DISSERTATION SUBMITTED FOR THE MASTER'S DEGREE IN MEDICAL BIOCHEMISTRY

A STUDY OF PROTEIN CARBONYL CONTENT AND HbA1c IN DIAGNOSED PATIENTS OF TYPE 2 DIABETES MELLITUS AND CONTROL SUBJECTS

SUBMITTED

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DISSERTATION SUBMITTED

In partial fulfilment of the requirement for the award of degree of

Master of Science

In

Medical Biochemistry

By

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Enrollment Number- 2000102717

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This is to certify that **Miss Apoorva** student of **M.Sc. Medical biochemistry**. Integral University has completed her dissertation titled "**A STUDY OF CARBONYL PROTEIN CONTENT AND HbA1c IN DIAGNOSED PATIENTS OF TYPE 2 DIABETES MELLITUS AND CONTROL SUBJECTS"** successfully. She has completed this work from the Department of Biochemistry, Integral Institute of Medical Sciences and Research, Integral University under my supervision .This dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future.

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CONTENTS

List of Abbreviations

M	Protein Carbonyl Content
WHO	World Health Organisation
ROS	Reactive Oxygen Species
AGEs	Advanced Glycation End Products
T2DM	Type 2 Diabetes Mellitus
DNA	Deoxyribose Nucleic Acid
ADA	American Diabetes Association
SMBG	Self -monitoring of blood glucose
MDA	Malondialdehyde
HNE	4-Hydroxynonenal
CAT	Catalase
GP_x	Glutathione Peroxidase
ATP	Adenosine Triphosphate
PAM	Peptidoglycine alpha amidating monoxygenase
GADPH	Glyceraldehyde 3 Phosphate Dehydrogenase
SOD	Superoxide Dismutase
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine

SYMBOLS

mg	mili Gram
dl	deci Litre
cm	centimetre
min	minute
mmol/l	milimoles per litre
mm/Hg	milimeter of Mercury
kg/m^2	Kilogram per meter square
%	Percentage
nmol/mg	Nano mole per miligram
≥	Greater than or Equal

INTRODUCTION

INTRODUCTION

Hyperglycemia with irregularities in the metabolism of fats, carbohydrates and proteins brought on by problems in production of insulin, action of insulin, or both, characterises diabetes mellitus as a metabolic illness (Joslin et al., 2005). People with diabetes are more likely to acquire cataracts, erectile dysfunction, nonalcoholic fatty liver disease, heart, peripheral artery, and cerebrovascular disease in addition to these disorders .(WHO/UCN/NCD/20.1) It can cause early cardiovascular morbidity and mortality if untreated**. (Poretsky et al., 2015)**

According to WHO, 422 million people globally were suffering from diabetes in 2014.Age-adjusted prevalence in adults increased from 4.7% in 1980 to 8.5% in 2014, which is almost double, with low- and middle-income nations seeing the largest increases. By the year 2030, it is predicted that about 440 million people worldwide falling in the age range of 20-79 years will be suffering from diabetes, hence the danger of more and more cases of diabetes mellitus seems inevitable and will cause stress on global healthcare systems. **(WHO Report, 1985)**

Type 2 diabetes mellitus has the following risk factors, according to WHO:-

1) Being overweight or obesity (BMI > 27 kg/m^2 or > 120% desired body weight).

2) Lack of exercise

3) Age

4) First-degree relatives with diabetes

5) Gestational diabetes history

6) Cardiovascular diseases

7) Ethnicity: Hispanic, Afro-Caribbean, or South Asian

The symptoms of Type 2 Diabetes Mellitus include abnormal lipid metabolism, increased hepatic glucose synthesis brought on by insulin resistance at peripheral receptors, and decreased insulin secretion as a result of beta cell malfunction.

Measuring glycated haemoglobin is an excellent indicator for maintaining long-term blood glucose management. Under hyperglycemic conditions, proteins may undergo glycation, a non-enzymatic process in which the N terminal amino group of proteins generate a schiff's base with glucose that undergoes an amadori rearrangement .Once connected, glucose does not separate from haemoglobin and stays in the erythrocyte for the duration of its life. **(Sherwani et al., 2016)**

The average glucose level over the past 10–12 weeks is shown by the HbA1c level. Recent food intake or changes in blood sugar levels had little impact. Patients with diabetes should do the estimation every three months. When HbA1c is less than 6%, diabetes is being exceptionally well controlled.7% indicates adequate control, 8% inadequate control, and 9% very inadequate control values above 6% necessitate close monitoring, whereas numbers between 6.5 to 7% are regarded to suggest impaired glucose tolerance **(Bennett et al., 2007)**

When there is a disparity between the body's ability to eliminate reactive oxygen species (ROS) from the body or repair the damage they have produced, the body experiences oxidative stress. Free radicals and other highly reactive oxygen molecules known as ROS are produced naturally as byproducts of regular cellular metabolism **(Sies et al., 2000)**

Under normal circumstances, the body's antioxidant defense mechanisms and ROS generation are balanced by the actions of several low molecular weight antioxidants, like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and others. However, some substances can disturb this balance and lead to an excessive ROS buildup, which causes oxidative stress. These components could include trans-fat rich diets, sedentary lifestyles, smoking, or the ageing process

naturally. **(Halliwell et al., 1999)**

Oxidative stress has adverse consequences because of the high reactivity of ROS, which has the potential to harm cells' metabolic components. The development of a number of diseases like cancer, diabetes, Parkinson's disease, cardiovascular and neurological problems, may be caused by such damage, which can impede cellular activities. **(Garrido et al., 2004)**

Since they are present in practically all biological processes, proteins are particularly vulnerable to these reactive oxygen species. They can occasionally be subjected to oxidation, generating protein carbonyls. Under the influence of oxidative stress, which is caused by a chemical alteration occurs when proteins come into contact with ROS. Carbonylation is an irreversible, non-enzymatic post translation alteration that proteins can experience **(Fedorova et al., 2014)**

Certain amino acid residues, including as lysine, arginine, proline, and threonine, are carbonylated and then oxidised **(Dalle-Donne et al., 2006)** Loss of protein function: Carbonylation can disrupt the structure and function of proteins, impairing their normal physiological roles. This can lead to a dysfunction of enzymes, receptors, transporters, and other proteins essential for cellular processes. **(Stadtman et al., 1998)**

Altered protein-protein interactions: Carbonylated proteins may have reduced binding affinity or altered interactions with other proteins. This can interfere with signaling pathways, regulatory mechanisms, and overall cellular homeostasis. **(Davies et al., 1999)**

Protein aggregation: Carbonylated proteins are more prone to aggregate, leading to the formation of protein aggregates and insoluble deposits. Numerous neurodegenerative illnesses, of the likes of Alzheimer's and Parkinson's, can be due to the secondary effects of protein aggregation. **(Dean et al., 1997)**

Proteolytic susceptibility: Carbonylated proteins are often more susceptible to proteolytic degradation. This can result in increased turnover rates of carbonylated proteins and dysregulation of cellular protein balance. **(Davies et al., 2005)**

In a number of ways, PCOs excel at lipid peroxidation. Oxidised proteins are often more stable as compared to other oxidative stress markers like malondialdehyde and glutathione disulphide. As their elevation in serum persists for more than four hours, PCOs circulates in the bloodstream for longer periods of time and develop early. **(Pantke et al., 1999)**

Protein carbonylation is known to increase in diabetes due particularly in conditions of chronic hyperglycemia and oxidative stress. The following factors contribute to the increased protein carbonylation in diabetes:

1. Increased formation of ROS: Diabetes can cause non-enzymatic protein glycation, which results in the generation of AGEs. Diabetes is characterised by persistently elevated blood glucose levels, Because of AGEs' capacity to induce oxidative stress and trigger the generation of ROS, protein carbonylation is made easier. Additionally, the production of ROS by proteins modified by AGEs could serve as a source, exacerbating oxidative damage. **(Rabbani et al., 2012)**

2. Impaired antioxidant defense system: Antioxidant enzyme deficiencies, such as those in superoxide dismutase, catalase, and glutathione peroxidase, are frequently linked to diabetes. Oxidative stress and protein carbonylation are caused by cells having insufficient antioxidant capacity, which reduces their ability to combat the increased ROS generation. **(Brownlee et al., 2005)**

3. Activation of pro-inflammatory pathways: Chronic low-grade inflammation is a feature of diabetes. Oxidative stress can be exacerbated by inflammatory mediators such cytokines and chemokines, which can increase the generation of ROS. Protein carbonylation is further facilitated by this inflammatory environment. **(Hotamisligil et al., 2006)**

In diabetes, the buildup of carbonylated proteins can negatively impact cellular performance and lead to the emergence of diabetic complications. Carbonylated proteins have been linked to endothelial dysfunction, diabetic nephropathy, retinopathy, and neuropathy pathogenesis. They can also interfere with insulin signalling pathways.

In conclusion, research on protein carbonylation in Type 2 diabetes mellitus should be emphasised because it is of critical importance. The genesis and progression of problems associated with T2DM are influenced by the negative effects of protein carbonylation at the cellular level. We can learn a lot about the mechanisms that underlies protein carbonylation, as well as how it affects -cell function and insulin regulation, which may help us find new treatment targets and understand the pathophysiology of T2DM. According to earlier research **(Telci et al., 2000)**, oxidative stress, inflammation, and -cell dysfunction associated with T2DM are all impacted by protein carbonylation. **(Dayanand et al., 2012)** Further research on protein carbonylation in T2DM is therefore essential for expanding our understanding of the condition and creating focused therapies to improve patient outcomes.

REVIEW

OF

LITERATURE

REVIEW OF LITERATURE

Diabetes Mellitus is a diverse and intricate metabolic illness characterised by hyperglycemia and complications from early cardiovascular disease and small artery disease that result in renal failure, eye damage, and neuropathy. **(Poretsky et al., 2015)**

Epidemiological data reveal concerning trends and future projections for Type 2 diabetes mellitus (T2DM). In 2019, diabetes accounted for 4.2 million deaths globally and affected 453 million adults of middle age. It is expected that by 2045, this number will rise to approximately 700 million. T2DM also accounted for a significant healthcare expenditure of at least 720 billion USD in 2019. Moreover, the actual burden of T2DM may be higher as one in three individuals with diabetes are undiagnosed, which corresponds to around 232 million people. People with diabetes are most affected when they are between the ages of 40 and 59. Over 80% of T2DM patients reside in low- to middle-income countries, which makes it challenging to deliver the necessary care. T2DM incidence and prevalence vary by geographic area. When compared to people without diabetes, those with T2DM have a higher risk of up to 15% all-cause mortality, with cardiovascular illnesses being the main cause. **(I.D.F. et al., 2019)**

Although diabetes usually manifests in adulthood, younger people are now being diagnosed with it as a result of rising obesity rates, adopting a more westernized diet, and sedentary lifestyles. **(A.D.A. 2021)**

Insulin resistance, which occurs when cells become less receptive to the actions of insulin, and decreased insulin release by the beta cells of pancreas are both factors in type 2 diabetes. **(Norhammar et al., 2002)**

Complex interactions between environmental, genetic, and behavioural factors play a role in the development of type 2 diabetes. The difference between the consumption and expenditure of energy is caused by long-term overeating, inactivity, and excess body fat, which causes adipose tissue to malfunction and generate pro-inflammatory cytokines. These elements affect the absorption and

utilisation of glucose and largely cause insulin resistance in the liver, skeletal muscles and adipose tissue. **(Galicia-Garcia et al., 2020)**.

The pancreas tries to create more insulin as a compensatory approach to combat insulin resistance. But gradually, the beta cells might run out of energy, resulting in decreased insulin output and worsened glucose regulation. **(Di Pino et al., 2019)**.

Multiple organ systems are affected by the consequences of type 2 diabetes. The development of microvascular problems such diabetes retinopathy, diabetic kidney disease, and neuropathy due to diabetes may result from long-term hyperglycemia damaging small blood vessels **(American Diabetes Association, 2021)**. People with type 2 diabetes frequently experience macrovascular consequences, such as peripheral artery disease, cardiovascular disease, and stroke. **(Abaci et al., 1999)**.

