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Comparative Analysis of Fructosamine and Glycosylated Hemoglobin in Diabetic Patients with Polycythemia

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Abstract

Background: The standard biomarker for long-term glycemic control in diabetes mellitus is Glycosylated Hemoglobin (HbA1c). However, in conditions like polycythemia, due to altered red blood cell (RBC) turnover, its accuracy can be compromised. Fructosamine, a marker of short-term glycemic control, may serve as a more reliable alternative in such cases.

Objective: This study aimed to evaluate the reliability of fructosamine compared to HbA1c in diabetic patients with varying degrees of polycythemia and to assess the impact of RBC parameters on HbA1c levels.

Methods: A prospective comparative study was conducted at Padmashree Diagnostic Centre, Bangalore, over a six-month period, involving 28 type 2 diabetic patients. Participants were categorized into three groups: normal RBC count (control), mild polycythemia, and moderate polycythemia. By using HPLC (D-10 Analyzer), Hemoglobin A1c levels were measured, and using a colorimetric method, fructosamine levels were measured. Correlations included in the Statistical analysis are ANOVA and Pearson.

Results: HbA1c and fructosamine levels showed a strong positive correlation in the control ($r = 0.7$) and mild polycythemia ($r = 0.8$) groups, while the correlation was moderate in the moderate polycythemia group ($r = 0.6$). Statistically significant difference was not observed in HbA1c or fructosamine values among the groups ($p > 0.05$), but in the polycythemia group, Hb levels and RBC counts were significantly elevated ($p < 0.005$).

Conclusion: In patients with polycythemia, HbA1c measurements may be unreliable due to altered RBC lifespan. Fructosamine offers a valuable alternative for monitoring glycemic control, especially in cases of elevated RBC counts. With larger associates, including severe polycythemia cases, further studies are recommended to confirm these findings.

Keywords: Polycythemia, Glycemic Control, Red Blood Cells, Diabetes Mellitus, HbA1c, Fructosamine.

1. Introduction

Increased red cell mass is indicated by the evaluation of laboratory findings as increased hemoglobin and hematocrit levels in conditions like polycythemia, which is also known as erythrocytosis. Polycythemia vera represents a clonal myeloproliferative neoplasm characterized by the autonomous overproduction of erythroid precursors, often alongside elevated leukocyte and platelet counts, reflecting a dysregulated hematopoietic process within the bone marrow. The clinical significance of polycythemia includes the risk of thrombotic events due to hyperviscosity of blood. Also, there is a possibility of the development of leukemia in cases of polycythemia vera.[1]

The underlying pathophysiology of the disease can be classified as Polycythemia. Primary erythrocytosis consequences from an intrinsic cellular defect, foremost to the augmented proliferation of erythroid progenitors.[2,3]

The heterogeneous group of conditions in which red cell mass increases, resulting in either the physiologically inappropriate production of erythropoietin or other erythropoietic factors and a reduced tissue oxygenation response to physiologically appropriate conditions, is known as secondary polycythemia.

Relative polycythemia (stress or spurious erythrocytosis) is characterized by an increased hematocrit with a normal to high red cell mass and low normal or reduced plasma volume. Even though true erythrocytosis does not exist in this disorder, these patients are at high risk for thromboembolic complications.[2,4]

However, Hypoxia is a momentary process in which the erythropoietin is increased; such increased EPO concentration ultimately will return to normal even if hypoxia continues, and even primary or secondary polycythemia will not completely conquer EPO production. Measuring serum EPO in a single sample may be misleading; thus, it is not worth it.[5]

Patients with T2DM are known to have more readily than those of normal subjects due to red blood cells. More accumulation of red blood cells is the most projected feature in diabetes patients with poor control of glycemia. It is considered that the accumulation of RBCs directly affects the viscosities of whole blood.[6,7]

Glycosylated hemoglobin A1c (HbA1c) is a significant biomarker, which signifies the fasting or post-prandial blood glucose average throughout the previous 2-3 months. Hemoglobin A1c is observed as a significant tool in diabetes diagnosis and management. Elevated HbA1c levels indicate long-term insulin resistance, which may accompany hyperglycemia, dyslipidemia, hypercoagulability, and systemic inflammatory responses.[6,8]

The degree of blood glucose control in patients with diabetes mellitus can be efficiently measured by measuring glycosylated hemoglobin (HbA_1). It has been newly shown.

It has also been shown that HbA1 has an altered affinity for oxygen. In adult hemoglobin (HbA), the β-chains can undergo non-enzymatic covalent modification at their N-terminal valine residues through glycation, particularly in the presence of elevated glucose concentrations. This modification results in the formation of glycated hemoglobin (HbA1c), which exhibits reduced interaction with 2,3-diphosphoglycerate (2,3-DPG), a key allosteric effector that normally lowers hemoglobin's affinity for oxygen. As a consequence, the oxygen affinity of glycated hemoglobin is increased, leading to altered oxygen delivery dynamics. This biochemical alteration is particularly significant in individuals with diabetes mellitus, where chronically elevated blood glucose levels result in a higher proportion of HbA1c. Thus, red blood cells in diabetics demonstrate a uniquely elevated oxygen affinity compared to non-diabetics, highlighting a critical physiological adaptation with potential clinical implications.[9,10]

Analysis of glycated hemoglobin (HbA1c) in the blood suggests signs about a person's average blood glucose levels over the previous two to three months and the projected half-life of red blood cells (RBCs). As per the Standard of Care (SOC), HbA1c is advised for the execution and monitoring of diabetes, chiefly type 2 diabetes.[11]

