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TO STUDY THE DETERMINATION OF PATTERNS OF GLYCEMIC CONTROL IN PATIENTS WITH DIABETES CONTROL BY GLYCOSYLATED IN JAMSHEDPUR

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ABSTRACT

Hemoglobin A (Hb A) in adults and in infants that are above the age of 6 months represents about 90% of the hemoglobin level. Glycosylated hemoglobin helps to identify the average plasma glucose concentration over an extended period of time. In the past decade a better insight into the problems associated and clinical uses with glycated hemoglobin measurement have been studied. Elevated levels of glycated hemoglobin have been attributed along with various diseases such as nephropathy, retinopathy and cardiovascular disease in diabetes mellitus. Diabetes mellitus is often referred by the doctors as diabetes (Lal, 2016). It is moreover, described as a group of metabolic diseases wherein the person is observed to suffers with elevated blood sugar (blood glucose) levels. Also, other reasons could be because there is inadequate insulin production or due to the lack of body to respond adequately to insulin, or both. This section will give a brief discussion on the prognosis and factors that cause elevated level of blood glucose levels in humans.

KEY WORDS: *Hemoglobin, Glycosylated, Diabetes mellitus, blood glucose etc.*

INTRODUCTION

Hemoglobin is the iron-containing oxygen-transport metalloprotein that is present in the red blood cells of almost all vertebrates as well as in the tissues of some invertebrates (Maton et al., 1993). The hemoglobin present in the blood carries oxygen from lungs or gills to the rest of the body. There are various types of hemoglobin present in the normal human body. Hemoglobin A (Hb A) is the first type which makes up about 95%-98% of hemoglobin found in adults; it contains two alpha (α) chains and two beta (β) protein chains; A2 (Hb A2) is the second in type which makes up about 2%-3% of hemoglobin that is observed in adults; it has two alpha (α) and two delta (δ) protein chains and third is Hemoglobin F (Hb F), which is observed up to 2.5% in adults. Some of the basic functions performed by hemoglobin molecule is the transportation of oxygen from lungs to tissues; delivering carbon dioxide from tissues to lungs; transportation of hydrogen ion from tissues to lungs and in kidney; to acts as an intracellular buffer and is thus involved in acid base balance as well ("Hemoglobin: Introduction and Abnormality," n.d.). Protein glycosylation is imperative in maintaining the integrity of the plasma membranes and thereby facilitating the protein secretion into the extracellular space. These specific actions occur in the presence of enzymes which are regulated under precisely controlled environment in the body. It has been observed that certain proteins may undergo non enzymatic glycosylation that is governed by the presence of high concentration of free sugar and non-physiological incubation conditions (Bunn, Gabbay & Gallop, 1978). It was until recently, that the focus has been centered on the non- enzymatic glycosylation of human hemoglobin. Moreover, hemoglobin is a useful tool to study non enzymatic glycosylation of other proteins as it may be involved in the long-term complications associated with the disease. Glycated hemoglobin (HbA1c) was identified primarily as an "unusual" hemoglobin in patients with diabetes over 40 years ago (Rahbar, Blumenfeld & Ranney, 1969). It was only after the discovery of numerous small studies that were conducted pertaining to glucose measurements that resulted in the idea that Hb A1c could be used as an effective measure for glycaemic control. The A1c-derived average glucose study was performed that included 643 participants who represented a range of A1c levels in them. Thereby, a validated relationship was established between A1c and average glucose across a range of diabetes types and patient populations (Nathan et al., 2008). HbA1c was thus introduced into the clinical use back in the year 1980s and has consequently become a corner stone of