Management of type 2 diabetes entails lifestyle adjustments, including adopting a nutritious diet, increasing physical exercise, and achieving weight loss if overweight or obese. Pharmacotherapy is often required to establish glycemic control, with many types of drugs available in the market. **(Rendell et al., 2004)**

In the process of producing insulin, beta cells are essential. Pre-proinsulin, which is converted into proinsulin in the endoplasmic reticulum, or ER, and Golgi apparatus, is the starting material for the synthesis of insulin**. (Cerf et al., 2013)** Up until it becomes active in hyperglycemic situations, mature insulin is kept in granules. Exocytosis of insulin is induced by calcium. Additionally, cAMP and extracellular ATP play a role in the release of insulin. These systems make sure that beta cells are able to properly control glucose levels. **(Boland et al., 2017)**

Beta-cell malfunction in type 2 diabetes mellitus seems intricate and not fully attributable to beta-cell death. It results from a complex interaction of environmental factors, biochemical pathways, and genetic predisposition. Diseases like being overweight or obesity, high blood sugar levels, and hyperlipidemia lead to insulin resistance, chronic inflammation, and cellular stress. Beta-cell destruction results from a combination of endoplasmic reticulum (ER) damage and the activation of

apoptotic pathways, which are caused by excessive quantities of unbound fatty acids along with elevated glucose levels. **(Christensen et al., 2019)** Sustained high glucose levels also increase the production of misfolded insulin and islet amyloid polypeptides, further disrupting cellular function and promoting inflammation. These disruptions in islet integrity impair communication between cells and contribute to insulin secretory dysfunction, a key factor in beta-cell failure and the development of T2DM **(Hoang Do et al., 2015)**

What is Protein Carbonylation?

Proteins that are the result of the chemical process of carbonylation, in which carbonyl groups (-C=O) are incorporated into the protein structure, are referred to as carbonylated proteins. When proteins are subjected to oxidative stress, this modification occurs as a result of the oxidation of specific amino acid residues, including lysine, arginine, proline, and threonine, which results in the formation of carbonyl groups on these residues **(Berlett et al., 1997)**. Due to their role as the main component of the majority of biological systems, proteins are particularly vulnerable to the destruction caused by oxidative stress. **(Dalle-Donne et al., 2006)**

Carbonylated proteins are distinguished by the presence of carbonyl groups, which can be detected and measured using specific analytical techniques such as mass spectrometry or immunochemical tests targeting carbonyl groups. The oxidation reaction due to carbonylation which is recognised as a characteristic of oxidative damage of proteins, has been linked to a number of pathological states and age-related diseases, including cancer, cardiovascular disease, and neurodegenerative disorders.. **(Dalle-Donne et al., 2003)**

Increased Protein Carbonylation in Type 2 Diabetes

Protein carbonylation is known to increase in diabetes due particularly in conditions of chronic hyperglycemia and oxidative stress. The following factors contribute to the increased protein carbonylation in diabetes:

1. Oxidative stress and increased reactive oxygen species (ROS) production: Diabetes may result in

abnormal protein compounds formed by glycation, which generates AGEs. AGEs' capacity to induce oxidative stress and the production of ROS enhance protein carbonylation. Additionally, the production of ROS by AGE-modified proteins directly may act as a source, exacerbating oxidative damage. **(Rabbani et al., 2012)**.

2. Impaired antioxidant defence system: Antioxidant deficiencies, such as those in enzymes superoxide dismutase, glutathione peroxidase, are frequently linked to diabetes. Oxidative stress and protein carbonylation are caused by cells having insufficient antioxidant capacity, which reduces their ability to combat the increased ROS generation. **(Brownlee et al., 2005)**

3 .Activation of pro-inflammatory pathways: Diabetes is characterized by persistant low-grade inflammation .cytokines and chemokines are inflammatory mediators that can stimulate ROS production and promote oxidative stress. This inflammatory environment further contributes to protein carbonylation **(Hotamisligil et al., 2006)**

Adverse Effects due to Protein Carbonylation

The accumulation of carbonylated proteins in diabetes can have severe effects on cellular function and add to the development of diabetic complications. Carbonylated proteins can impair insulin signaling pathways, promote endothelial dysfunction, and may increase the chances of diabetic kidney disorders, retinopathy, and neuropathy. **(Miyata et al., 1999)**

Protein carbonylation at the cellular level can lead to a cascade of physiological issues, culminating in larger complications. These can be of the following types-

1. Structural modifications: Protein carbonylation can result in structural changes, including alterations in protein conformation, stability, and solubility. Carbonylation can induce protein cross-linking, which can cause proteins to aggregate and form amyloid fibrils, which are associated with diabetic neuropathy and nephropathy. **(Butterfield et al., 2002)**

2. Enzymatic activity disruption: Carbonylation of enzymes can impair their catalytic activity and substrate binding capacity. For example, carbonylation of key enzymes involved in glucose

metabolism, such as pyruvate kinase, can lead to decreased enzymatic activity and dysregulated glucose metabolism in diabetes. **(Dalle-Donne et al., 2003)**

3. Protein-protein interactions: Carbonylation can interfere with protein-protein interactions, disrupting critical cellular signaling pathways. For instance, if insulin receptor substrate-1 (IRS-1) undergoes carbonylation, it will impair its interaction with insulin receptor, hampering insulin signaling and contributing to insulin resistance in diabetes. **(Stadtman et al., 2000)**

4. Oxidative modification: Protein carbonylation often occurs as a result of increased oxidative stress. Carbonylated proteins can undergo further oxidative modifications, such as amino acids side chains undergoing direct oxidation and formation of AGEs. These modifications can affect protein-protein interactions, enzymatic activity, and protein turnover processes. **(Miyata et al., 2000)**

5. Impaired protein degradation: Carbonylation can interfere with protein degradation pathways and autophagy. Carbonylated proteins may be less efficiently recognized and targeted for degradation, leading to their accumulation and the formation of protein aggregates, which are commonly observed in diabetic complications. **(Stolzing et al., 2001)**

Mechanisms involved in Protein Carbonylation

Having explored the causes and consequences of protein carbonylation in Type 2 diabetes mellitus (T2DM), it becomes evident that studying this phenomenon is of utmost importance. Protein carbonylation can occur in the following ways-

Direct Oxidation of Protein side chains

Reactive oxygen species (ROS) modify certain side chains of amino acids, mainly lysine (Lys) and arginine (Arg), during the direct oxidation method of protein carbonylation. ROS can be a result of normal physiological processes or as a result of increased oxidative stress. Hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•) and examples of such species. **(Valko et al., 2007)**.They can directly interact with certain amino acid side chains, particularly Lys and Arg, through oxidation reactions. These reactions involve the transfer of an oxygen atom or an oxygen-containing group to the amino

acid side chain, resulting in the formation of a carbonyl group (-C=O) **(Requena et al., 2003)** Protein carbonyl groups are created when the side chains of the impacted amino acids undergo chemical changes. These carbonyl groups serve as indicators of oxidative damage that can be found and measured**. (Dalle-Donne et al., 2006)**

Oxidation of Proteins to yield reactive carbonyl derivatives that result from Alpha Amidation Pathway

The alpha amidation pathway is responsible for the enzymatic conversion of carboxylic acid groups at the C-terminus of peptides into amides. This pathway involves several enzymatic steps, including the activation of peptidylglycine alpha-amidating monooxygenase (PAM) and subsequent catalysis of the alpha amidation reaction **(Eipper et al., 1988)**.These enzymes being essentially proteins are also susceptible to ROS attack which specifically target lysine and arginine residues. **(Requena et al., 2003)** reactive carbonyl derivatives, such as aldehydes and ketones are generated by direct oxidation of proteins by ROS, through the modification of amino acid side chains. These reactive carbonyl derivatives are highly reactive and can further react with nearby amino acid residues or other biomolecules, leading to the formation of carbonylated proteins **(Dalle-Donne et al., 2006)**

Metal catalysed oxidation of PUFA

Omega-3 and omega-6 fatty acids, are examples of PUFAs which have have many double bonds. Metal-catalyzed oxidation is a method whereby PUFAs can undergo oxidative processes with the help of metal ions, such as iron (Fe) and copper (Cu). **(Poli et al., 2004)**. This process involves production of ROS as intermediates. Fe and Cu, can react with hydrogen peroxide (H_2O_2) or molecular oxygen (O_2) to generate ROS, including hydroxyl radicals (OH•) and lipid peroxyl radicals (LOO•) **(Davies et al., 2005).** A chain lipid peroxidation is started by these hydroxyl and lipid peroxyl radicals. When PUFAs are degraded by oxidation, lipid hydroperoxides and a variety of reactive intermediates, such as aldehydes and ketones, are produced. **(Esterbauer et al., 1991)**. Malondialdehyde (MDA) and 4-hydroxynonenal (HNE), reactive carbonyl substances produced during lipid peroxidation, can

covalently alter proteins. In particular, these reactive carbonyl compounds can react with the residues of the amino acids lysine, arginine, and proline to produce adducts and carbonylated proteins. **(Dalle-Donne et al., 2006)**

Reducing sugars forming AGEs

In the procedure known as "non-enzymatic glycation," sugars with free aldehyde or ketone groups interact with proteins' amino groups to form Schiff bases, which undergo further rearrangements to create Amadori products**. (Vistoli et al., 2013)**.The Amadori products can then undergo a series of chemical reactions, including oxidation and dehydration, leading to the formation of reactive carbonyl compounds **(Monnier et al., 1984)**

Reactive carbonyl compounds, such as glyoxal, methylglyoxal, and 3-deoxyglucosone, can covalently modify proteins by forming stable adducts through nucleophilic reactions with amino acid residues, particularly lysine and arginine **(Ahmed et al., 2003)**. This process is referred to as protein carbonylation.

The formation of carbonylated proteins through the reaction of reducing sugars with proteins and subsequent formation of AGEs is a result several factors, including the concentration of reducing sugars, the duration of exposure, and the increase in oxidative stress **(Singh et al., 2001)**.

Use of HbA1c as Glycemic marker

HbA1c, or glycated hemoglobin, is a clinically significant biomarker used in the monitoring and diagnosis of diabetes mellitus. It provides an estimation of the average blood glucose levels over a specific period, typically the preceding 2-3 months. HbA1c reflects the percentage of hemoglobin A (HbA) that has glucose molecules attached to it. **(Petersmann et al., 2019)**.It is expressed as a percentage of total hemoglobin in the blood. It represents the proportion of glycated hemoglobinrelative to the total hemoglobin present. The normal range for individuals without diabetes is usually below 5.7%, whereas higher levels indicate poorer blood glucose control and the presence of diabetes.