A metabolic disorder characterized by raised blood glucose levels, called hyperglycemia, is mainly seen in diabetes mellitus. [12]

Classification of diabetes is mainly into two types, 5% incidence is mainly due to type 1, and among 95% diabetics, the incidence is type 2. For better treatment of hyperglycemia and other risk factors related to metabolic disorders, this calls. Both micro and macrovascular complications can significantly possible to lower the risk.[12,13]

Persistent elevations in blood sugar increase the risk for long-term vascular complications of diabetes such as stroke, heart failure, kidney failure, blindness, neuropathy (loss of sensation, especially in the feet), and gastroparesis (slowed emptying of the stomach). Increased risk of short-term surgical complications, such as poor wound healing, is due to poor blood glucose control.[12]

The non-enzymatic glycation of circulating proteins with albumin, globulins, and lipoproteins has changed to be a reasonable alternative is Fructosamine, to HbA1c measurement in situations where HbA1c is unreliable. Since albumin is the most plentiful of the serum proteins, fructosamine is mainly glycated albumin (GA), which is the percentage of glycated albumin. Latent role in diagnosing, monitoring, and managing diabetes: Fructosamine and Glycated albumin

Fructosamine (1-amino-1-deoxy fructose) is a constant ketoamine formed by the protein and glucose amino group. A movable product, Schiff base, is the aldimine intermediate, which is shaped by the attachment of the aldehyde group of the carbohydrate with the N-terminal amino acid of the protein. The initially formed Schiff base can either revert to its original constituents, protein and glucose, yielding a relatively stable fructosamine complex, or proceed through an Amadori rearrangement, marking a critical early step in the non-enzymatic glycation pathway.

2. Aim of the study

To evaluate fructosamine levels and glycosylated hemoglobin levels in diabetic patients with polycythemia and to study the influence of red blood cell parameters on the HbA1c level.

3. Review of literature

3.1. Polycythemia

Polycythemia, derived from the Greek roots *poly* (many) and *cythemia* (cells in the blood), refers to a hematological condition marked by an abnormal elevation in red blood cell (RBC) mass. This increase contributes to heightened blood viscosity, reduced hemodynamic flow, and a predisposition to thrombotic events. Clinically, it is frequently associated with an elevated packed cell volume (PCV), reflecting the expanded erythrocytic component within the circulatory system.[22.23]

The mass of RBCs is 26 to 32 mL/kg in men and 23 to 29 mL/kg in women with normal health.[22,24] Usually, high RBC mass is associated with patients having more than 51% and 48% hemoglobin and values of hematocrit more than 185g/L and 165 g/L/ in males and females, respectively.

Erythrocytosis is not identical to Polycythemia. Absolute erythrocytosis is dissimilar as an RBC mass of more than 125% of the predicted value adjusted for gender and body weight.[22]

3.1.1. Classification of Polycythemia

1. **Primary Polycythemia:** Primary polycythemia is a myeloproliferative disorder marked by an autonomous overproduction of erythroid progenitor cells due to intrinsic abnormalities in hematopoietic stem cell regulation. This dysregulated erythropoiesis occurs despite suppressed levels of erythropoietin, distinguishing it from secondary causes of polycythemia. Primary polycythemia is broadly categorized into two principal subtypes: polycythemia vera (PV) and familial/congenital polycythemia, each defined by distinct genetic mutations and underlying pathophysiological mechanisms. These differences not only guide diagnosis and management but also highlight the heterogeneity within this hematologic condition, making it a compelling focus for research and clinical investigation.[2,22]

- **Polycythemia Vera: -** This is the neoplastic disorder in which erythroid progenitor cells are increased as well, and sensitivity to erythropoietin secondary to a mutation known as the JAK mutation is also increased.[22] People with polycythemia vera (PV) have an exceptionally ruddy appearance with red discoloration of the face, and they may have dizziness, skin changes(bruises), headaches, and enlargement of the spleen. Though Certain blood clotting factors are not produced in adequate amounts or hemorrhage, there is the chance of ulcers or minor wounds.[2,22,23]
- **Pure Erythrocytosis: -** It is the subsection of patients with pure erythrocytosis with an increased RBC mass without any other precipitation factor.[22]

2. **Secondary Polycythemia:** It is an uncommon disease that includes the overproduction of RBCs, due to various reasons ranging from genetic irregularities to other diseases. It leads to hyperviscosity associated with other complications, which include ischemic events in particular.[22] Secondary polycythemia is also classified into two types: congenital or acquired, based on when the individual developed the defect.[22,24,25] Some studies exposed the presence of about 100 mutation consequences in more than 50 variants of alpha and beta globulin genes, accompanied by high oxygen affinity. This mutation also affects the affinity of 2,3-bisphosphoglycerates (2,3-BPG).[22,26] Familial erythrocytosis results from a mutation in hypoxia-inducible factor (HIF). A mutation in the HIF transcription factor leads to abnormal oxygen sensing, which results in elevated production of erythropoietin; hence, red cell mass is increased.[22,27,28] The erythropoietin is accompanied by the growth factor 166-amino-acid glycoprotein, and its molecular weight is about 39000d Da. It is a hydrophobic molecule that needs an intact disulfide bond for action.[5] Erythropoietin is produced in the kidney by the endothelial, epithelial tubular cells, and peritubular cells, with a small contribution from the liver. It is a required growth factor for the proliferation and differentiation of dedicated erythroid progenitor cells.[5,29] Serum erythropoietin concentration can be measured by radioimmunoassay (RIA), which is commercially available. Two studies were observed for the efficacy of this analysis. In one study, EPO levels were found to be useful for classifying primary and secondary erythrocytosis, but a more recent study was unable to classify them. Overall, higher EPO levels recommend secondary erythrocytosis.[2,30]
3. **Relative Polycythemia (**Gaisbock syndrome, Spurious or stressed erythrocytosis**):** Patients with relative polycythemia have a higher risk of thromboembolic complications.[2,22] It is due to numerous factors responsible for the increased hematocrit. Reduction in drinking alcohol and smoking cessation can be helpful, and adequate blood pressure control is an important factor.[2,32]
4. **Chuvash Polycythemia** is a recessively inherited congenital polycythemia named after the Chuvash people in central Russia. In this case, the EPO level will be higher than the normal level.[22]