clinical practice (Massi-Benedetti, 2006). Ideally, about five percent of the Hemoglobin population of normal human red blood cells are covalently linked to glucose, that result in the formation of a minor components designated as hemoglobin A1c (Hb A1c) by Allen et al, (1958). Interest in Hb A1c was considerably augmented by the scholars as there was two to threefold increase in the glycoprotein in patients with diabetes mellitus (Vogel & Waterbolk, 1973). It was the formation of condensation in glucose and hemoglobin that indicated the structural analysis of hemoglobin A1c. Other minor component is Hemoglobin A2 ($\alpha 2\delta 2$) and Hb F ($\alpha 2$-$\gamma 2$) that comprises about 2.5 percent and 0.2 percent of the total hemoglobin content present in the human body. These two minor components are synthesized that are controlled by two other globin chain genes (δ and γ). Other minor hemoglobin content is post-translational modifications of hemoglobin A (Bunn, Gabbay & Gallop, 1978). HbA1c reflects average plasma glucose from previous eight to twelve weeks in human body (Nathan, Turgeon & Regan, 2007). The advancement and breakthroughs in treating patients has observed meteoritic changes in recent years due to the practice of medicine along with science and its applications. The significant aspect is the use science concerning to health and disease. One such new concept was the cautious control of blood glucose levels that would assist in preventing or delaying the dread complications of angioplasty and neuropathy in the course of diabetes mellitus. The restrains faced to a greater acceptance of this is lack in controlling the blood sugar level in human body. There were various prospective studies that stated that there is a positive correlation between the frequency and extent of late complications with the degree of metabolic control of diabetes mellitus (Ronald, 1987). The term diabetes mellitus recounts a metabolic disorder with diverse etiologies that are characterized by chronic hyperglycemia and disturbance of carbohydrate, fat and protein metabolism that is a result from defects in secreting insulin, action of insulin or both (WHO, 1999).

Glycosylation and its effect on hemoglobin associated with Diabetes Mellitus patients

Glycosylated hemoglobin occurs at a specific site whose derivatives are both natural and synthetic thereby providing an excellent opportunity to explore structure and functional relationships. As canvased HB's A1a1, A1a2 and A1c are modified at the NH2-terminal amino group of the β chains, a site normally involved in the binding of the organic phosphates

(Pomponi, 2000). 2,3-Diphosphoglycerate (2,3-DPG) is an imperative regulator for hemoglobin function at intracellular level. It has also been observed in mammals that their red cells do contain the presence of 2,3-DPG in high concentrations that are equivalent approximately to the concentration of hemoglobin tetramer. The binding to deoxyhemoglobin is more strong as compared to oxyhemoglobin by 2,3-DPG, thus causing pronounced reduction in the affinity of hemoglobin for oxygen. As the negatively charged groups form salt bonds with positively charged residues on the two β chains at the entrance of the central cavity of the hemoglobin molecule, inclusive of the NH2-terminal amino groups. This site if gets blocked by a covalent attachments with hexose, or an acetyl or carbamyl group the reactivity of hemoglobin with 2,3-DPG is markedly observed to reduce (Bunn & Briehl, 1970; Kilmartin, Fogg, Luzzana, Rossi & Biol; 1973).

The identification of increased level of even a minor hemoglobin component exist in patients with diabetes perceived its identification as hemoglobin A1c. It was Humisam and Doxy in 1962 who observed initially the two to threefold increase in hemoglobin A1abc in four diabetic patients that were given tolbutamide in treatment. Erythrocytes were observed to have abnormal membrane properties in diabetic patients. Jain, Mcvie, Duett and Herbst (1969) examined in-vivo membrane lipid peroxidation in erythrocytes of diabetic subjects and their possible correlation with hyperglycemia. A significant increase in membrane lipid peroxidation in diabetic erythrocytes compared with non-diabetic erythrocytes of diabetic patients. The level of damage to the membrane lipid peroxidation with degree of glycosylated hemoglobin was thus found to be evident from their study. The further examined diabetic patients and observed the same abnormal pattern in each case. Subsequently, a two fold increase in chromatographically separated hemoglobin A1c in a small number of diabetic patients. A larger study that showed a twofold increase of hemoglobin A1c over values found in normal subjects. The other facts that came into light were the increase in the levels of hemoglobin did not have any correlation to the duration of disease, age of the patients, and type of therapy that they were taking nor the presence of the complications of diabetes mellitus. Additionally, there was no attempt made to relate the increase in hemoglobin A1c to the blood glucose concentrations. Similar findings were obtained by Paulsen in the year 1973 with children related to overt insulin-dependent diabetes