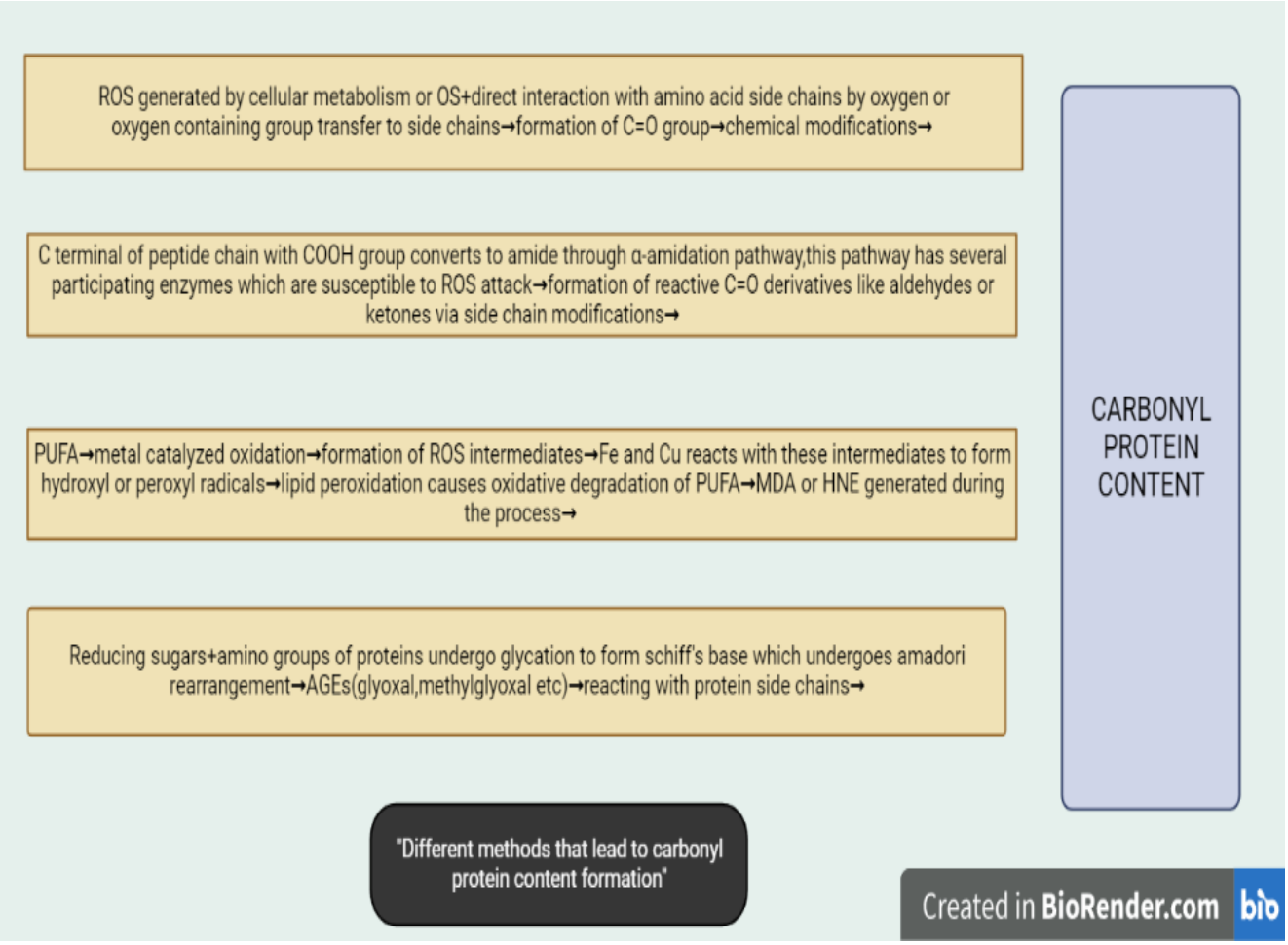


Fig.1 Different methods leading to protein carbonyl content formation.

In contrast to SMBG, which provides instantaneous results, HbA1c provides information on mean blood glucose levels over a lengthy period of time, often 2-3 months. It aids medical personnel in assessing the success of diabetes management techniques and modifying treatment approaches accordingly **(Sacks et al., 2013)**

HbA1c reflects the attachment of glucose to hemoglobin molecules in red blood cells. During hyperglycemia, more glucose molecules react non-enzymatically to hemoglobin, resulting in glycation and elevated HbA1c levels. As red blood cells have a lifespan of approximately 120 days, HbA1c measurement provides an estimation of average blood glucose levels over that time period.to ensure

consistent and reliable measurements, HbA1c assays are standardized across laboratories. IFCC established a reference method based on mass spectrometry, which provides accurate and comparable results across different laboratories. **(The Diabetes Control and Complications Trial Research Group, 1993)**

HbA1c is a useful test for evaluating and keeping track of glycemic management in diabetics. It assists in determining the efficiency of medicine, insulin therapy, and lifestyle changes in sustaining desired blood glucose levels. Healthcare professionals can make well-informed judgements about therapy modifications and interventions thanks to routine HbA1c testing. Several professional associations, notably the **American Diabetes Association (ADA)**, have recognized it as a diagnostic standard for diabetes mellitus. Diabetes is indicated by HbA1c result of 6.5% or above. When making a diagnosis, it's crucial to take other clinical criteria like symptoms and further diagnostic procedures into account.

HbA1c targets are established based on individual patient factors, such as age, comorbidities, and risk of hypoglycemia. The ADA recommends a general target of less than 7% for most non-pregnant adults with diabetes. However, individualized goals should be determined in consultation with healthcare providers to optimize outcomes while considering the patient's overall health status**.** **(American Diabetes Association 2022)**

Limitations of using HbA1c as a glycemic marker

Using HbA1c as a tool for diabetes mellitus management has several limitations and challenges that need to be considered. HbA1c levels can be influenced by factors other than average blood glucose levels, such as erythrocyte lifespan, hemoglobin variants, and certain medical conditions (e.g., anemia, hemoglobinopathies).

These factors can lead to inaccurate HbA1c measurements and affect the interpretation of glycemic control. **(Little et al., 2013)**, It also does not any provide real-time information about glucose fluctuations. It cannot capture short-term glycemic variability or detect hypoglycemic or

hyperglycemic episodes that may occur between HbA1c measurements. **(Sacks et al., 2013)**.Moreover the relationship between HbA1c and average blood glucose levels can vary among individuals. Factors such as age, race, ethnicity, and certain medical conditions can influence this relationship. Therefore, relying solely on HbA1c may not fully capture individual variations in glycemic control **(Weykamp et al., 2008)**. Certain medical conditions, such as chronic kidney disease and liver disease, can also affect HbA1c levels independently of glycemic control. In these cases, HbA1c may not accurately reflect the true average blood glucose levels. Despite immense international efforts, there is still a lack of standardization among different laboratory methods and equipment. This lack of standardization can lead to differences in reported HbA1c values, making it challenging to compare results from different laboratories **(Little et al., 2001)**.

HbA1c may also have limited applicability in certain populations, such as pregnant women, children, and individuals with hemoglobinopathies or conditions affecting erythrocyte turnover. Alternative measures, such as continuous glucose monitoring (CGM), may be more suitable for monitoring glycemic control in these populations. **(Chehregosha et al., 2019)**

It is important to consider these limitations and challenges when interpreting HbA1c results and making clinical decisions. Healthcare providers should integrate HbA1c measurements with other clinical information, such as SMBG, patient symptoms, and individual factors, to ensure comprehensive diabetes management.

In conclusion, the development of type 2 diabetes mellitus and the consequences linked to it have been linked to the buildup of protein carbonyls. The importance of protein carbonylation in T2DM has been clarified by a number of prior studies, emphasising the necessity for more research in this field. The study conducted by **Oriquat et al., (2013)** demonstrated a positive relationship between serum protein carbonyl content and glycated hemoglobin (HbA1c) levels in Egyptian T2DM patients, highlighting the potential role of protein carbonylation in glycemic control. Similarly, In T2DM patients with vascular problems, **Adeshara et al., (2017)** found a connection between plasma

glycation, membrane modification, and oxidative stress, demonstrating the role of protein carbonylation in diabetic vascular injury.

Furthermore, the research conducted by **Pandey et al. in 2010** and **Tupe et al. in 2014** highlighted the existence of protein oxidation biomarkers and the presence of oxidative stress in T2DM patients, emphasising the need of looking at protein carbonylation as a potential indicator of oxidative damage. Protein carbonylation negatively affects diabetes-related difficulties, as **Bigagli et al. (2012)** found a link between lipid and protein oxidation products, levels of antioxidants, and vascular complications in poorly managed T2DM.

Furthermore**, Chawla et al. (2014)** clarified the significance of AGE-induced receptor expression in diabetic vascular problems, raising the possibility of a connection between protein carbonylation and the emergence of diabetes difficulties. The importance of protein carbonylation as a factor to the aetiology of T2DM difficulties was supported by **Liu et al.'s 2022** investigation of fluorescent advanced glycation end products and their connection with diabetes duration, HbA1c, and diabetic comorbidities. In addition, **Ye et al., (2016)** and **Indyk et al., (2021)** offered additional insights into the serum's advanced glycation end products and their receptors, highlighting their role in T2DM.

When taken in entirety, these findings offer convincing proof of the importance of protein carbonylation in T2DM and related problems. Our research intends to advance understanding of the function of protein carbonylation in T2DM pathogenesis by analysing the protein carbonyl content in diagnosed T2DM patients and control subjects.

AIM

&

OBJECTIVES

AIM:

To find an association between protein carbonyl content and HbA1c in diagnosed Type 2 Diabetes Mellitus patients and control subjects

OBJECTIVES:

1. To determine the level of protein carbonyl content in diagnosed patients of Type 2 Diabetes Mellitus and control subjects.

2. To determine the level of HbA1c in diagnosed patients of Type 2 Diabetes Mellitus and control subjects.

3. To determine the correlation between level of protein carbonyl content and HbA1c of diagnosed Type 2 Diabetes Mellitus patients, if any

MATERIALS

&

METHODS

RESEARCH QUESTION:

Is there any significant association between the level of protein carbonyl content and HbA1c in diagnosed patients of Type 2 Diabetes Mellitus and control subjects?

NULL HYPOTHESIS (H_0):

There is no significant association between the level of protein carbonyl content and HbA1c in diagnosed patients of Type 2 Diabetes Mellitus and control subjects.

ALTERNATE HYPOTHESIS (H_1):

There is a significant association between the level of protein carbonyl content and HbA1c in diagnosed Type 2 Diabetes Mellitus patients and co

METHODOLOGY

Type of study - Case-Control study.

Study Design: -Prospective

PLACE OF STUDY:

Department of Biochemistry, Integral Institute of Medical Science and research, Lucknow (U.P).

COLLABORATING DEPARTMENT –

Department of General Medicine, OPD at IIMS&R, Integral University, Lucknow.

SUBJECTS SELECTION-:

SELECTION OF CONTROL SUBJECTS

1. Age between 35- 65 years.

2. Apparently healthy subjects.

3. Individuals who have agreed to sign the consent form.

SELECTION OF CASES:

Inclusion Criteria (Cases):

1. Subjects within the age of 35 to 65 year.

2. Diagnosed Patients of Type 2 Diabetes Mellitus (diabetes duration less than 10 years)

3. Known Diabetic cases without complications.

4. Patients who have agreed to sign the consent form.

Exclusion Criteria

1. Type 1 Diabetes Mellitus patients

2. History of smoking and alcoholism.

3. History of chronic illnesses

4. History of renal or liver disorders

Enrollment of participants:

Cases were enrolled from Type 2 Diabetes Mellitus Patients attending the Integral Hospital.

Data collection

A detailed clinical history including age, occupation, socio-economic status, and any associated risk factors contributing to the illness was elicited from Type 2 Diabetes Mellitus patients and control subjects.

Sampling Method: Purposive sampling.

Collection of samples: -

Under aseptic conditions, 2 ml of venous blood was collected in the Lavender top (EDTA whole blood vial) from the subjects.

Storage of samples:-

The samples for estimation of Protein carbonyl content and HbA1c were stored at -20℃ until Testing in Central Clinical Laboratory, Department of Biochemistry, IIMS&R, Lucknow (U.P).

Sample Size Estimation

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2(Z_\beta + Z_{\alpha/2})^2}{(d\text{ifference})^2}$$

{Charan et al., (2013)}

r = 1 (control to case ratio)

$\mathbf{Z}_{\boldsymbol{\beta}}$ = it has desired power (0.84 for 80% power)

$\mathbf{Z}\boldsymbol{\alpha}_{/2}$ = critical value and a standard value for the corresponding value for confidence (at 95% CI it is 1.96)

$\boldsymbol{\sigma}^2$ = 0.47 standard deviation

d = 0.33 effect size (expected difference in the mean)

n = 30

Group 1 = 30

Group 2 = 30

Total sample size =60

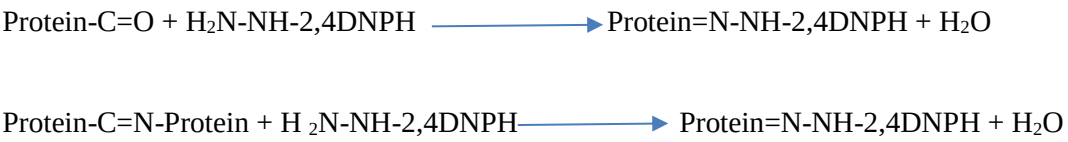
Reference paper: Dayanand et al., (2012)

LABORATORY INVESTIGATION:

1) PROTEIN CARBONYL CONTENT ESTIMATION

Protein carbonyl content was estimated according to the method described by **Levine et al., (2000)** which is a highly sensitive assay.