3.1.2. Epidemiology of Polycythemia

In a study conducted by Galeus et al. As of 2015, severe pulmonary hypertension, congenital heart anomalies, and chronic obstructive pulmonary disease (COPD) have been identified as some of the most prevalent underlying causes of secondary polycythemia. Despite these associations, comprehensive epidemiological data on secondary polycythemia remain limited and underreported. In contrast, polycythemia vera, a primary myeloproliferative neoplasm, has an estimated prevalence of approximately 22 cases per 100,000 individuals, reflecting its relatively rare but clinically significant occurrence in the general population. Polycythemia vera shows a male preponderance in all races and civilizations, with a male-to-female ratio of around 2:1. Polycythemia vera (PV) typically presents in individuals in their sixth decade of life, aligning with its classification as a disorder of aging hematopoiesis. However, early-onset PV, defined by diagnosis before the age of 40, remains an uncommon clinical entity, often raising diagnostic challenges due to its atypical demographic presentation and potential overlap with hereditary erythrocytosis. This rare subset may exhibit distinct biological behavior and molecular characteristics, underscoring the need for tailored diagnostic and therapeutic approaches.. In contrast, polycythemic conditions arising from hemoglobinopathies and congenital cyanotic heart diseases tend to emerge at a significantly younger age, reflecting their genetic and developmental origins.[1]

3.1.3. Pathophysiology of Polycythemia

The generation and maintenance of red blood cell (RBC) mass lie at the core of physiological erythropoiesis, a tightly regulated process that ensures adequate oxygen delivery to tissues by balancing RBC production with cellular turnover and systemic oxygen demand.. This process is controlled by a variation of hormones, factors, and receptors.[22,33] EPO is the most significant of all the RBC mass controllers. It is formed by the kidneys and the erythroid progenitor cells as well.[22,26,31]Normally, EPO is formed as a response to anemia and hypoxemia. Hypoxia-inducible factors are produced by the stimulation of hypoxia, which leads to high gene expression of erythropoietin.[22,26,31]RBCs are important for oxygen transport. An increased RBC mass will lead to a raised oxygen-carrying capacity of the blood. Secondary polycythemia patients have a physiologically appropriate response, which is required to increase RBC mass, the standard for proper tissue oxygen delivery.[2,22]It has been reported that a higher than 45% of hematocrit is accompanied by hyperviscosity in normovolemic subjects and is also related to decreased cerebral blood flow, as revised after phlebotomy. The small balance between hyperviscosity and normal tissue oxygenation in such patients is the therapeutic goal.[2,22] The pathophysiology also would differ, depending on the cause in thought. High erythropoietin levels are a cellular hypoxia, which can occur due to any causes that activate the release of EPO from the renal peritubular lining capillary cells, as well as a small amount of EPO produced by the liver. Erythropoietin (EPO) exerts its effect by binding to receptors on erythroid progenitor cells, thereby promoting their survival, proliferation, and differentiation. In polycythemia vera (PV), however, erythropoiesis becomes largely independent of EPO regulation. Approximately 95% of PV cases harbor an acquired JAK2 V617F mutation, a valine-to-phenylalanine substitution in exon 14 of the *JAK2* gene, leading to constitutive activation of the JAK-STAT signaling pathway. This mutation drives uncontrolled erythroid proliferation despite characteristically suppressed EPO levels, forming the molecular hallmark of the disease.. Mutations have similarly been considered in exon 12 of JAK2. These mutations consequence in a loss of the auto-inhibitory pseudo-kinase domain of JAK2, after its fundamental activation.[1]

3.1.4. Evaluation of Polycythemia

The diagnostic evaluation includes a complete blood count (CBC), which typically reveals elevated hemoglobin concentration and hematocrit levels cardinal laboratory features indicative of polycythemia.

These hematologic elevations reflect an increased red cell mass and serve as the initial clue prompting further investigation into the etiology of erythrocytosis.

