

## GLYCATED HEMOGLOBIN: LINK BETWEEN DIABETES AND PERIODONTICS

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### Abstract

Diabetes mellitus (DM) is a complex metabolic syndrome manifested by abnormally high levels of glucose in the blood. It is now recognized that chronic hyperglycemia leads to long term damage to different organs including the heart, eyes, kidney, nerves and vascular system. In recent years, a bilateral relationship has been postulated between periodontitis and systemic conditions. One such condition is diabetes mellitus. Several studies have demonstrated a close relationship between diabetes mellitus and chronic periodontitis. Either of them may have effect on the other. Early diagnosis with strict control of blood glucose level is essential and of utmost importance in preventing or reducing the numerous complications associated with diabetes mellitus including periodontitis. Glycosylated haemoglobin assay, a novel modality of testing blood glucose level, does not require fasting and does not rely on patient's compliance and gives an indication of blood glucose level over an extended period of time.

So, various articles were analysed and interpreted to assess the bilateral relationship between diabetes mellitus and periodontitis through the assessment of a metabolic parameter i.e. glycated haemoglobin (HbA1c). Numerous studies quoted that scaling and root planning resulted into reduction in clinical parameters as well as in HbA1c level which were statistically significant. It indicates a positive correlation between the improvement in periodontal status and reduction in HbA1c level after periodontal therapy (SRP). So, periodontal treatment regimen should be included in the overall management of the diabetic patients.

**Key Words:** - Diabetes mellitus, Glycated haemoglobin, Advanced glycation end products, Inflammatory mediators, Adipocyte metabolism, Non-surgical periodontal therapy.

### Introduction

Diabetes mellitus (DM) is a clinically and genetically heterogenous group of metabolic disorders manifested by abnormally high levels of glucose in the blood. It is a syndrome and it is, now recognized that chronic hyperglycemia leads to long term damage to different organs including the heart, eyes, kidney, nerves and vascular system.<sup>1</sup> Diabetes mellitus is an extremely common metabolic disorder affecting approximately 4% of the population, almost half of those being unaware of the condition.<sup>2</sup>

There are several etiologies for diabetes and although establishing the type of diabetes for each patient is important, understanding the pathophysiology of various forms of disease is the key to appropriate treatment.<sup>1</sup>

In recent years, there has been an emerging interest in the link between the periodontal disease and systemic conditions. Periodontal disease has been cited as the 6<sup>th</sup> complication of diabetes mellitus.<sup>2,3</sup>

Oral complications of diabetes have been widely described and include an increased incidence and severity of periodontitis. In addition to periodontitis, uncontrolled or poorly controlled diabetics are at an increased risk for a variety of systemic complications including micro and macroangiopathy, neuropathy and nephropathy.<sup>4,5</sup>

Early diagnosis with strict control of blood glucose level is imperative in preventing or mitigating the numerous complications associated with diabetes mellitus including periodontitis.<sup>6</sup> <sup>7</sup>Historically, the primary methods used to diagnose diabetes mellitus and monitor blood glucose levels

have been the fasting blood glucose and oral glucose tolerance tests.<sup>5</sup> While accurate, these tests require fasting by the patient, tend to be highly dependent on patient compliance and are useful only for the immediate time period in which the test is administered.<sup>8,9</sup>

Over a last few years, a glucose assay has been developed which is known as glycosylated haemoglobin assay. This assay offers important advantages over traditional testing methods. Glycosylated haemoglobin assay does not require fasting and does not rely on patient's compliance and gives an indication of blood glucose level over an extended period of time.<sup>10</sup>

The glycosylated haemoglobin assay was originally used only to monitor the blood glucose levels of diabetics but with improvements in the assay, it currently affords an additional means of diagnosing the diabetes.

### Definition

The process of non-enzymatic addition of carbohydrate to polypeptides and proteins is called glycosylation/ glycation and the products that are formed are called as advanced glycated end products (AGE's).