Principle- 2, 4 Dinitrophenyl hydrazine (DNPH) reacts with protein carbonyls to form a Schiff base to produce 2, 4 dinitrophenylhydrazone products measured spectrophotometrically at 370 nm



Reagents:

1. HCl, 2 M
2. 2, 4-dinitrophenylhydrazine 10 mM, in 2 M HCl
3. Trichloroacetic acid, 20% (w/v)
4. Guanidine, 6 M, with 20 mM potassium phosphate, adjusted to pH 2.3 with trifluoroacetic Acid.
5. Streptomycin sulfate, 10% in 50mM HEPES (Ph 7.2)

Procedure:

1. Prepare the extract of bacterial or mammalian cells as desired; the final protein concentration should be no greater than 5 mg/ml.
2. Centrifuge to remove debris. Add 1 volume streptomycin solution to 9 volumes of

Supernatant and allow it to stand for 15 min. Centrifuge at 11,000 g for 10 min and discard The pellet. Use the supernatant for assay of protein-bound carbonyl groups.

3. Pipette the protein solution into 1.5-ml centrifuge tubes (e.g., Sarstedt or Eppendorf) and Either dry in a vacuum centrifuge or precipitate with trichloroacetic acid).

4. To each tube, add 500/~1 of 10 mM 2, 4 -dinitrophenylhydrazine in 2 M HC1 and allow it to stand at room temperature for 1 hr, with vortexing every 10—15 minutes.

5. Then add 500 ~1 of 20% trichloroacetic acid, centrifuge the tubes in a tabletop microcentrifuge (11,000 g) for 3 min, and discard the supernatant.

6. Wash the pellets 3 times with 1 ml ethanol-ethyl acetate (1: l) to remove free reagent, allowing the sample to stand 10 min before centrifugation and discarding the supernatant each time.

7. Redissolve the precipitated protein in 0.6 ml guanidine solution. Proteins usually redissolve within 15 min at 37 °. Remove any insoluble material by centrifugation in the microcentrifuge for 3 min.

8. Obtain the spectrum, read against the complementary blank in the case of cruder samples or against water in the case of purified proteins. Calculate the carbonyl content from the maximum absorbance (360-390 nm) using a molar absorption coefficient 37 of 22,000 M -~cm-L

2) HbA1c ANALYSIS USING ERBA SEMI AUTO ANALYZER KIT

Principle- Total Hb and HbA1c in hemolyzed blood bind with the same affinity to particles in R1.The amount of binding is proportional to the relative concentration of both substances in the blood. Mouse anti-human HbA1c monoclonal antibody (R2a) binds to particle bound HbA1c. Goat anti- -mouse IgG polyclonal antibody (R2b) interacts with the monoclonal mouse anti-human HbA1c antibody and agglutination takes place. The measured absorbance is proportional to the HbA1c bound to particles,

which in turn is proportional to the percentage of HbA1c in the sample.

Reagent Composition-

R1: Buffer 20 mmol/l,Latex 1.5 %

R2a: Buffer 10 mmol/l,Mouse anti-human HbA1c monoclonal antibody 5.5 mg/dl

R2b: Buffer 1 mmol/l,Goat anti-mouse IgG polyclonal antibody 67 mg/dl,Stabilizers

R3: Hemolyzing solution.

Reagent Preparation- Transfer 4 ml of R2b into bottle R2a and mix well immediately.

Ratio between R2a and R2b must be 2/1.

Stability of premixed R2a/R2b: One month stored at 2–8°C.

Assay Procedure-

Wavelength 660 nm

Optical path 1 cm

Temperature 37 °C

Measurement Against air

Calculation- The results are automatically calculated by the machine, and need to be noted down.

SAMPLE OR CALIBRATOR	20 microlitres
REAGENT 1	750 microlitres
Mix, incubate for 3 min, then add,	
REAGENT 2a	250 microlitres
Mix, incubate for 3 min ,then add,	
REAGENT 2b	125 microlitres
Mix ,read absorbance after exactly 5 minutes	

ETHICS REVIEW

Permission from the Integral University ethics committee was taken **(IEC/IIMS&R 2023/71)**

STATISTICAL ANALYSIS

Statistical analysis was performed using IBMSPSS software (version 16), Graph Pad Software (version 6.0) and Microsoft – Excel (2007). All the data was expressed as mean ±standard deviation. An unpaired t-test was performed to compare the study parameters between cases and controls. Karl Pearson's correlation analysis was employed to determine the relationship between variables. p-value <0.05 was considered statistically significant.

OBSERVATION
&
RESULTS

Total 60 subjects (30 cases and 30 controls) were enrolled for this study. Results showed that Protein carbonyl content was remarkably increased in cases as compared to controls (p=0.004) (Fig.2) Similarly HbA1c levels were also elevated significantly in cases as compared to controls (p= 0.002)(Fig.3).

Table.1 Demographics and biochemical parameters in control subjects and cases.

Parameters	**Cases(n=30)**	**Control(n=30**	**p-value**	**Significance**
BMI	28.23±2.94	29.50±2.42	0.074	Not Significant
AGE	49.03±7.91	47.9±8.22	0.588	Not Significant
PCC	0.65±0.103	1.48±0.389	**0.004**	Significant
HbA1c	5.37±0.27	8.20±2.033	**0.002**	Significant

n=number of samples, $p < 0.05$ considered statistically significant

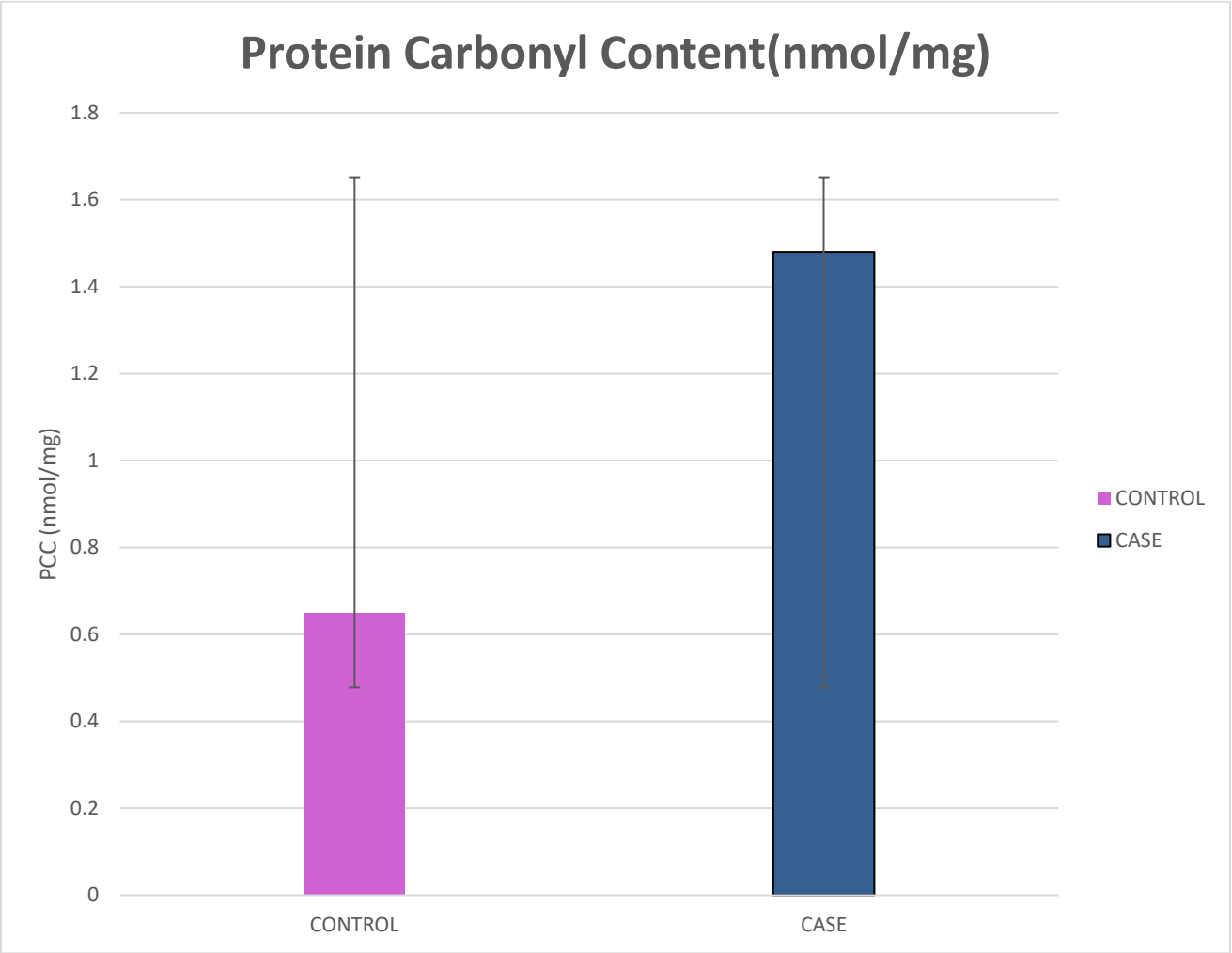


Fig.2 Comparison of Protein Carbonyl Content (nmol/mg) between Control and Cases

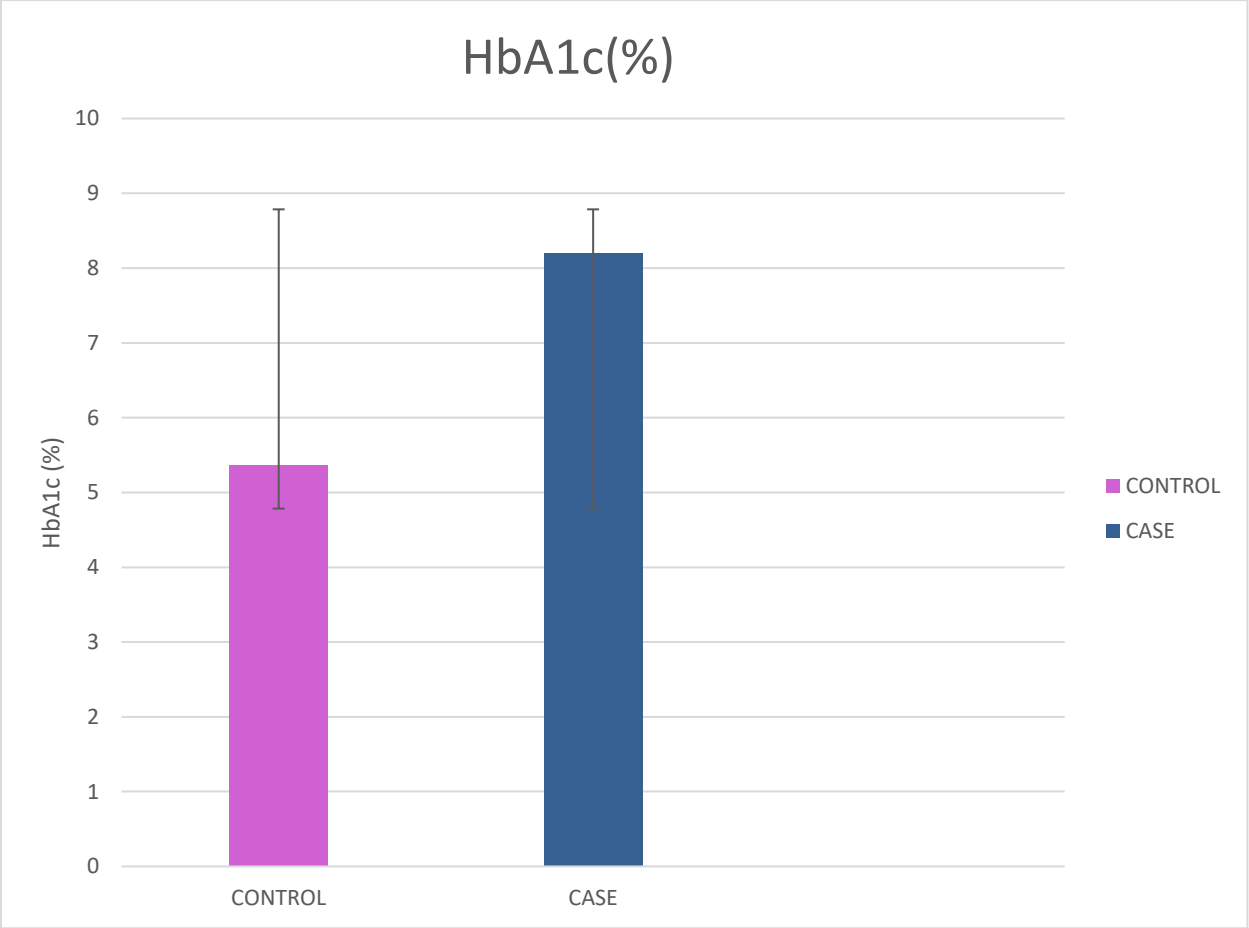


Fig. 3 Comparison of HbA1c (%) between Control and Cases

Table.2 Karl Pearson's Correlation Coefficient among the study parameters in cases.