Additional testing with liver function tests, kidney function tests, ferritin levels, abdominal ultrasonography, and a chest x-ray should also be performed to diagnose polycythemia.[22,25] Chromium 51-red cell mass can be assessed along with plasma volume. However, the test is not broadly available and is infrequently performed in routine clinical practice. [22]Originally, in the analytical examination, an erythropoietin analysis was completed to measure the levels of EPO. It differentiates primary polycythemia and secondary polycythemia.[22,25]An increased EPO level is used to diagnose secondary polycythemia, but a normal level does not rule out secondary polycythemia, as the rise in EPO can be intermittent. We can do an in vitro culture of erythroid progenitor cells to investigate secondary polycythemia.[22]In addition to these, a JAK2 mutation must be considered for polycythemia vera.[25] When an elevated red cell mass is detected, it is necessary to rule out the presence of polycythemia vera. To examine polycythemia vera (PV), iron levels and cytogenetic investigation are vital. Bone marrow examination plays a pivotal role in the diagnostic workup of polycythemia vera, often revealing hypercellularity with panmyelosis, a hallmark of this myeloproliferative neoplasm. In parallel, pulse oximetry serves as a crucial non-invasive tool to assess arterial oxygen saturation, helping to distinguish primary from secondary polycythemia. An oxygen saturation level below 92% is indicative of tissue hypoxia, a key driver of secondary erythrocytosis through compensatory elevation of erythropoietin production. Measurement of arterial oxygen saturation is essential for patients with carboxyhemoglobinemia. This is frequently seen in patients who smoke. Pulmonary function tests (PFTs) are essential in evaluating the underlying cause of hypoxemia, particularly when investigating secondary polycythemia driven by chronic lung disease. In patients with suspected hemoglobinopathies, assessment of hemoglobin-oxygen affinity, often inferred through shifts in the oxygen dissociation curve, can be diagnostically revealing. A leftward shift, indicating high oxygen affinity, may point toward rare hereditary conditions where reduced oxygen delivery triggers compensatory erythrocytosis despite normal or elevated oxygen saturation.

An improved test in these patients is a PaO2-50, which is less than 20 in these patients. PaO2-50 is the fractional pressure of oxygen, where 50% of hemoglobin is saturated.[22]Low ferritin and low folate levels have been associated more with primary polycythemia.[1,34] Raised vitamin B12 levels are often conspicuous, may be observed due to increased secretion of transcobalamin by leukocytes.[1] Gene mutation testing done for EPOR, PHD2, VHL, and HIF2A can help to identify a congenital etiologic factor.[22,25]

3.1.5. Complications Associated with Polycythemia [1,22]

- Strokes due to elevated erythrocytosis (and it is also reported that anabolic steroids are used in athletes).
- Venous thromboembolism (low dose of aspirin used for prevention)
- Pulmonary hypertension leading to cardiomegaly
- Impaired oxygenation and blood supply
- Bleeding: - Persistent epistaxis or gastrointestinal bleeding is often seen, which might be the chief symptom of iron deficiency anemia; it can also include bone marrow appearance.
- Poor exercise tolerance
- Increased whole blood viscosity
- Generalized weakness and fatigue

- Decreases mentation

3.1.6. Laboratory Findings [34]

The workup of polycythemia initiates with ruling out polycythemia vera (PV), which is a neoplastic clonal blood disorder with autonomous (EPO-independent) erythroid proliferation that results in down-regulation of EPO production and low or normal serum EPO levels. All patients should primarily be tested for the V617F mutation in exon 14 of the Janus kinase 2 (JAK2) gene.[34]

Laboratory tests that help in the diagnosis of polycythemia include:

- Complete Blood Count (CBC)
- Red Cell Mass Volume
- Peripheral Blood Smear
- Comprehensive Metabolic Panel
- Bone Marrow Test
- Molecular Testing

3.2 Diabetes Mellitus

Diabetes was first recognized by the Egyptians and is characterized by loss of weight and polyuria.[35] Diabetes is a heterogeneous complex with metabolic disorder that is characterized by raised blood glucose concentrations secondary to either resistance to the action of insulin, inadequate insulin secretion, or both[36,37,38]. The main clinical appearance of the diabetic state is hyperglycemia. However, lack of insulin or insulin resistance is also related to abnormalities in lipid metabolism and protein metabolism, and with mineral and electrolyte instabilities.[38]

3.2.1. Etiologic Classification of Diabetes Mellitus

- **Type 1 Diabetes Mellitus (T1DM):-** Type 1 diabetes results from autoimmune destruction of the pancreatic beta-cells. [38,39,40]Markers of immune destruction of the beta-cell exist at the time of diagnosis in 90% of persons.[38]
- **Type 2 Diabetes Mellitus (T2DM):-** Type 2 diabetes is considered to be caused by insulin resistance and, originally, a lack of insulin secretion.[38,41,42] In terms, the plasma insulin concentration is usually raised, whereas relative to the insulin resistance, the concentration of the plasma insulin is insufficient to maintain standard glucose homeostasis.[38,43,49]
- **Gestational Diabetes Mellitus (GDM)**:- Gestational diabetes mellitus is defined as glucose intolerance first documented during pregnancy. In most women who progress with GDM, the illness is introduced in the third trimester of pregnancy. At least 6 weeks after the pregnancy ends, the woman should take an oral glucose tolerance test.[38]

3.2.2. Erythrocyte Changes In Patients With Diabetes Mellitus

Morphology of RBCs: -The morphology of normal erythrocytes is important for their survival and capability to carry oxygen. Spherocytes are seen in type 1 diabetes mellitus (T1DM), whereas both spherocytes and echinocytes were seen in the peripheral blood smears of type 2 diabetes mellitus (T2DM).[43] The authors have stated that spherocytosis, which is distinguished in both types of diabetes, looks to be accompanied by hyperglycemia.[43,44]

In assumption, when the inner environment of the body changes, the number of normal, biconcave disc erythrocytes diminishes as the number of deformed erythrocytes gradually rises, which additionally increases the risk of diabetic complications.[43,45]

