillary tube into the sterilized Eppendorf tube containing 250 µL of phosphate buffer (pH-7) and stored at -20°C until it was analyzed.

**β Glucuronidase Assay:**

β Glucuronidase activity was determined by the method of Fishman et al using phenolphthalein glucuronide as substrate. [20], [21] The assay system consisted of 0.2 ml of acetate buffer (0.2M, pH 5.0), 1.0 ml of glycine buffer (0.6M, pH 11.7), 0.1 ml of sample, 0.1 ml of phenolphthalein glucuronide [15 mM] as substrate with appropriate serum and reagent blanks. After incubation at 37°C for 2 hours, absorbance was read at 546 nm in UV Spectrophotometer against water blank. Standard curve was plotted. The activity is expressed in Mili Units.

**Results:-****Table 1:-** Descriptive Statistics for β Glucuronidase in all Fluids.

Parameter	Group	Salivary Enzymes	GCF Enzymes	Serum Enzymes
β Glucuronidase (mili Units)	Control Group	0	5.02	.260
	Diabetes	2.18	9.17	2.450
	Periodontitis	4.38	17.32	1.670
	Diabetes + Periodontitis	7.29	36.07	3.550

**Table 2:-** Multiple Comparisons of β glucuronidase between control and study groups (Using Tuckey's POST HOC Test).

Dependent Variable	Group	Group	p-value
Salivary β Glucuronidase	Control Group	Diabetes	.032*
		Periodontitis	< 0.010**
		Diabetes with Periodontitis	< 0.010**
	Diabetes	Periodontitis	.030*
		Diabetes with Periodontitis	< 0.010**
	Periodontitis	Diabetes with Periodontitis	.003**
GCF β glucuronidase	Control Group	Diabetes	.923
		Periodontitis	.265
		Diabetes with Periodontitis	< 0.010**
	Diabetes	Periodontitis	.612
		Diabetes with Periodontitis	.001**
	Periodontitis	Diabetes with Periodontitis	.037*
Serum β Glucuronidase	Control Group	Diabetes	< 0.010**
		Periodontitis	0.002**
		Diabetes with Periodontitis	< 0.010**
	Diabetes	Periodontitis	0.160

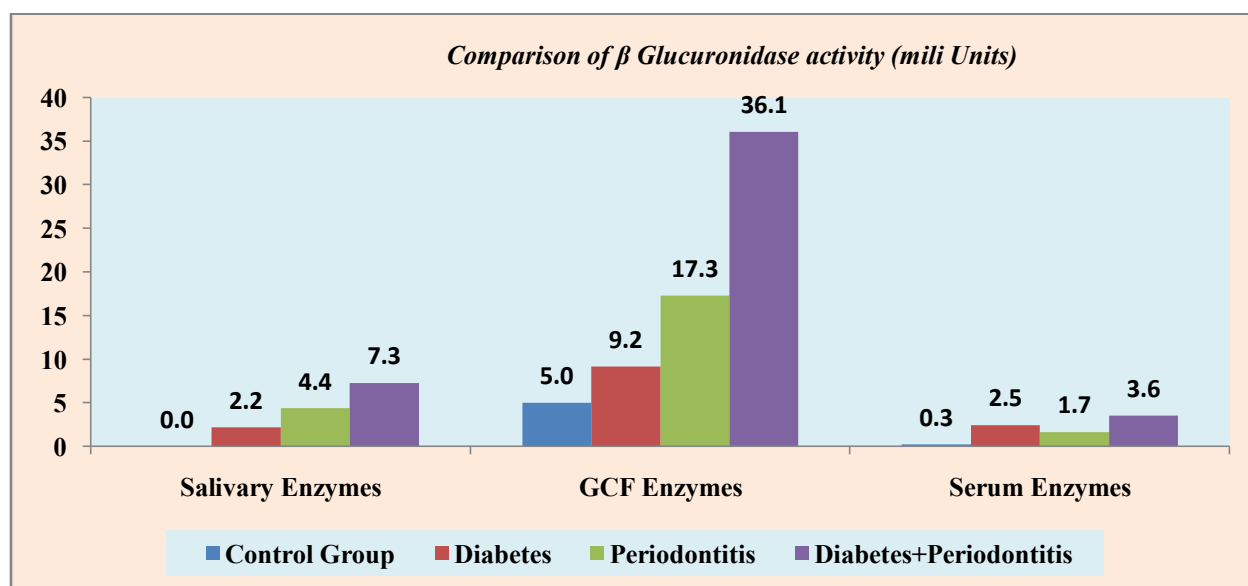
		Diabetes with Periodontitis	0.023*
	Periodontitis	Diabetes with Periodontitis	< 0.010**

\*:p value  $\leq 0.05$ : fairly significant; \*\*:p value  $\leq 0.01$ : highly significant

**Table 3:-** Correlation between Clinical Parameters and Glucuronidase activity in all Fluids.

	PPD		PI		GI	
Parameter	CC	p-value	CC	p-value	CC	p-value
Saliva Glucuronidase	0.443	0.05*	-0.022	0.93	0.164	0.49
GCF Glucuronidase	0.409	0.07	0.3	0.19	0.154	0.51
Serum Glucuronidase	0.445	0.05*	0.503*	0.02*	0.44	0.05*

CC: Correlation Coefficient; \*:p value  $\leq 0.05$ : fairly significant; \*\*:p value  $\leq 0.01$ : highly significant