Correlation is Significant at the 0.01 level (2-tailed)

	BMI	**PCC**	**HbA1c**
BMI	1	**.0535**	**.560**
Sig. 2 tailed		.002	.001
n	30	30	30
PCC	**.535**	1	**.897**
Sig. 2 tailed	.002		<.001
n	30	30	30
HbA1c	**.560**	**.897**	1
Sig. 2 tailed	.001	<.001	
n	30	30	30

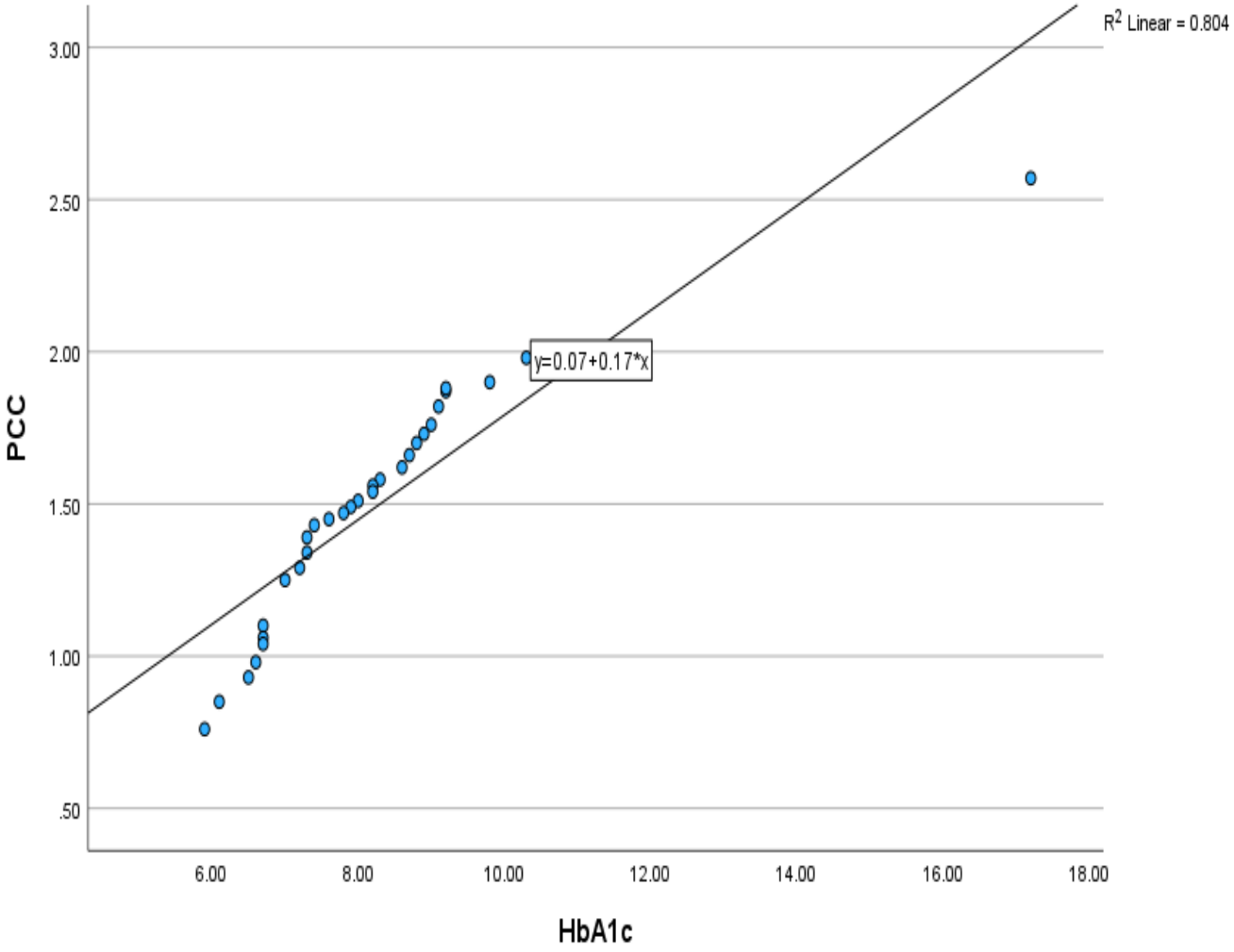


Fig.4 A scatter plot between levels of HbA1c and Protein carbonyl content (PCC) of cases.

RESULT

This study was designed to check for an association, if any between Protein carbonyl content and HbA1c in Type 2 diabetes mellitus patients and controls.

The parameters estimated were:

1) Protein Carbonyl Content (nmol/mg)

2) HbA1c (%)

The parameters levels were compared with control levels, who are apparently healthy individuals.

The study observations were as follows-:

1) The level of HbA1c (%) was significantly increased in cases ($p=0.002$) as compared to control subjects.

2) The level of Protein Carbonyl Content (nmol/mg) was significantly increased in cases ($p=0.004$) as compared to control subjects.

3) There was a significant positive correlation between Protein Carbonyl content and HbA1c among cases.

DISCUSSION

DISCUSSION

The purpose of the current study was to compare the oxidative stress indicators HbA1c and protein carbonyl concentration in individuals with type 2 diabetes mellitus (T2DM) and apparently healthy controls. Our study's results demostrated a positive correlation between protein carbonyl concentration and HbA1cwhich was statistically significant, suggesting a possible connection between glycemic management and oxidative damage in T2DM. These results align with previous studies conducted in the field.

Dayanand et al., (2012) demonstrated the validity of protein carbonyl content as a reliable marker of oxidative damage in type 2 diabetes. Their findings support the hypothesis that the pathophysiology of T2DM is associated with increased protein carbonyl content, a sign of increased oxidative stress. Similar to this, Sharma et al. (2021) demonstrated the importance of oxidative stress in T2DM by discovering a significant correlation between protein carbonyl content and glycated haemoglobin.

Mahmoud et al., (2013) examined the activity of glucose-6-phosphate dehydrogenase and protein modification due to oxidative stress in T2DM patients, highlighting the significance of oxidative stress in the disease. Their findings provide more evidence for the significance of protein the carbonylation process as a biological indicator of oxidative injury in T2DM.

The results of our investigation have significant importance for ongoing research and clinical practice. Firstly, our results support the potential utility of protein carbonyl content as a biomarker for assessing oxidative stress levels in T2DM patients. Monitoring protein carbonyl levels may aid in evaluating disease progression, assessing treatment efficacy, and identifying high risk individuals for developing diabetes-related complications.

In addition, our study highlights the need for further investigations with larger sample sizes and diverse geographical populations. The generalizability of the results is constrained by the study's limited sample size, as was acknowledged. Future studies should aim to include a larger and more

diverse cohort to validate the observed correlations and provide a comprehensive understanding of the relationship between HbA1c and protein carbonyl content in T2DM.

Our study's findings, which show a significant positive association of HbA1c with protein carbonyl concentration in T2DM patients, raise the possibility that oxidative stress plays a role in the development of the condition. The results confirm the significance of evaluating the extent of carbonylation in proteins as a sign of excessive oxidative damage and the possible application of this biomarker in the treatment of T2DM. However, the shortcomings of our investigation, such as the small sample size and geographical restrictions previously noted, call for additional research to confirm our findings and offer more thorough insights into the function of protein carbonylation in T2DM.

SUMMARY

AND

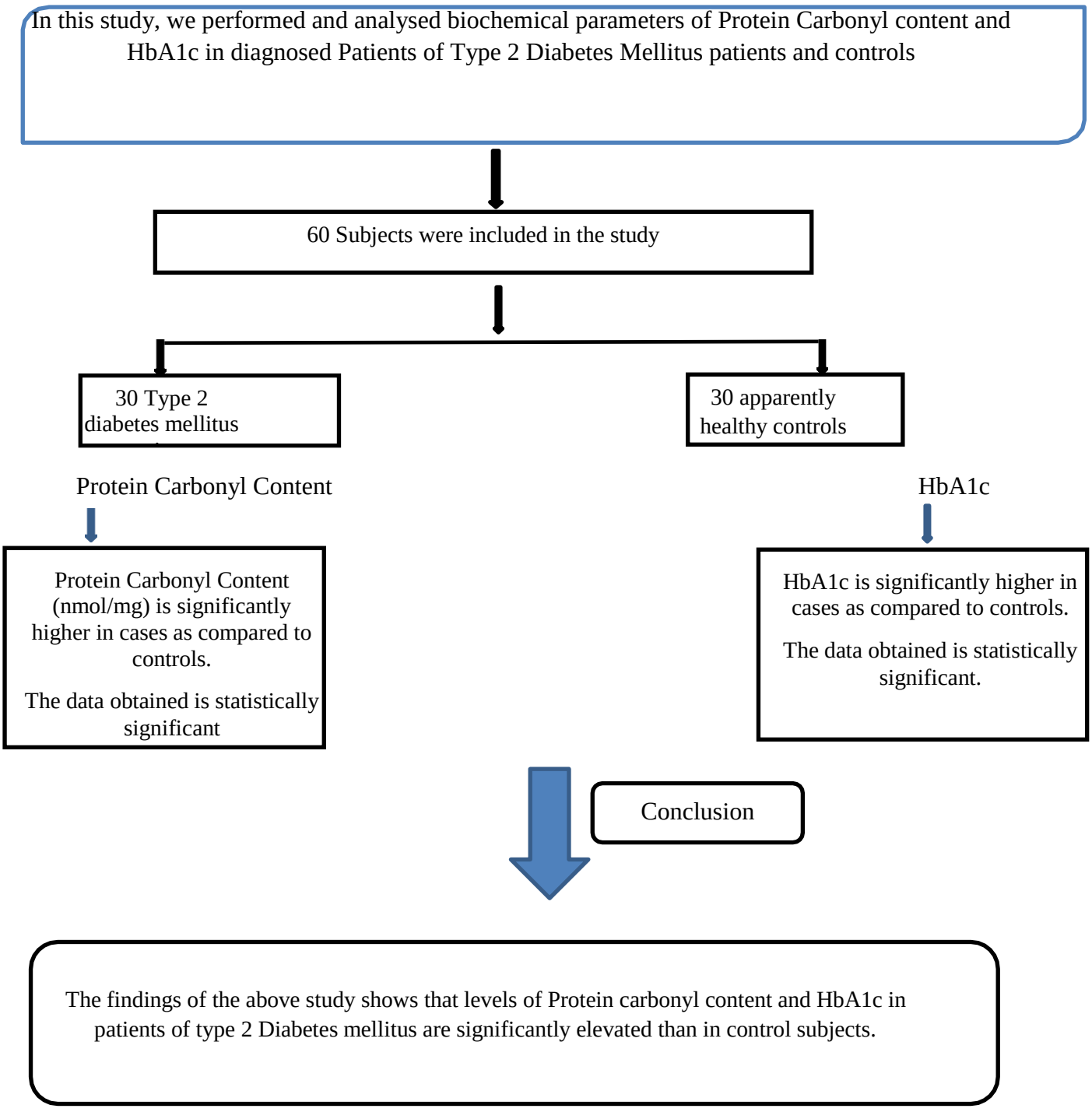
CONCLUSION

SUMMARY

The goal of the study was to examine the carbonyl protein content and percentage of HbA1c in type 2 diabetic mellitus patients and control individuals. The study used a manual Levine method to detect protein carbonyl levels, with a total of 30 cases and 30 controls. The results showed a significant positive link between HbA1c and protein carbonyl concentration, suggesting that glycemic management and oxidative damage in T2DM may be related. The study was constrained, nonetheless, by the small sample size and constrained geographic distribution. It is necessary to conduct a more thorough research with a bigger sample size and a broader location.