Red Cell Distribution Width (RDW) and Mean Corpuscular Value (MCV):- MCV refers to the normal volume of an erythrocyte and is usually calculated indirectly. RDW is the coefficient of variation of MCV; higher RDW values replicate better heterogeneity in MCV, which is typically caused by agitations in erythrocyte maturation or degradation.[43,46]

Blaslov et al. presented a study in which both MCV and RDW were completely associated with HbA1c and the rate of diabetic retinopathy.[43,47]

Hemoglobin: - When glucose concentration is elevated in the blood, it binds to the Hb in erythrocytes. Once HbA1c is formed, it does not easily decay. HbA1c has an improved affinity toward O2; therefore, higher HbA1c concentrations chief to difficulties in releasing oxygen to cells and the condensed oxygen-transporting function of erythrocytes.[43,48]

Red Blood Cell Count (RBC): -RBC count is the number of erythrocytes per microliter of blood and can be used to monitor the action of blood diseases or medications that affect RBCs. A low erythrocyte count shows a reduction in oxygen-carrying cells in the blood, also known as anemia; vice versa, in some high erythrocyte count cases, this may show that the body is recompensing for some disorder that is depriving the body of oxygen.[43,49]A study presented that low erythrocyte counts in patients with T2DM were associated with microvascular complications.[43,50]

Erythrocyte Sedimentation Rate (ESR): ESR denotes the sedimentation rate of erythrocytes in the blood. It varies within a fine range in healthy individuals and increases in many pathological circumstances.[43,51]In patients with T2DM, Guo et al. reported that the ESR was independently related to the rate and severity of diabetic kidney disease. Hence, ESR can also be used as an indicator to assess the development of patients with diabetes.[43,52]

3.3 Glycosylated Hemoglobin

Glycated hemoglobin (HbA1c) is a form of hemoglobin used primarily to detect the average plasma glucose concentration over long periods. It is formed in a non-enzymatic pathway by hemoglobin's normal contact with high plasma levels of glucose.[12,53,54,55]Haemoglobin, which is conclusively glycated at one or both N-terminal valines of the ß chain, is defined as HbA1c.[53,56] HbA1c has been the most used and accepted test for monitoring glycemic control in individuals with diabetes. Once a hemoglobin molecule is glycated, it remains in the red blood cell for the rest of its life span (120 days).[53]Monitoring the degree of control of glucose metabolism by HbA1c in diabetic patients was first projected in 1976.[12,53,57 The clinical significance of the HbA1c test was flagged by the Diabetes Control and Complications Trial (DCCT) in type 1 diabetes and the United Kingdom Prospective Diabetes Study (UKPDS) in type 2 diabetes.[12,58]The studies showed that HbA1c is a significant marker in measuring a patient's risk of microvascular complications and hypoglycemia. The quantity of glycated hemoglobin is recommended for both (a) checking blood sugar control in individuals who might be pre-diabetic and (b) monitoring blood sugar regulators in patients with additionally elevated levels.[12]

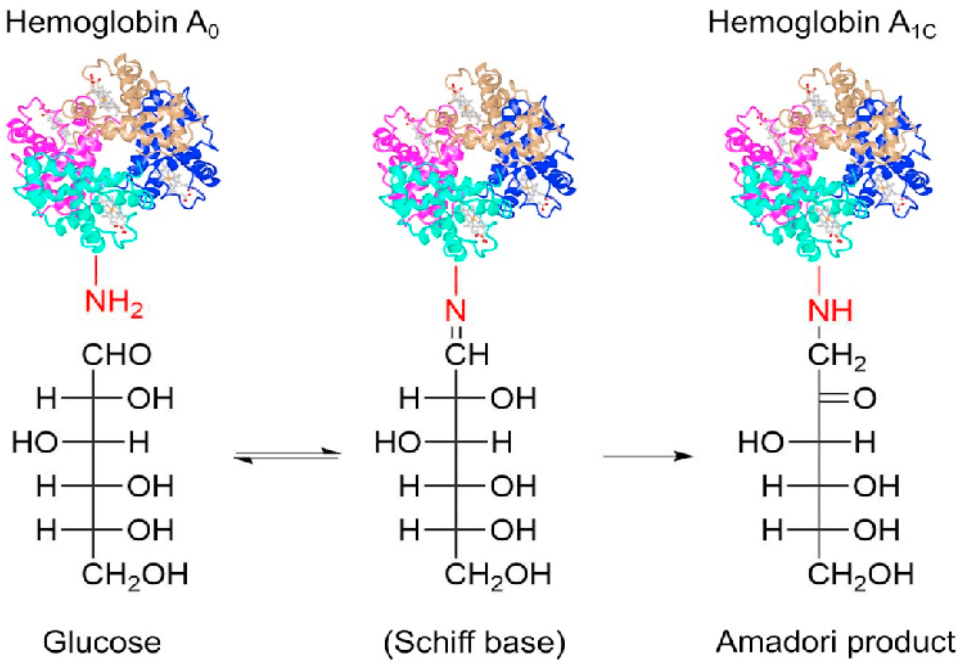
3.3.1.History of HbA1c

Historically, in 1955, investigators for the first time considered that adult hemoglobin contains heterogeneous molecules.[53] HbA1c was first isolated by Huisman et al.3 in 1958 and considered by Bookchin and Gallop in 1968 as a glycoprotein.[11,59,60] The increased levels of HbA1c in diabetic patients were testified by Rahbar et al.5 in 1969. Bunn et al.6 acknowledged the pathway important to the establishment of HbA1c in 1975.[11,61,62]Using the HbA1c as a biomarker for checking the values of

glucose between diabetic patients was first projected by Koenig et al.7 in 1976. By the early 1980s, the HbA1c test was extensively accepted in clinical practice.[11,63]