Numerous proteins in the body including collagen are capable of being glycated. AGE's are heterogenous class of compounds such as: Carboxymethyllysine, Pyralline, Pentosidine etc.

### Formation

Advanced glycated end products are non-enzymatically glycated proteins and lipids which can form spontaneously in healthy individuals. However, a chronically elevated

glucose level leads to accelerated AGE formation and accumulation, especially in vessel walls.

Glycohemoglobin is formed continuously in erythrocytes as a product of the non-enzymatic reaction between the haemoglobin protein, which carries oxygen molecules, and glucose. Binding of glucose to haemoglobin is highly stable; thus haemoglobin remains glycosylated for the life span of the erythrocytes, approximately 120 days. There are other serum proteins like albumin beside haemoglobin that becomes glycosylated in the presence of hyperglycemia but because of their shorter half-life, can give the impression of glycosylation for two to three weeks only.<sup>11</sup>

### **Pathogenic Mechanisms**

Traditionally, diabetics have been considered more susceptible to severe periodontal disease due to metabolic and host response imbalances that result in impaired wound healing, microangiopathy and diminished salivary flow.<sup>12</sup> More recently, defects in neutrophil function have been cited as possible mechanisms for the increased severity of periodontal disease associated with diabetes mellitus. The relationship between Diabetes and Periodontal disease can be described under the following heads:<sup>13</sup>

1. Epidemiological basis
2. Biologic basis
3. Clinical basis

### **Epidemiological Basis**

It is generally accepted that periodontal disease is more prevalent and more severe in individuals with diabetes than in non-diabetics. Both epidemiological studies and case reports have shown diabetes to be a major risk factor for periodontitis. These and other controlled studies reported at least a 2-fold increase in the risk of or periodontal disease in diabetics when compared with healthy controls.<sup>14,15</sup>

Various studies have concluded that both type 1 and type 2 DM are predictors of periodontal disease and periodontal disease is considered an additional complication of DM.

It has been proposed that periodontal therapy for uncontrolled diabetes may reduce their insulin requirement and improve their metabolic control. Stewart *et al.* designed a study to investigate the effect of periodontal therapy on glycemic control in subjects with type-2 DM. The treatment included oral hygiene instructions, full mouth scaling, root planning, subgingival curettage under local anaesthesia and extractions of teeth deemed unsalvageable. The results of this study suggested that following periodontal therapy, there was a marked improvement in glycemic control in individuals with type 2 DM when compared to the non-treatment control group.

Grossi and co-workers studied the effects of periodontal treatment along with doxycycline on the level of glycemic metabolic control in 113 Pima Indians suffering from type 2 DM and periodontal disease. All groups showed clinical and microbial improvement with the doxycycline treated groups showing the greatest probing depth reduction and

subgingival *P. gingivalis* reduction as compared to the control group. This study, thus, concluded that effective treatment of periodontal infection and reduction of periodontal inflammation was associated with the reduction in the level of glycosylated hemoglobin.

It should be noted that doxycycline which has antimicrobial and anti-collagenase activity, may be the reason for the improved results rather than periodontal therapy per se. It has been hypothesized that TNF- $\alpha$  produced due to periodontal inflammation and from adipose tissues synergistically affects insulin resistance as a result of which there was a significant reduction in HbA1c values and the number of microorganisms in periodontal pockets secreting TNF- $\alpha$ .

### **Biological Basis**

Different researchers have correlated in the background of biologic basis, the effect of hyperglycemia on the tissues.

#### *Effects of hyperglycemia*

Periodontitis is a multifactorial disease. The development of periodontitis is dependent on many factors, like the microbial challenge and genetic risk factors, environmental and acquired risk factors.