CONCLUSION

The results of the current investigation supports the alternate hypothesis that increased oxidative stress may play a role in the pathogenesis of T2DM by showing a positive connection between HbA1c and protein carbonyl concentration. The findings support earlier research, highlighting the importance of protein carbonylation as a biomarker of oxidative damage in T2DM. Screening protein carbonyl concentrations may have consequences for determining those at risk of problems, tracking the effectiveness of treatment, and gauging the development of the disease. Future research should, however, address the study's shortcomings, including the small number of participants and constrained geographic scope. To confirm the observed associations and offer a thorough knowledge of the link between HbA1c and protein carbonyl concentration in T2DM, larger and more diverse cohorts are required.


In this study, we performed and analysed biochemical parameters of Protein Carbonyl content and HbA1c in diagnosed Patients of Type 2 Diabetes Mellitus patients and controls
60 Subjects were included in the study
30 Type 2 diabetes mellitus
30 apparently healthy controls
Protein Carbonyl Content
HbA1c
Protein Carbonyl Content (nmol/mg) is significantly higher in cases as compared to controls.
The data obtained is statistically significant
HbA1c is significantly higher in cases as compared to controls.
The data obtained is statistically significant.
Conclusion
The findings of the above study shows that levels of Protein carbonyl content and HbA1c in patients of type 2 Diabetes mellitus are significantly elevated than in control subjects.

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ANNEXURES

Unique Identification No:

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH LUCKNOW -226026

INCLUSION AND EXCLUSION CRITERIA -CASES

Inclusion Criteria

S.N.	Criteria	YES	NO
1.	Diagnosed cases of T2DM		
2.	Subjects within the age of 35 to 65 years		
3.	Not on insulin therapy		
4.	Diagnosed patients of Type 2 Diabetes mellitus without complications		

Exclusion Criteria

S.N.	Criteria	YES	NO
1.	Type 1 Diabetes Mellitus patient		
2.	Diagnosed Diabetes mellitus patient on insulin therapy/diuretics therapy		
3.	History of chronic illnesses		
4.	Below 35 years of age		

Subject is eligible for the study, if all **INCLUSION** criteria are **YES** and all **EXCLUSION**

Criteria are No.

INVESTIGATOR STATEMENT

I have verified the data entered in the case report form and have determined that it is complete, accurate and compatible with the source documents

Investigator's name Investigator's signature Date

Unique Identification No

IDENTIFIERS- CASES

Registration No:

Contact No:

Name:

Father's Name /Husband's Name:

Address:

DEMOGRAPHICS- CASES

Age:

Sex: Male Female

Place of Residence: Urban Rural

Social / Economical Status: a) Upper b) Upper Middle c) Lower Middle

d) Upper Lower e) Lower

Education: a) Illiterate b) Primary c) Middle d) High School e) Intermediate

f) Graduation g) Post-graduation & above

ANTHROPOMETRIC PARAMETERS- CASES

Body Mass Index (kg/ m^2): Physical activity (Sedentary/Moderate/Active):

Unique Identification No:

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH LUCKNOW -226026

INCLUSION AND EXCLUSION CRITERIA -CONTROLS

Inclusion Criteria

S.N.	Criteria	YES	NO
1.	Apparently healthy individuals		
2.	Subjects within the age of 35 to 65 years		

Subject is eligible for the study, if all **INCLUSION** criteria are **YES** and all

EXCLUSION

Criteria are **No.**

INVESTIGATOR STATEMENT

I have verified the data entered in the control report form and have determined that it is complete, accurate and compatible with the source documents

Investigator's name Investigator's signature
Date

Unique Identification No:

IDENTIFIERS- CONTROL

Registration No:

Contact No:

Name:

Father's Name /Husband's Name:

Address:

DEMOGRAPHICS- CONTROL

Age:

Sex: Male Female

Place of Residence: Urban Rural

Social / Economical Status:

Education: a) Illiterate b) Primary c) Middle d) High School e) Intermediate

f) Graduation g) Post-graduation & above

ANTHROPOMETRIC PARAMETERS- CONTROL

Body Mass Index (kg/ m^2)

Physical Activity (Sedentary/Moderate/Active):

ANNEXURE I (A)

INFORMED CONSENT FORM

1. I am Apoorva,an MSc Medical Biochemistry 3rd year student, IIMS&R Lucknow.
2. For this study, I will take your 2 ml blood sample for the estimation of HbA1c and Protein carbonyl content.
3. The blood is only subjected for estimation of HbA1c and Protein carbonyl content. and not for any other purpose.
4. There will be no charges /fees/any consideration will be given or taken for the study.
5. Your identity will be confidential and information and the result of your blood test will not be revealed to any other except you if you desire.
6. This study has nothing to do with your treatment nor is it going to hamper the same if you refuse to participate.
7. The study has nothing to do with your current treatment but may improve the knowledge and understanding of the disease process and that knowledge may or may not be helpful in future.
8. After knowing all the above details, would you like to participate in our study? YES / NO

CONSENT FORM

I……..…age.....W/OD/OS/O.....
.....
R/O.....…… here with state that I have been duly informed about the study Titled: "A STUDY OF PROTEIN CARBONYL CONTENT AND HbA1c IN DIAGNOSED PATIENTS OF TYPE 2 DIABETES MERLLITUS AND CONTROL SUBJECTS" its prospects and consequences.

I hereby give informed and written consent for the collection of my blood sample for the above said study only.

Signature/thumb impression of the patient:

Signature/thumb impression of the witness Signature of research scholar:

PATIENT'S MEDICAL HISTORY

Name of the Patient-

Age-

1.) Currently used medications

2.) Duration of Diabetes mellitus

3.) Any first relative with Diabetes

Mellitus

4.) BMI

5.) History of any Chronic Illnesses

6.) History of Smoking or Alcoholism

अनुबंध I (ए)

सूचित सहमति फॉर्म

मैं अपूर्वा एमएससी बायोकैमिस्ट्री तृतीय वर्ष की छात्रा मेडिकल, आईआईएमएस और आर लखनऊ हूं।

इस अध्ययन के लिए, मैं प्रोटीन कर्बोनील कंटेंट एंड HbA1c के आकलन के लिए आपके 2 मिलीलीटर रक्त का नमूना लूंगी
रक्त केवल प्रोटीन कर्बोनील कंटेंट एंड HbA1c के आकलन के अधीन है और किसी अन्य उद्देश्य के लिए नहीं।
कोई शुल्क/शुल्क नहीं होगा/अध्ययन के लिए कोई विचार किया जाएगा या लिया जाएगा।

आपकी पहचान गोपनीय और जानकारीपूर्ण होगी और यदि आप चाहें तो आपके रक्त परीक्षण का परिणाम आपके अलावा किसी अन्य को प्रकट नहीं किया जाएगा।
इस अध्ययन का आपके उपचार से कोई लेना-देना नहीं है और न ही यदि आप भाग लेने से इंकार करते हैं तो इससे इसमें बाधा नहीं आएगी।
अध्ययन का आपके वर्तमान उपचार से कोई लेना-देना नहीं है, लेकिन यह रोग प्रक्रिया के ज्ञान और समझ में सुधार कर सकता है और यह ज्ञान भविष्य में सहायक हो भी सकता है और नहीं भी।
उपरोक्त सभी विवरणों को जानने के बाद, क्या आप हमारे अध्ययन में भाग लेना चाहेंगे? हां नहीं

सहमति प्रपत्र

मैं ……आयु……डब्ल्यू/ओडी/ओएस/ओ…… ....आर/ओ..... यहाँ यह बताने के साथ कि मुझे अध्ययन के बारे में विधिवत सूचित किया गया है: " आ स्टडी ऑफ प्रोटीन कर्बोनील कंटेंट एंड HbA1c इन दिग्नोसेड पेशेंट ऑफ टाइप टू डाबेटेस् मलिट्स एंड कंट्रोल सब्जेक्ट "
संभावनाएं और परिणाम।
मैं केवल उपरोक्त अध्ययन के लिए अपने रक्त के नमूने के संग्रह के लिए सूचित और लिखित सहमति देता हूं।

रोगी के हस्ताक्षर/अंगूठे का निशान:

साक्षी के हस्ताक्षर/अंगूठे का निशान शोधार्थी के हस्ताक्षरः

INSTITUTIONAL ETHICS COMMITTEE (IEC)

IIMS&R INTEGRAL UNIVERSITY, LUCKNOW

IEC/IIMS&R/2023/71

CERTIFICATE

This is to certify that research work entitled "**A Study of Protein Carbonyl Content and HbA1c in Diagnosed Patients of type 2 Diabetes Mellitus And Control Subjects**" submitted by **Apoorva, Dr. Roshan Alam** for ethical approval before the Institutional Ethics Committee IIMS&R.

The above mentioned research work has been approved by Institutional Ethics Committee, IIMS&R with consensus in the meeting held on **30th December 2022.**

Dr.Q.S.Ahmed
(Member Secretary)
IRC/IEC
IIMS &R

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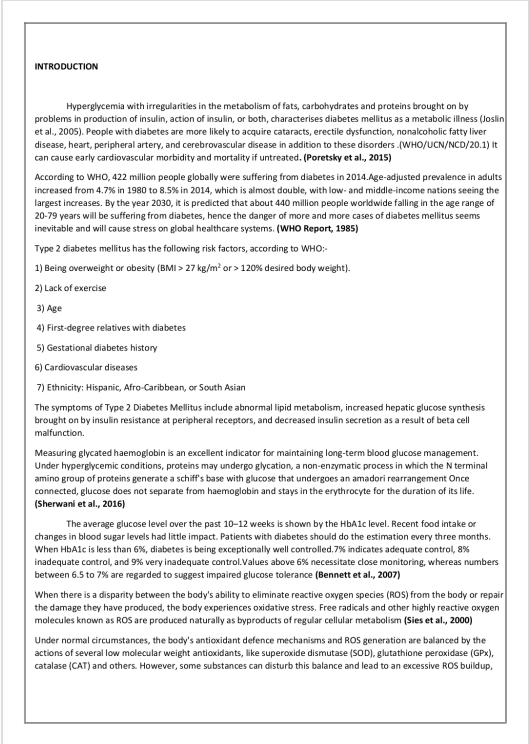

INTRODUCTION

Hyperglycemia with irregularities in the metabolism of fats, carbohydrates and proteins brought on by problems in production of insulin, action of insulin, or both, characterises diabetes mellitus as a metabolic illness (Joslin et al., 2005). People with diabetes are more likely to acquire cataracts, erectile dysfunction, nonalcoholic fatty liver disease, heart, peripheral artery, and cerebrovascular disease in addition to these disorders .(WHO/UCN/NCD/20.1) It can cause early cardiovascular morbidity and mortality if untreated. **(Poretsky et al., 2015)**

According to WHO, 422 million people globally were suffering from diabetes in 2014.Age-adjusted prevalence in adults increased from 4.7% in 1980 to 8.5% in 2014, which is almost double, with low- and middle-income nations seeing the largest increases. By the year 2030, it is predicted that about 440 million people worldwide falling in the age range of 20-79 years will be suffering from diabetes, hence the danger of more and more cases of diabetes mellitus seems inevitable and will cause stress on global healthcare systems. **(WHO Report, 1985)**

Type 2 diabetes mellitus has the following risk factors, according to WHO:-

1) Being overweight or obesity (BMI > 27 kg/m^2 or > 120% desired body weight).