3.3.2. Formation of HbA1c

Excessive invention of the early glycation products is an acute, reversible change made by hyperglycemia. These glycation products are designed inside and outside the cells, as glucose quickly features in the amino groups of the proteins through the non-enzymatic reaction of nucleophilic addition, to form Schiff base adducts. Within hours, these adducts influence equilibrium levels that are related to the blood glucose concentration and then undergo reordering to form more stable early glycation products, which reach equilibrium over several weeks.[12,64]One of the proteins glycated in this way is glycated hemoglobin. Once a hemoglobin particle gets glycated, an accumulation of glycated hemoglobin within the red cell reflects the average glucose level to which the cell has been exposed during its life cycle.[12,64]The volume of glycated hemoglobin can measure therapy efficiency by monitoring long-term serum glucose regulation. The HbA1c level is associated with the average blood glucose concentration over the previous three to four weeks.[12,64]



In the first irreversible step, glucose binds to the N-terminal valine of the hemoglobin ß chain and forms a labile Schiff Base, which rearranges to a stable ketamine bond, yielding HbA1c.[11].The factors disturbing the Hb glycation rate and the mean age of RBCs in circulation could cause imprecise HbA1c quantities. Of these causes, iron deficiency anemia is most common in particular. However, in the other extreme, polycythemia, the interaction between HbA1c and erythrocytosis was unknown, and investigations were limited. Thus, this study aimed to evaluate the influence of polycythemia on HbA1c levels.[65]

3.4. Fructosamine

Fructosamine is glycated albumin (GA) that refers to the formation of ketamine specifically involving the most circulating protein albumin (3.5g/dl to 5g/dl). Due to albumin is the most abundant of the serum proteins, fructosamine primarily measures the glycated albumin. The development of fructosamine and

glycated albumin is a post-translational modification that occurs in proteins.[14]The amount of fructosamine or GA provides information on glucose control within 2-3 weeks.HbA1c is also the rate of nonenzymatic glycation of albumin, which is around 9 to 10-fold more than that of HbA1c.[14,66,67]Fructosamine analysis is similar to HbA1c, which is also a marker of evaluation of glycemic control. This test calculates the fraction of serum proteins, primarily albumin, that becomes glycosylated. As the half-life of albumin is twenty days, serum fructosamine regulates glycemic control over 2-3 weeks.[68,69] Fructosamine is a rapid and theoretically simple test. However, it is very carefully used in clinical practice, and the aim of this is not very clearly discussed. Fructosamine also has some other limitations, as its value differs with the alteration in serum albumin level. Thus, it can be falsely low in circumstances with rapid albumin turnover and low albumin states.[68]Fructosamine, being an alternative marker for the valuation of glycemic control, can be used to substitute HbA1C in special clinical situations stated above, whereas HbA1C would be unreliable. Fructosamine gives us glycemic control in a small period (2-3 weeks) in evaluations to HbA1C levels (2-3 months), hence it could be used as a more suitable test for monitoring the initial response of treatment.[68,70]

4. Materials And Methods

The study was conducted at Padmashree Diagnostic Centre, Vijaynagar, for six months. It involved 28 samples, which were divided into 3 groups based on their varying levels of RBC count by evaluating their complete blood count.

4.1. Type of Study: A prospective comparative study conducted in the biochemistry and hematology department of Padmashree Diagnostic Center.

4.2. Type of Sampling: Purposive sampling of a sample size (n) of 28, in which these patients were divided into three groups.

- Test group 1 consists of 12 Normal samples (normal RBC count).
- Test group 2 consists of 10 Mild polycythemia samples (RBC count between 4.9 to 5.1 mil/cm3).
- Test group 3 consists of 6 Moderate polycythemia samples (RBC count more than 5.2 million/cm3).

In this study, samples were taken from 14 males and 14 females in the 25-80 age group.

4.3. Selection criteria

- **Inclusion criteria:** Type II diabetes mellitus cases diagnosed a minimum 1 year ago.Hemoglobin levels greater than 16.5 g/dL in men and greater than 16.0 g/dL in women with identified diabetic patients.In women,>48% and in men,>49% Hematocrit levels.Patients who consent to the study.
- **Exclusion criteria:** Hemolyzed or icteric sample. Wrongly labelled sample. The sample was collected in an inappropriate anticoagulant. Cases of dehydration and Cases of polycythemia vera should be contained within the run once per 24 hours. A repeat run is shown when likely control values are not obtained.

5. Statistical Analysis

5.1. Statistical Tools Applied: All the values are expressed as Mean ± SD. One way Annova was applied to find the inter-group comparison, and p=<0.05 is considered significant. Correlation(r): 0.1 to 0.3: Poor correlation, 0.4 – 0.6: Moderate correlation, and >7: Strong correlation.