and normal amounts in nine children with asymptomatic hyperglycemia and concluded that such alterations may be specific to genetic markers associated for diabetes mellitus. Study was performed to check the same in chemically induced diabetic mice. The study manifested that adult diabetic mice (C57BL/KsJ-db/db) had increased amounts of minor fast-moving hemoglobin component compared to wild-type non-diabetic mice. Samples of these mice when runned chromatographically showed mobility to human hemoglobin A1c and also contained sodium borohydride reducible linkage on the β chains. Their study showed that db/db animals had normal level of mouse hemoglobin A1c at weaning showed an increase approximately four weeks after the onset of hyperglycemia. However, the amount of hemoglobin A1c was normal in the non-diabetic obese C57BL/6J-ob/ob mouse and only did the transient increase was noted in the transiently diabetic C57BL/6J-db/ db mouse. Thus rejected any relationship between the ob or db gene and hemoglobin A1c levels. This observation was clarified by inceptioning the structure of hemoglobin A1c, as a glucose adduct of hemoglobin A by using Schiff base and a subsequent Amadori rearrangement approach. Ketoamine configuration stability along with kinetic data invivo and the subsequent measurements of Hb A1c in young and old red cells made it clear that hemoglobin A1c accumulated throughout the lifespan of the circulating red blood cells. Koenig and their co-workers demonstrated that the hemoglobin A1c concentration correlated significantly with both the maximal response to a glucose tolerance test and to that of the group that was assessed for fasting blood glucose of diabetic patients treated with diet, insulin or oral hypoglycemic. However, no significant correlation observed amongst hemoglobin A1c levels and muscle capillary basement membrane thickness; it was this distinguishing lack in correlation amongst the results over the period of several years that lead to an development of detectable basement membrane thickening.

Recent findings and studies conducted by scholars in other groups have confirmed that amounts of Hb A1c provides an independent assessment of diabetic control. Gonen et al., in 1977 illustrated the state of control on their patients on an arbitrary scale and established a significant correlation amongst the concentration of glycosylated hemoglobin and the clinical assessment of control. Many clinical reports have correlated diabetes between the blood sugar of fasted patients to the amount of Hb A1c. Schwartz, King, Schwartz, Edmunds & Schwartz (1976) also illustrated on the reduction of Hb A1c that occurs when diabetic women becomes pregnant might

be due to reduction in hyperglycemia or perhaps to an influx of juvenile red cells during pregnancy.

Incidence and prognosis of diabetes mellitus:

It is the presence of sugar level in the urine that is looked up to as an earnest symptom and that is an imperative feature of diabetes mellitus (Lyon, 1922). The researcher further stated that glycosuria is the excretion of sugar in the urine which is often discovered accidentally without any impairment to their health. There are various conditions that lead to the presence of sugar in the person's urine. However, there are very limited mode of mechanisms through which glycosuria can be produced. Firstly, the cases where the amount of blood-sugar is normal or is observed to be reduced, sugar could elude in the urine. The "renal threshold" is low which is called as "renal diabetes" or negligible glycosuria. Second condition, when adrenaline hydrochloride producing a sudden and frequent rise in the blood-sugar level accompanied by glycosuria in certain cases. Subsequently, an eminent condition observed in experimental animals is the adrenalin glycosuria. Further, the ability of the patients to store away the mobilized sugar and the quantity of glycogen stored in the liver are the factors at an extent that decide the degree of response. A normal renal threshold is about 17%, any rise above the renal threshold scale leads to occurrence of glycosuria in the blood-sugar level. Increased mobilization (breaking down) of glycogen that is stored in the liver could lead to this type of hyperglycemic condition. There are many emotional factors that elicit sympathetic system and are followed by elevated liberation of adrenaline and glucose in the blood (Cannon, 1916). Transient and intermittent characteristics are exhibited by adrenalin or sympathetic glycosuria. Lastly, it's the secondary and hyperglycaemic glycosuria that occur as sugar is being absorbed from the bowel at a higher level which can stored in the muscles and liver. Such a condition is called as postprandial or alimentary glycosuria. This can be processed in a normal person by giving them an adequate dose of carbohydrate in assimilable form. Further, it has been observed to occur more efficiently in cases of exophthalmic goitre and in the lesions of ductless glands. It's the same mechanism observed to work in diabetes mellitus.