2) Lack of exercise

3) Age

4) First-degree relatives with diabetes

5) Gestational diabetes history

6) Cardiovascular diseases

7) Ethnicity: Hispanic, Afro-Caribbean, or South Asian

The symptoms of Type 2 Diabetes Mellitus include abnormal lipid metabolism, increased hepatic glucose synthesis brought on by insulin resistance at peripheral receptors, and decreased insulin secretion as a result of beta cell malfunction.

Measuring glycated haemoglobin is an excellent indicator for maintaining long-term blood glucose management. Under hyperglycemic conditions, proteins may undergo glycation, a non-enzymatic process in which the N terminal amino group of proteins generate a schiff's base with glucose that undergoes an amadori rearrangement Once connected, glucose does not separate from haemoglobin and stays in the erythrocyte for the duration of its life. **(Sherwani et al., 2016)**

The average glucose level over the past 10–12 weeks is shown by the HbA1c level. Recent food intake or changes in blood sugar levels had little impact. Patients with diabetes should do the estimation every three months. When HbA1c is less than 6%, diabetes is being exceptionally well controlled.7% indicates adequate control, 8% inadequate control, and 9% very inadequate control.Values above 6% necessitate close monitoring, whereas numbers between 6.5 to 7% are regarded to suggest impaired glucose tolerance **(Bennett et al., 2007)**

When there is a disparity between the body's ability to eliminate reactive oxygen species (ROS) from the body or repair the damage they have produced, the body experiences oxidative stress. Free radicals and other highly reactive oxygen molecules known as ROS are produced naturally as byproducts of regular cellular metabolism **(Sies et al., 2000)**

Under normal circumstances, the body's antioxidant defence mechanisms and ROS generation are balanced by the actions of several low molecular weight antioxidants, like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and others. However, some substances can disturb this balance and lead to an excessive ROS buildup,

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Under normal circumstances, the body's antioxidant defense mechanisms and ROS generation are balanced by the actions of several low molecular weight antioxidants, like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and others. However, some substances can disturb this balance and lead to an excessive ROS buildup,

which causes oxidative stress. These components could include transfat-rich diets, sedentary lifestyles, smoking, or the ageing process naturally. **(Halliwell et al., 1999)**

Oxidative stress has adverse consequences because of the high reactivity of ROS, which has the potential to harm cells' metabolic components. The development of a number of diseases like cancer, diabetes, Parkinson's disease, cardiovascular and neurological problems, may be caused by such damage, which can impede cellular activities. **(Garrido et al., 2004)**

Since they are present in practically all biological processes, proteins are particularly vulnerable to these reactive oxygen species. They can occasionally be subjected to oxidation, generating protein carbonyls. Under the influence of oxidative stress, which is caused by a chemical alteration occurs when proteins come into contact with ROS. Carbonylation is an irreversible, non-enzymatic post translation alteration that proteins can experience **(Fedorova et al., 2014)**

Certain amino acid residues, including as lysine, arginine, proline, and threonine, are carbonylated and then oxidised **(Dalle-Donne et al., 2006)** Loss of protein function: Carbonylation can disrupt the structure and function of proteins, impairing their normal physiological roles. This can lead to a dysfunction of enzymes, receptors, transporters, and other proteins essential for cellular processes. **(Stadtman et al., 1998)**

Altered protein-protein interactions: Carbonylated proteins may have reduced binding affinity or altered interactions with other proteins. This can interfere with signaling pathways, regulatory mechanisms, and overall cellular homeostasis. **(Davies et al., 1999)**

Protein aggregation: Carbonylated proteins are more prone to aggregate, leading to the formation of protein aggregates and insoluble deposits. Numerous neurodegenerative illnesses, of the likes of Alzheimer's and Parkinson's, can be due to the secondary effects of protein aggregation. **(Dean et al., 1997)**

Proteolytic susceptibility: Carbonylated proteins are often more susceptible to proteolytic degradation. This can result in increased turnover rates of carbonylated proteins and dysregulation of cellular protein balance. **(Davies et al., 2005)**

In a number of ways, PCOs excel at lipid peroxidation. Oxidised proteins are often more stable as compared to other oxidative stress markers like malondialdehyde and glutathione disulphide. As their elevation in serum persists for more than four hours, PCOs circulates in the bloodstream for longer periods of time and develop early. **(Pantke et al., 1999)**

Protein carbonylation is known to increase in diabetes due particularly in conditions of chronic hyperglycemia and oxidative stress. The following factors contribute to the increased protein carbonylation in diabetes:

1. Increased formation of ROS: Diabetes can cause non-enzymatic protein glycation, which results in the generation of AGEs. Diabetes is characterised by persistently elevated blood glucose levels, Because of AGEs' capacity to induce oxidative stress and trigger the generation of ROS, protein carbonylation is made easier. Additionally, the production of ROS by proteins modified by AGEs could serve as a source, exacerbating oxidative damage. **(Rabbani et al., 2012)**

2. Impaired antioxidant defense system: Antioxidant enzyme deficiencies, such as those in superoxide dismutase, catalase, and glutathione peroxidase, are frequently linked to diabetes. Oxidative stress and protein carbonylation are caused by cells having insufficient antioxidant capacity, which reduces their ability to combat the increased ROS generation. **(Brownlee et al., 2005)**

3. Activation of pro-inflammatory pathways: Chronic low-grade inflammation is a feature of diabetes. Oxidative stress can be exacerbated by inflammatory mediators such cytokines and chemokines, which can increase the generation of ROS. Protein carbonylation is further facilitated by this inflammatory environment. **(Hotamisligil et al., 2006)**

In diabetes, the buildup of carbonylated proteins can negatively impact cellular performance and lead to the emergence of diabetic complications. Carbonylated proteins have been linked to endothelial dysfunction, diabetic nephropathy, retinopathy, and neuropathy pathogenesis. They can also interfere with insulin signalling pathways.

In conclusion, research on protein carbonylation in Type 2 diabetes mellitus should be emphasised because it is of critical importance. The genesis and progression of problems associated with T2DM are influenced by the negative effects of protein carbonylation at the cellular level. We can learn a lot about the mechanisms that underlies protein carbonylation, as well as how it affects -cell function and insulin regulation, which may help us find new treatment targets and understand the pathophysiology of T2DM. According to earlier research **(Telci et al., 2000)**, oxidative stress, inflammation, and -cell dysfunction associated with T2DM are all impacted by protein carbonylation. **(Dayanand et al., 2012)** Further research on protein carbonylation in T2DM is therefore essential for expanding our understanding of the condition and creating focused therapies to improve patient outcomes

REVIEW OF LITERATURE

Diabetes Mellitus is a diverse and intricate metabolic illness characterised by hyperglycemia and complications from early cardiovascular disease and small artery disease that result in renal failure, eye damage, and neuropathy. **(Poretsky et al., 2015)**

Epidemiological data reveal concerning trends and future projections for Type 2 diabetes mellitus (T2DM). In 2019, diabetes accounted for 4.2 million deaths globally and affected 453 million adults of middle age. It is expected that by 2045, this number will rise to approximately 700 million. T2DM also accounted for a significant healthcare expenditure of at least 720 billion USD in 2019. Moreover, the actual burden of T2DM may be higher as one in three individuals with diabetes are undiagnosed, which corresponds to around 232 million people. People with diabetes are most affected when they are between the ages of 40 and 59. Over 80% of T2DM patients reside in low- to middle-income countries, which makes it challenging to deliver the necessary care. T2DM incidence and prevalence vary by geographic area. When compared to people without diabetes, those with T2DM have a higher risk of up to 15% all-cause mortality, with cardiovascular illnesses being the main cause. **(I.D.F. et al., 2019)**

Although diabetes usually manifests in adulthood, younger people are now being diagnosed with it as a result of rising obesity rates, adopting a more westernized diet, and sedentary lifestyles. **(A.D.A. 2021)**

Insulin resistance, which occurs when cells become less receptive to the actions of insulin, and decreased insulin release by the beta cells of pancreas are both factors in type 2 diabetes. **(Norhammar et al., 2002)**

Complex interactions between environmental, genetic, and behavioural factors play a role in the development of type 2 diabetes. The difference between the consumption and expenditure of energy is caused by long-term overeating, inactivity, and excess body fat, which causes adipose tissue to malfunction and generate pro-inflammatory cytokines. These elements affect the absorption and utilisation of glucose and largely cause insulin resistance in the liver, skeletal muscles and adipose tissue. **(Galicia-Garcia et al., 2020)**.

The pancreas tries to create more insulin as a compensatory approach to combat insulin resistance. But gradually, the beta cells might run out of energy, resulting in decreased insulin output and worsened glucose regulation. **(Di Pino et al., 2019)**.

Multiple organ systems are affected by the consequences of type 2 diabetes. The development of microvascular problems such diabetes retinopathy, diabetic kidney disease, and neuropathy due to diabetes may result from long-term hyperglycemia damaging small blood vessels **(American Diabetes Association, 2021)**. People with type 2 diabetes frequently experience macrovascular consequences, such as peripheral artery disease, cardiovascular disease, and stroke. **(Abaci et al., 1999)**.

Management of type 2 diabetes entails lifestyle adjustments, including adopting a nutritious diet, increasing physical exercise, and achieving weight loss if overweight or obese. Pharmacotherapy is often required to establish glycemic control, with many types of drugs available in the market. **(Rendell et al., 2004)**

In the process of producing insulin, beta cells are essential. Pre-proinsulin, which is converted into proinsulin in the endoplasmic reticulum, or ER, and Golgi apparatus, is the starting material for the synthesis of insulin**. (Cerf et al., 2013)** Up until it becomes active in hyperglycemic situations, mature insulin is kept in granules. Exocytosis of insulin is induced by calcium. Additionally, cAMP and extracellular ATP play a role in the release of insulin. These systems make sure that beta cells are able to properly control glucose levels. **(Boland et al., 2017)**

Beta-cell malfunction in type 2 diabetes mellitus seems intricate and not fully attributable to beta-cell death. It results from a complex interaction of environmental factors, biochemical pathways, and genetic predisposition. Diseases like being overweight or obesity, high blood sugar levels, and hyperlipidemia lead to insulin resistance, chronic inflammation, and cellular stress. Beta-cell destruction results from a combination of endoplasmic reticulum (ER) damage and the activation of apoptotic pathways, which are caused by excessive quantities of unbound fatty acids along with elevated glucose levels. **(Christensen et al., 2019)** Sustained high glucose levels also increase the production of misfolded insulin and islet amyloid polypeptides, further disrupting cellular function and promoting inflammation. These disruptions in islet integrity impair communication between cells and contribute to insulin secretory dysfunction, a key factor in beta-cell failure and the development of T2DM **(Hoang Do et al., 2015)**

What is Protein Carbonylation?

Proteins that are the result of the chemical process of carbonylation, in which carbonyl groups (-C=O) are incorporated into the protein structure, are referred to as carbonylated proteins. When proteins are subjected to oxidative stress, this modification occurs as a result of the oxidation of specific amino acid residues, including lysine, arginine, proline, and threonine, which results in the formation of carbonyl groups on these residues **(Berlett et al., 1997)**. Due to their role as the main component of the majority of biological systems, proteins are particularly vulnerable to the destruction caused by oxidative stress. **(Dalle-Donne et al., 2006)**

Carbonylated proteins are distinguished by the presence of carbonyl groups, which can be detected and measured using specific analytical techniques such as mass spectrometry or immunochemical tests targeting carbonyl groups. The oxidation reaction due to carbonylation which is recognised as a characteristic of oxidative damage of proteins, has been linked to a number of pathological states and age-related diseases, including cancer, cardiovascular disease, and neurodegenerative disorders.. **(Dalle-Donne et al., 2003)**

Increased Protein Carbonylation in Type 2 Diabetes

Protein carbonylation is known to increase in diabetes due particularly in conditions of chronic hyperglycemia and oxidative stress. The following factors contribute to the increased protein carbonylation in diabetes:

1. Oxidative stress and increased reactive oxygen species (ROS) production: Diabetes may result in abnormal protein compounds formed by glycation, which generates AGEs. AGEs' capacity to induce oxidative stress and the production of ROS enhance protein carbonylation. Additionally, the production of ROS by AGE-modified proteins directly may act as a source, exacerbating oxidative damage. **(Rabbani et al., 2012)**.