Results

Variables	Control	Case(1) Mild Polycythemia	Case(2)Moderate Polycythemia	*P*	Remarks
Age (years)	60 ± 15	53 ± 13.7	55 ± 11	0.4	Not significant
Hemoglobin (g%)	13.9 ± 0.7	13.7 ± 1.68	15.2 ± 0.6	0.009	Significant
RBC (millions/cumm)	4.8 ± 0.2	5 ± 0.08	5.8 ± 0.2	<0.005	Significant
PCV (%)	42.2 ± 2.1	43 ± 3.8	47 ± 2.7	<0.005	Significant
HbA1c (%)	7.2 ± 1.7	7.8 ± 3.4	8.4 ± 2	0.5	Not significant
MBG (mg/dl)	161.7 ± 50	178 ± 98	194 ± 58	0.5	Not significant
Fructosamine (µM/L)	327 ± 101	380 ± 152	367.7 ± 142.8	0.6	Not significant

Table 8: Represents the mean ± SD of variables in study groups

Age:

Correlation (*r*) between HbA1c and Fructosamine:

		HbA1c	Remarks
Control	**Fructosamine**	0.7	Strong positive correlation
Case(1) Mild Polycythemia	**Fructosamine**	0.8	Hematologyitive correlation
Case(2) Moderate Polycythemia	**Fructosamine**	0.6	Moderate positive correlation

Table 9: Represents the correlation between Fructosamine and HbA1c in the Case and Control groups.

Fructosamine levels in the Case and control groups:

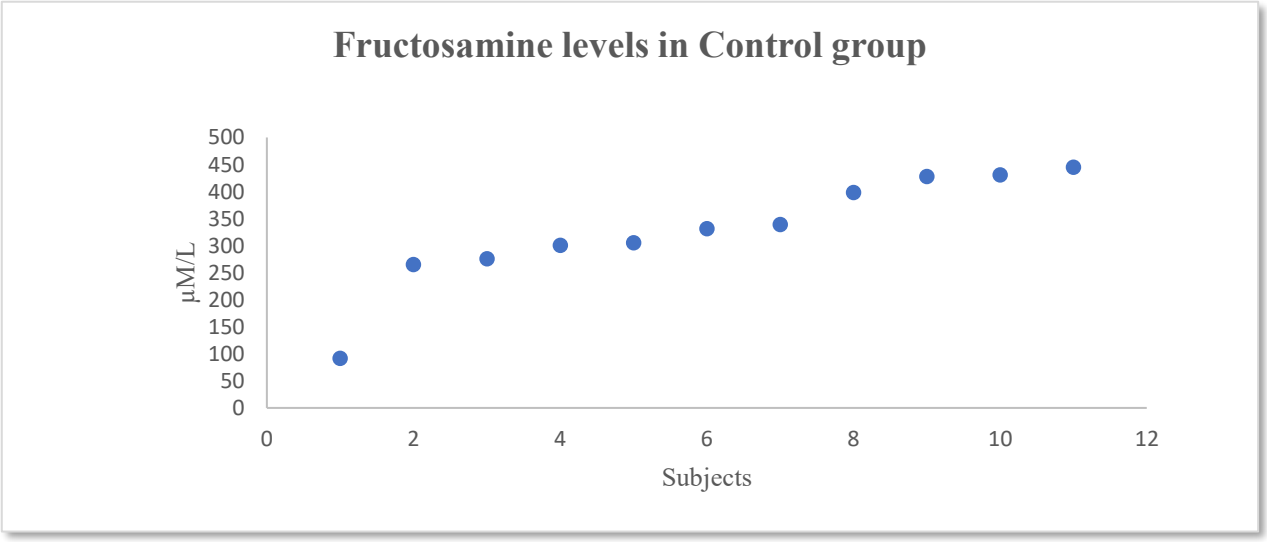


Fig 1: Scatter plot represents the distribution of Fructosamine in the control group

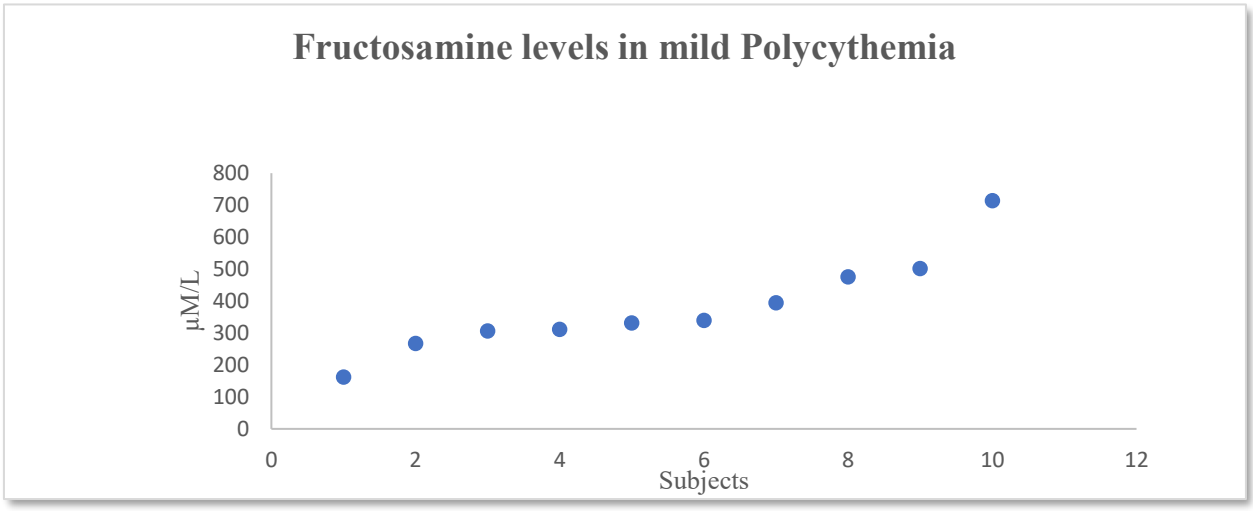


Fig 2: Scatter plot represents the distribution of Fructosamine in Mild polycythemia *group*