### **Cellular response**

Polymorphonuclear leukocyte (PMN) is important in the maintenance of gingival and periodontal health. They are the first line of host's defense mechanism in the inflammatory process. They are primarily protective against periopathogenic bacteria and play a role in periodontal wound healing. However, there is an impaired PMN function in patients with diabetes, e.g.- defects in PMN functions like chemotaxis, phagocytosis, intracellular bactericidal activity and serum opsonin activity, thus reducing the diabetic patient's ability to combat infections, including periodontal infections.

Thus, impaired neutrophil functions rather than the control of glucose abnormalities, predisposes diabetic patients to the development of periodontitis.

The advanced periodontal disease in diabetics has been ascribed to a number of structural and functional hyperglycemia related alterations. In a hyperglycemic environment, numerous proteins including collagen undergo a non-enzymatic glycosylation process to form advanced glycosylated end products (AGE's). Formation of these stable carbohydrate-containing proteins is a major link between the various diabetic complications.<sup>16</sup>

AGE formation changes the function of numerous extracellular matrix components affecting collagen stability and vascular integrity. AGE formation on collagen causes increased cross linking between collagen molecules resulting in reduced solubility and decreased turnover rate.

The AGE mediated events are of primary importance in the pathogenesis of diabetic complications. In the blood vessel wall, advanced glycosylated-end-product-modified collagen

accumulates resulting into thickening of the vessel wall and narrowing of the lumen. This modified vascular collagen can immobilize and covalently cross-link circulating low-density lipoprotein, causing an accumulation of low-density lipoprotein and contributing to atheroma formation in the large blood vessels. Hyperglycemia causes thickness of vascular basement membranes (microangiopathy), which leads to deterioration of the microcirculation in the periodontal tissue and consequently to decreased supply of oxygen and nutrition to the tissues and accumulation of harmful metabolites and migrations of leukocytes. Advanced glycated end-product formation occurs in both central and peripheral arteries, and is thought to contribute greatly to macrovascular complications of diabetes, in part by up-regulation of vascular adhesion molecules.

Monocytes, macrophages and endothelial cells possess high affinity receptors for AGEs. Binding of AGE to macrophages and monocyte receptors results in a hyper-responsive cellular state, which leads to increased cellular oxidant stress and activation of transcription factor nuclear factor- $\kappa$ B, altering the phenotype of the monocyte/macrophage and resulting in increased production of pro-inflammatory cytokines such as IL-1, Insulin like growth factor, MMP's and TNF- $\alpha$ , and may therefore, be a possible candidate mechanism to explain severe expression of periodontal disease in diabetics.<sup>2,3</sup> This is evident by the use of doxycycline which has anti-collagenase activity, improvements in periodontal health have been observed in diabetic patients.

Collagenase activity is increased in the gingival tissues of diabetic patients as well. This could partly explain why patients given doxycycline showed improved periodontal health.

### **Microbiological response**

The bacteria involved in periodontitis are usually anaerobic gram negative bacteria. The pathogenic effect of gram negative bacteria which causes direct and indirect damage to the periodontal supporting tissues via its toxic products and activation of a series of inflammatory reactions is well documented. Gram negative bacteria derived lipopolysaccharide (LPS) is also considered a potent inducer of TNF- $\alpha$  from monocytes and macrophages. LPSs are endotoxins present in the bacterial cell walls of the periodontal pathogens.<sup>13</sup>

#### Role of TNF- $\alpha$

TNF- $\alpha$  has been reported to be a strong candidate responsible for insulin resistance.<sup>13</sup>

In a normal situation, when insulin binds to the insulin receptor on muscle and fat cells, a tyrosine residue of cytoplasmic domain of the insulin receptor is autophosphorylated. A signal is transduced and translocated to the cell membrane to take up glucose. However, in the presence of TNF- $\alpha$ , this process is inhibited, thus influencing glucose uptake by cells resulting in insulin resistance.<sup>13</sup>

TNF- $\alpha$  is the major cytokine responsible for inducing insulin resistance at receptor level. It prevents auto-phosphorylation of the insulin receptor and inhibits second messenger signalling the inhibition of the enzyme tyrosine kinase. Thus, a signal which is transduced and translocated to the cell membrane to take up glucose is inhibited, thereby, resulting in insulin resistance.<sup>17</sup>

The increased cytokine levels (IL-6, TNF- $\alpha$ ) also lead to increased c-reactive production, which may impact insulin resistance as well.