RESEARCH METHODOLOGY

A methodology is characterized to be associated with the drawing up a research plan by every student. It is generally confined to questionnaire writing, a collection of a limited set of data and, thereby, learning to apply some vital statistics in the field of academics. As this theory is budding and representing the outlook of the research, thus, this perspective is strengthened by the terminological confusion about the word methodology and its underlying connotations. According to Jonker and Pennink (2010), terms such as 'methodology' and 'method' are often frequently used arbitrarily. The following chapter aims at presenting the philosophical premises and the rationale of the present research study, as well as to acquaint the readers with the research strategy and the practical techniques employed. The chapter further iterates the scope and limitations of the research design and the approaches utilized in the study to perform the analysis. Also, it dwells the research amongst existing periodicals, medical journals, reports, research papers and book accumulating the glycosylated hemoglobin assay information by understanding the levels of glycosylated hemoglobin of diabetes in Jamshedpur, Jharkhand are in good or poor control. Additionally, this research aims to use the glycosylated hemoglobin to determine the patterns of glycemic control in patients with diabetes and further, identify the relationship between the glycosylated hemoglobin and plasma glucose in diabetic patients in Jamshedpur.

RESEARCH APPROACH:

Research is seldom mistaken to be the method for collecting the information, documentation, etc. however, it is the process of collecting the data, analyzing it, and subsequently analyzing the collected data to understand the particular process (Hennink, Hutter, & Bailey, 2010). For conducting the research and identifying the solution to the research question three different approaches are adopted like quantitative, qualitative, and mixed. However, the implementation of the research approach depends on the demand of the research (Williams, 2007). The research approach is described as a proper plan which includes methods like data collection, analysis and interpretation. This is based on the identified research problem in the previous chapter and is divided into main categories like:

a) Data collection approach
b) Data analysis or reasoning approach

RESULTS AND DISCUSSION

ON THE BASIS OF AGE

From the following table, we observe that 35% of the respondents belonged to the age group **30 to 45 years**. The following bar chart also shows the taller bar corresponding to the same.

Table 1 Age factor

	Frequency	Percent	Valid Percent	Cumulative Percent
Below 30 years	25	12.5	12.5	12.5
30 years - 45 years	70	35.0	35.0	47.5
45 years - 60 years	56	28.0	28.0	75.5
60 years - 75 years	41	20.5	20.5	96.0
75 years - 90 years	8	4.0	4.0	100.0
Total	200	100.0	100.0	