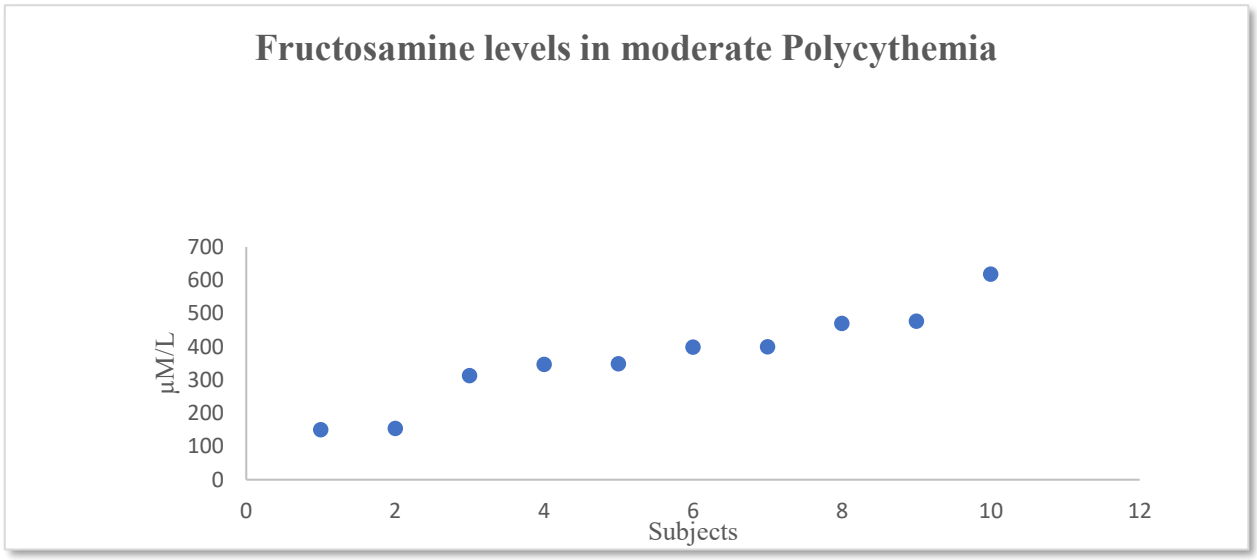


Fig 3: Scatter plot represents the distribution of Fructosamine in the Moderate polycythemia group

6. Discussion

Hemoglobin A1c (HbA1c) has been broadly used to measure glycemic control in diabetes mellitus patients. It can be used to estimate long-term average glycemia and design a dosage regimen for diabetes mellitus.

A study conducted by S Ayyappan et al. shows that due to the consistency, technical simplicity, low cost, and reduced analytical time of fructosamine assay, it can be used as a choice of first-line monitoring for diabetes patients, and the fructosamine determination is more reliable when compared to HbA1c.

Another study conducted by Neal Mittman et al. showed that two indices of glycemic control, HbA1c and serum fructosamine, correlate with each other and the mean blood glucose levels in diabetic patients.

The study by J.J. Graham et al. showed that patients with non-diabetes have higher ranges of RBC count of the same age and sex in diabetic patients. It also shows that in poorly controlled diabetes, as determined by HbA_1, the total red cell count is higher than in patients with good control.
A study conducted by us shows that the levels of HbA1c in Polycythemia may not be reliable if the RBC count is high, and the correlation was found to be stronger in control and mild polycythemic groups; hence, we concluded that fructosamine is a better indicator for monitoring diabetes.

7. Summary

An observational Case-Control study was conducted at Padmashree Institute of MLT, Kommaghatta, Bangalore. The study consists of a total of thirty-one participants (n=31), consisting of 10 subjects each in the mild and moderate polycythemia groups, and the remaining were in control. Age group, Hemoglobin profile, HbA1c, MBG, and Fructosamine levels of all the participants are given in Table 8. No significant inter-group difference was observed concerning Age, HbA1C, MBG, and Fructosamine levels (p=>0.05). Considering the correlation (r), a Strong correlation was observed in the control and Mild Polycythemic group concerning the major variables (ie, r=0.7 and 0.8, respectively), whereas the correlation was moderate in the Moderate Polycythemic group (r=0.6) (Table 9)

8. Limitations of the Study

- The inter-group correlation of the major variables would be better demonstrated if the sample size were higher.
- Another study group, with severe polycythemia, was required to determine the usefulness of Fructosamine in such patients.

9. Conclusion

Hemoglobin A1c is widely used to monitor glucose homeostasis in diabetic mellitus patients, but it can be potentially influenced by the quantity of RBCs.
The levels of HbA1c in Polycythemia, where there is a higher RBC count, the value may not be reliable. The best alternative in that case is Serum Fructosamine.
In the current study, we correlated both the values in normal, mild, and moderate Polycythemic patients. The correlation was found to be stronger in control and mild polycythemic groups (ie, 0.7 and 0.8, respectively). Compared with the moderate polycythemia group, the strength of the correlation (r) reduced to 0.6, but still, there is still a moderate positive correlation.
With this evidence, we can conclude that Fructosamine is a better indicator of blood glucose homeostasis in Polycythemia patients. Further studies with a higher sample size and a severe Polycythemia group are required to validate our results.

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