### **Clinical Evidence**

The clinical evidence that supports a bilateral relationship between periodontal disease and diabetes can be reinforced as following:

1. Role of infection in glycemic control
2. Response of DM patients to conventional periodontal therapy
3. Effects of periodontal therapy on glycemic control

### **Role of infection on glycemic control**

Acute infections alter the endocrinologic metabolic state leading to difficulty in controlling blood sugar and increased insulin resistance.<sup>18</sup>

Grossi *et al.* suggested that chronic gram negative infections and chronic endotoxemia, like those in such periodontal disease, resulted in altered secretions of IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and PGE-2. These cytokines could induce insulin resistance and the worsening of metabolic control in diabetic patients. For e.g. - the presence of TNF- $\alpha$  inhibits phosphorylation on insulin receptor substrate one, resulting in insulin resistance.<sup>19</sup>

Thus, good control of serum glucose in diabetic patients appears to be a desirable goal in preventing certain infections and to ensure the maintenance of normal host defence mechanism that determines resistance and response to infection.

### **Response of patients with diabetes to conventional periodontal therapy**

1. Controlled studies have shown that the response of diabetes to non-surgical and surgical periodontal therapy is similar to that of non-diabetics.<sup>20</sup>
2. A study of 57 young adult type-I diabetic subjects found no differences from controls for gingival inflammation and plaque index at baseline, and one week after scaling and oral hygiene instructions.<sup>20,21,22,23</sup>
3. DM subjects with severe periodontitis presented with more rapid deterioration without periodontal intervention.
4. There is no substantial evidence that suggests individual with diabetes require a more thorough and aggressive periodontal therapy than standard periodontal therapy. However, with poorly controlled diabetes, periodontal health appears to deteriorate more rapidly than in healthy individuals.

5. Therefore, assessment of patient's metabolic status is important in determining the prognosis and the recall interval for the periodontal therapy.

#### **Effects of periodontal therapy on glycemic control**

An important aspect related to periodontal therapy in diabetics is the effect of control of periodontal infection on the metabolic status. However, to date, there are several intervention studies that examine this effect.

Periodontal intervention trials suggest a significant potential metabolic benefit of periodontal therapy in people with diabetes. Several studies of diabetic subjects with periodontitis have shown improvements in glycemic control following scaling and root planning combined with adjunctive systemic doxycycline therapy, the magnitude of change is often about 0.9%-1.0% in the haemoglobin A1c test.<sup>24, 25</sup>

There are some studies in which the periodontal treatment was associated with improved periodontal health, but minimal impact was seen on glycemic control.<sup>26, 27</sup> Most of these studies used scaling and root planing alone, without adjunctive antibiotic therapy. Conversely, a recent study of well-controlled type 2 diabetic patients who had only gingivitis or mild localized periodontitis examined the effects of scaling and localized root planning without systemic antibiotics.<sup>28</sup> A diabetic control group with a similar level of periodontal disease received no treatment. Following therapy, the treated subjects had a 50% reduction in the prevalence of gingival bleeding and a reduction in mean haemoglobin A1c from 7.3% to 6.5%. The control group, which received no periodontal treatment, had no change in gingival bleeding, as expected, and no improvement in haemoglobin A1c. These results suggest that changes in the level of gingival inflammation after periodontal treatment may be reflected by changes in glycemic control.