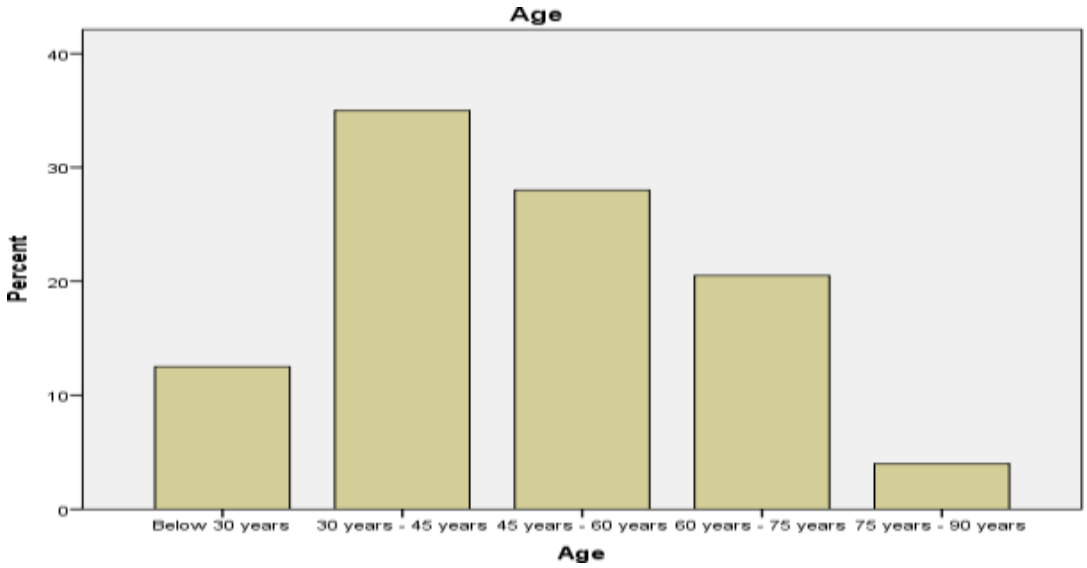

Figure 1 : on the basis of diabetes

ON THE BASIS OF DURATION OF DIABETES

From the following table, we can observe that 41.5% of the respondents belong to the >10 up to 15 years age group. The following bar chart also shows the taller bar corresponding to the same.

Table 2 ***Duration of diabetes***

	Frequency	Percent	Valid Percent	Cumulative Percent
<1 year	39	19.5	19.5	19.5
1–5 years	100.014	7.0	7.0	26.5
>5 up to 10 years	25	12.5	12.5	39.0
>10 up to 15 years	83	41.5	41.5	80.5
>15 up to 20 years	39	19.5	19.5	100.0
Total	200	100.0		