**Fig 1:-**

### Discussion:-

Diabetes mellitus is a risk factor for development of periodontal disease with progressive periodontal destruction seen in diabetic patients. Periodontal diseases are "silent" in nature; most patients do not realize they have such conditions until significant destruction has occurred. [22] Hence accurate detection of periodontal sites exhibiting disease progression or those at risk of future deterioration has always proven difficult. [23]

Traditional diagnostic procedures are inherently limited, in that only disease history, not current disease status, can be assessed. These conventional disease diagnosis techniques lack the capacity to identify highly susceptible patients who are at risk for future breakdown. [19] Advances in diagnostic research in oral and periodontal disease are moving towards methods whereby periodontal risk can be identified and quantified by objective measures by so called biomarkers. [14] Therefore we have attempted to study the levels of Glucuronidase in all the three fluids that are saliva, gingival crevicular fluid as well as serum and correlated them with clinical parameters, in order to establish both a suitable biomarker for periodontitis and to assess the suitable body fluid for its assay.

$\beta$ -glucuronidase is a lysosomal acid hydrolase enzyme which is capable of breaking down connective tissue ground substances. It is released during the inflammatory process by the degranulation of polymorphonuclear leukocytes (PMN). It has been used as a potential marker for periodontal disease activity. This acid glycohydrolase is used as a marker for primary granule release from polymorphonuclear leukocytes (PMN) and in GCF has been correlated with the number of PMN in gingival crevice. [24]

During active phase of periodontal disease there is influx of PMN cells into the gingival crevice. This denotes the body's self-defense against bacterial products and bacteria. Six fold increases in  $\beta$ -Glucuronidase activity has been found in periodontal diseases. [24] The activity of  $\beta$ -Glucuronidase is associated with severity of inflammation and also with the presence of putative pathogenic flora. This enzyme has been detected in GCF of patients with active site of periodontal disease, thus making  $\beta$ -Glucuronidase as an important biochemical marker for disease activity [25]

In our study, salivary  $\beta$ -Glucuronidase activity was not detected in control group but highest levels were found in Group IV ( $7.29 \pm 3.12$ ) as seen in table no.1. When salivary  $\beta$  Glucuronidase levels were compared between all four groups, significant increase was found in all study groups, with highest found between group IV and other groups ( $p < 0.01$ ) as seen in table no. 2

GCF  $\beta$ -Glucuronidase in study groups was higher than that in control group and was highest in Group IV as can be seen from table no.1. When the levels were compared between all four groups we found that diabetic patients and patients with periodontitis did not differ statistically within themselves and from healthy individuals. Patients with diabetes associated periodontitis showed highly significant increase in  $\beta$ -Glucuronidase levels when compared with control and diabetic groups ( $p < 0.01$ ) whereas fairly significant increase was observed when they were compared with the patients having periodontitis alone ( $p < 0.05$ ) as seen in table no.2.

The serum  $\beta$  Glucuronidase levels represented in table no.1 were increased in study groups compared to controls. This enzyme was noted to be highest in patients of group IV. When this enzyme levels in serum of all four groups were compared, diabetic subjects did not differ statistically from periodontitis group. Whereas the levels showed statistically significant differences in rest all the groups (table no.2)

Thus in the present study, there was a high level of  $\beta$ -glucuronidase activity in saliva and GCF compared to serum with highest activity in GCF as seen in table no.1 and figure no.1. Diabetes with Periodontitis patients had the highest  $\beta$ -glucuronidase activity in GCF. In control group (periodontal healthy subjects), low levels of  $\beta$ -glucuronidase activity was demonstrated in GCF, which was similar to the findings of Aarti et al and Lamster et al. [25] They observed that even in periodontal healthy individuals, some amount of subclinical inflammation was always present.

Serum  $\beta$ -glucuronidase levels correlated significantly with clinical indices; PPD ( $p=0.05$ ) and with PI ( $p = 0.024$ ) as seen in table no.3

There was higher level of salivary and GCF  $\beta$ -glucuronidase activity in patients of diabetes with periodontitis as compared to non-diabetic with periodontitis. Thus our findings are in accordance with study by Bacic et al., [25] who showed that periodontal disease is more frequent and severe in diabetics as compared to non-diabetics. Aarti et al demonstrated that there was higher level of GCF glucuronidase activity in patients of diabetes with chronic periodontitis as compared to non-diabetic with chronic periodontitis

Increased GCF  $\beta$ -glucuronidase activity may be due to hyperactivity and increased deregulation of lysosomes of polymorphonuclear leucocytes in diabetes mellitus. Hayden and Buckley [25] demonstrated that in diabetics with periodontitis, other than impaired glucose metabolism, genetic predisposition plays an important role in the progression of disease. Cimasoni and co-workers examined the lysosomal enzyme activity in gingival crevice and found a threefold increase in glucuronidase level from baseline during the course of progression of gingivitis. [18]

A recent cross-sectional study done by Lamster to determine the association between salivary  $\beta$ -glucuronidase activity and the whole-mouth clinical periodontal parameters, complete blood count, smoking status, and age demonstrating various levels of periodontal disease and the results suggested that a significant association exists between periodontal clinical parameters and salivary Glucuronidase activity, which is similar to role played by glucuronidase in gingival crevicular fluid [26]

### Conclusion:-

Oral fluids reflect the pathomechanism of periodontal diseases. They act as better diagnostic medium compared to blood as increased level of  $\beta$ -glucuronidase activity was observed in saliva and GCF compared to serum with highest activity in GCF.



Oral health assessment and treatment may be considered as common as the eye, foot, and kidney evaluations that are routinely performed as part of preventive medical therapies in Diabetes mellitus.

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