#### **Test for glycosylated haemoglobin**

Measurement of blood level of glycosylated haemoglobin (HbA1c) reflects the average blood glucose level over the past two to three months (mean life of RBC- 120 days), thereby, providing the valuable tool for monitoring the treatment of diabetes (ADA 1998). Estimation of blood concentration of HbA1c has also proved to be a valuable parameter for predicting the risk of later onset of diabetic complications (diabetes control and complications trial research group, 1995).

Glycosylated haemoglobin is expressed as a percentage of normal haemoglobin.

In clinical practice, the blood level of HbA1c are usually determined at least twice annually in order to make adjustments to treatment.<sup>29</sup>

The glycosylated haemoglobin assay is readily available in most commercial laboratories. It is comparable in price to other screening tests such as the fasting blood glucose assay and the two hour oral glucose tolerance test.

For diagnosis, recent evidence indicates that the glycosylated haemoglobin assay can identify diabetes with a sensitivity of 45% to 94%, a specificity of 83-98% and a predictive value of approximately 90%. The values compare favourably with the results reported using the more traditional methods of diagnosis.<sup>30, 31</sup>

Laboratory evaluation of diabetes control: glycosylated haemoglobin assay (HbA1c)

- 4-6%- normal
- <7%- good diabetic control
- 7-8%- moderate diabetic control
- >8% need to improve diabetic control.

There are other serum proteins beside haemoglobin that become glycated in the presence of hyperglycemia like albumin, fructosamine etc.. Measurement of these glycated proteins can be used as an alternative to HbA1c. Albumin is a serum protein with a half-life of 2-3 weeks; measurement of glycated albumin reflects glycemic control over a shorter interval than does haemoglobin A1c. This can be helpful when an objective is needed, as might occur during initiation of a new therapy, during a medical illness, during pregnancy, or when the haemoglobin A1c may not be reliable (for example, when anemia is present).

The normal range of fructosamine test is 2.0-2.8mmol/l. In cases of anaemia and during pregnancy, the levels of HbA1c may be erroneously low and the test therefore inappropriate. In such cases, measurement of glycosylated plasma fructosamine may be useful (ADA, 1998).<sup>29</sup>

#### **Advantages of Glycosylated haemoglobin assay over traditional testing methods**

This assay is based on the knowledge that the blood glucose becomes irreversibly bound to haemoglobin molecule (glycosylation). The higher the level of blood glucose, the greater the amount that biodynamically and irreversibly binds to the hemoglobin molecule.<sup>32</sup> Since the average life-span of a RBC is 120 days, the glycosylated haemoglobin assay will reveal the patient's glucose status over the half-life of RBC, or approximately 30 to 90 days. One of the haemoglobin fractions resulting from the glycosylation reaction is HbA1c.<sup>33, 34</sup>

Since, the assay measures the blood glucose level over a 1-3 month period, it provides an excellent indicator of the long term control of the patient's diabetes, whereas traditional monitoring methods indicate the glucose status at one point in time.

In addition, the glycosylated haemoglobin assay can be administered at any time of the day without prior fasting by the patient. This eliminates the need for patient's compliance prior to testing which some are unwilling or unable to do so. Conversely, other patients may make great efforts to practice proper diet control and take insulin or oral hypoglycemic agents as directed immediately prior to testing but are out of compliance most other times. Thus, giving false indication of good control to the health provider.

## Limitations

1. The sample size of the study was small so a larger scale study is required for adequate statistical power.
2. The study should have included a control group also so as to evaluate the differences in the metabolic status of the patients, one with the periodontal therapy (study group) and the other without the periodontal therapy (control group).

## Conclusion

Diabetic patients are commonly encountered in the dental office. Proper patient management requires close interaction between the dentist and the physician. Dentists and other oral health care providers should understand the diagnostic and therapeutic methodologies used in the diabetic care. Working with the diabetic patients can be challenging and rewarding when open lines of communication are established and thorough patient education is attained.

This paper has attempted to demonstrate the advanced stages of the glycosylated haemoglobin assay over traditional testing methods as well as offer indications for its use by the periodontist.

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