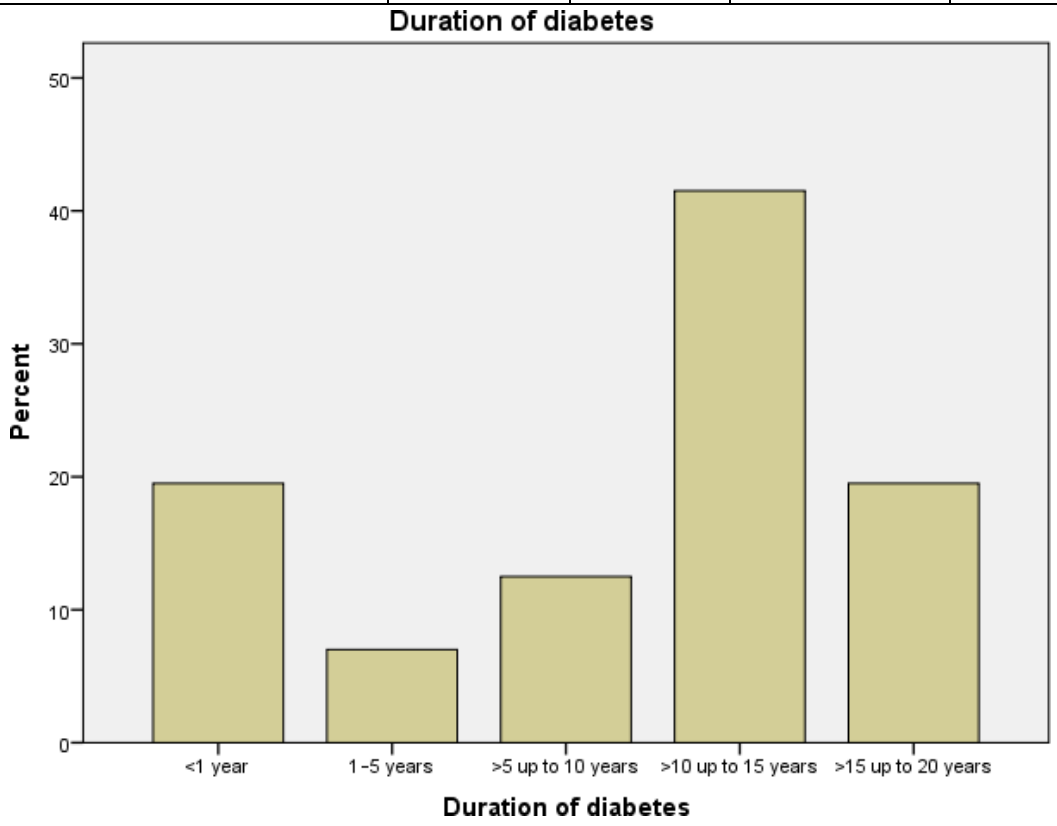

Figure 2 Duration of diabetes

Consists of certain statements related to the determination of patterns of glycemic control in patients with diabetes

Table 3 *Reliability Statistics*

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
.863	.865	*8*

Here, Cronbach's alpha = 0.863

It indicates a *high* level of internal consistency for the scale with statements regarding the variables for determination of patterns of glycemic control in patients with diabetes.

Table 4 ***Item-Total Statistics***

S.No.	***Questions***	***Analysis***
1.	Intensification of diabetes therapy helps in controlling the glycemic index of diabetes patients	*.87*
2.	Maintaining a paper logbook helps to keep the glycemic index in check	*.842*
3.	Maintaining a Meters with Add-On Devices or Applications helps to keep the glycemic index in check	*.852*
4.	Maintaining on the meter and off meter software helps to keep the glycemic index in check	*.846*
5.	Glycaemic control in diabetics can be better assessed with glycosylated hemoglobin and FPG together.	*.856*
6.	Medical nutrition therapy (MNT), exercise provides proper control of glycemic index	*.842*
7.	Self-monitoring of blood glucose can work to achieve and maintain specific glycemic goals	*.838*
8.	Elevated postprandial glucose (PPG) concentrations may contribute to suboptimal glycemic control.	*.850*

CONCLUSIONS

Hemoglobin is the iron-containing oxygen-transport metalloprotein that is present in the red blood cells of almost all vertebrates as well as in the tissues of some invertebrates (Maton et al., 1993). Based on the current data collected, the results mainly indicate the data which are collected based on the demographic factors. The study examined the results related to the age and showed that 35% of the respondents belonged to the age group 30 to 45 years and 58.5% of the respondents were female. Hence, it can be said that majority of the respondents are female who participated in the research. The study also examined the data based on the residence and it was examined that 58% of the respondents reside in urban areas and 63% of the respondents are married. Hence, it can be said that the majority of people are married and lived in urban areas. To collect the data based on religion, the study showed the result that 74% of the respondents are Hindus and 33.5% of the respondents have completed their graduation. The study also makes focus on the duration of diabetes among the respondents, the study showed the result that 41.5% of the respondents belong to the>10 up to 15 years age group who highly affect the diabetes diseases. In addition to this, the study also examined the result based on the body mass index in Kg/m2 and study showed the result that 60.5% of the respondents weigh>24.9–30 or are over- weight. Hence, it can be said that people who were overweight highly caused by diabetes diseases. The study also examined the current treatment based on the current status of the respondents and it showed the result that46% of the respondents take combined treatment such as Injectable & Oral for effective treatment of the diseases.

The study also defines the glycosylated hemoglobin and found the result that 46.5% of the respondents check their glycosylated hemoglobin twice a year. The study also showed the normal range of glycosylated hemoglobin and examined that 54% of the respondents think that the normal range of glycosylated hemoglobin is between 4% and 6%. To make focus on the level of glycosylated hemoglobin over the past 3 months showed the result in the study that 60% of the

respondents believed over the past 3 months, the level of their glycosylated hemoglobin is between 5.7% - 6.4%. In addition to this, 74.5% of the respondents believe that glycosylated hemoglobin is not curable.

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