2. Impaired antioxidant defence system: Antioxidant deficiencies, such as those in enzymes superoxide dismutase, glutathione peroxidase, are frequently linked to diabetes. Oxidative stress and protein carbonylation are caused by cells having insufficient antioxidant capacity, which reduces their ability to combat the increased ROS generation. **(Brownlee et al., 2005)**

3 .Activation of pro-inflammatory pathways: Diabetes is characterized by persistant low-grade inflammation .cytokines and chemokines are inflammatory mediators that can stimulate ROS production and promote oxidative stress. This inflammatory environment further contributes to protein carbonylation **(Hotamisligil et al., 2006)**

Adverse Effects due to Protein Carbonylation

The accumulation of carbonylated proteins in diabetes can have severe effects on cellular function and add to the development of diabetic complications. Carbonylated proteins can impair insulin signaling pathways, promote

endothelial dysfunction, and may increase the chances of diabetic kidney disorders, retinopathy, and neuropathy. **(Miyata et al., 1999)**

Protein carbonylation at the cellular level can lead to a cascade of physiological issues, culminating in larger complications. These can be of the following types-

1. Structural modifications: Protein carbonylation can result in structural changes, including alterations in protein conformation, stability, and solubility. Carbonylation can induce protein cross-linking, which can cause proteins to aggregate and form amyloid fibrils, which are associated with diabetic neuropathy and nephropathy. **(Butterfield et al., 2002)**

2. Enzymatic activity disruption: Carbonylation of enzymes can impair their catalytic activity and substrate binding capacity. For example, carbonylation of key enzymes involved in glucose metabolism, such as pyruvate kinase, can lead to decreased enzymatic activity and dysregulated glucose metabolism in diabetes. **(Dalle-Donne et al., 2003)**

3. Protein-protein interactions: Carbonylation can interfere with protein-protein interactions, disrupting critical cellular signaling pathways. For instance, if insulin receptor substrate-1 (IRS-1) undergoes carbonylation, it will impair its interaction with insulin receptor, hampering insulin signaling and contributing to insulin resistance in diabetes. **(Stadtman et al., 2000)**

4. Oxidative modification: Protein carbonylation often occurs as a result of increased oxidative stress. Carbonylated proteins can undergo further oxidative modifications, such as amino acids side chains undergoing direct oxidation and formation of AGEs. These modifications can affect protein-protein interactions, enzymatic activity, and protein turnover processes. **(Miyata et al., 2000)**

5. Impaired protein degradation: Carbonylation can interfere with protein degradation pathways and autophagy. Carbonylated proteins may be less efficiently recognized and targeted for degradation, leading to their accumulation and the formation of protein aggregates, which are commonly observed in diabetic complications. **(Stolzing et al., 2001)**

Mechanisms involved in Protein Carbonylation

Having explored the causes and consequences of protein carbonylation in Type 2 diabetes mellitus (T2DM), it becomes evident that studying this phenomenon is of utmost importance. Protein carbonylation can occur in the following ways-

Direct Oxidation of Protein side chains

Reactive oxygen species (ROS) modify certain side chains of amino acids, mainly lysine (Lys) and arginine (Arg), during the direct oxidation method of protein carbonylation. ROS can be a result of normal physiological processes or as a result of increased oxidative stress. Hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•) and examples of such species. **(Valko et al., 2007)**.They can directly interact with certain amino acid side chains, particularly Lys and Arg, through oxidation reactions. These reactions involve the transfer of an oxygen atom or an oxygen-containing group to the amino acid side chain, resulting in the formation of a carbonyl group (-C=O) **(Requena et al., 2003)** Protein carbonyl groups are created when the side chains of the impacted amino acids undergo chemical changes. These carbonyl groups serve as indicators of oxidative damage that can be found and measured**. (Dalle-Donne et al., 2006)**

Oxidation of Proteins to yield reactive carbonyl derivatives that result from Alpha Amidation Pathway

The alpha amidation pathway is responsible for the enzymatic conversion of carboxylic acid groups at the C-terminus of peptides into amides. This pathway involves several enzymatic steps, including the activation of peptidylglycine alpha-amidating monooxygenase (PAM) and subsequent catalysis of the alpha amidation reaction **(Eipper et al., 1988)**.These enzymes being essentially proteins are also susceptible to ROS attack which specifically target lysine and arginine residues. **(Requena et al., 2003)** reactive carbonyl derivatives, such as aldehydes and ketones are generated

by direct oxidation of proteins by ROS, through the modification of amino acid side chains. These reactive carbonyl derivatives are highly reactive and can further react with nearby amino acid residues or other biomolecules, leading to the formation of carbonylated proteins **(Dalle-Donne et al., 2006)**

Metal catalysed oxidation of PUFA

Omega-3 and omega-6 fatty acids, are examples of PUFAs which have have many double bonds. Metal-catalyzed oxidation is a method whereby PUFAs can undergo oxidative processes with the help of metal ions, such as iron (Fe) and copper (Cu). **(Poli et al., 2004)**. This process involves production of ROS as intermediates. Fe and Cu, can react with hydrogen peroxide (H_2O_2) or molecular oxygen (O_2) to generate ROS, including hydroxyl radicals (OH•) and lipid peroxyl radicals (LOO•) **(Davies et al., 2005).** A chain lipid peroxidation is started by these hydroxyl and lipid peroxyl radicals. When PUFAs are degraded by oxidation, lipid hydroperoxides and a variety of reactive intermediates, such as aldehydes and ketones, are produced. **(Esterbauer et al., 1991)**. Malondialdehyde (MDA) and 4-hydroxynonenal (HNE), reactive carbonyl substances produced during lipid peroxidation, can covalently alter proteins. In particular, these reactive carbonyl compounds can react with the residues of the amino acids lysine, arginine, and proline to produce adducts and carbonylated proteins. **(Dalle-Donne et al., 2006)**

Reducing sugars forming AGEs

In the procedure known as "non-enzymatic glycation," sugars with free aldehyde or ketone groups interact with proteins' amino groups to form Schiff bases, which undergo further rearrangements to create Amadori products**. (Vistoli et al., 2013)**.The Amadori products can then undergo a series of chemical reactions, including oxidation and dehydration, leading to the formation of reactive carbonyl compounds **(Monnier et al., 1984)**

Reactive carbonyl compounds, such as glyoxal, methylglyoxal, and 3-deoxyglucosone, can covalently modify proteins by forming stable adducts through nucleophilic reactions with amino acid residues, particularly lysine and arginine **(Ahmed et al., 2003)**. This process is referred to as protein carbonylation.

The formation of carbonylated proteins through the reaction of reducing sugars with proteins and subsequent formation of AGEs is a result several factors, including the concentration of reducing sugars, the duration of exposure, and the increase in oxidative stress **(Singh et al., 2001)**.

Use of HbA1c as Glycemic marker

HbA1c, or glycated hemoglobin, is a clinically significant biomarker used in the monitoring and diagnosis of diabetes mellitus. It provides an estimation of the average blood glucose levels over a specific period, typically the preceding 2-3 months. HbA1c reflects the percentage of hemoglobin A (HbA) that has glucose molecules attached to it. **(Petersmann et al., 2019)**.It is expressed as a percentage of total hemoglobin in the blood. It represents the proportion of glycated hemoglobinrelative to the total hemoglobin present. The normal range for individuals without diabetes is usually below 5.7%, whereas higher levels indicate poorer blood glucose control and the presence of diabetes.

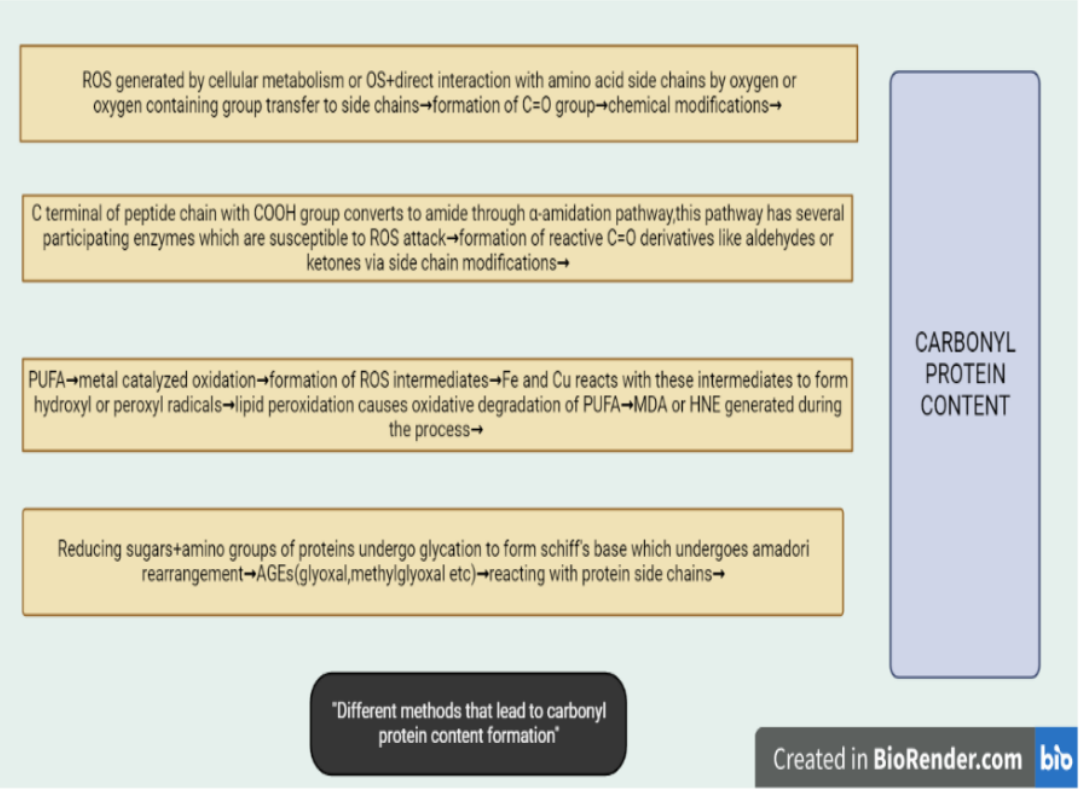


Fig.1 Different methods leading to protein carbonyl content formation

In contrast to SMBG, which provides instantaneous results, HbA1c provides information on mean blood glucose levels over a lengthy period of time, often 2-3 months. It aids medical personnel in assessing the success of diabetes management techniques and modifying treatment approaches accordingly **(Sacks et al., 2013)**

HbA1c reflects the attachment of glucose to hemoglobin molecules in red blood cells. During hyperglycemia, more glucose molecules react non-enzymatically to hemoglobin, resulting in glycation and elevated HbA1c levels. As red blood cells have a lifespan of approximately 120 days, HbA1c measurement provides an estimation of average blood glucose levels over that time period.to ensure consistent and reliable measurements, HbA1c assays are standardized across laboratories. IFCC established a reference method based on mass spectrometry, which provides accurate and comparable results across different laboratories. **(The Diabetes Control and Complications Trial Research Group, 1993)**

HbA1c is a useful test for evaluating and keeping track of glycemic management in diabetics. It assists in determining the efficiency of medicine, insulin therapy, and lifestyle changes in sustaining desired blood glucose levels. Healthcare professionals can make well-informed judgements about therapy modifications and interventions thanks to routine HbA1c testing. Several professional associations, notably the **American Diabetes Association (ADA)**, have recognized it as a diagnostic standard for diabetes mellitus. Diabetes is indicated by HbA1c result of 6.5% or above. When making a diagnosis, it's crucial to take other clinical criteria like symptoms and further diagnostic procedures into account.

HbA1c targets are established based on individual patient factors, such as age, comorbidities, and risk of hypoglycemia. The ADA recommends a general target of less than 7% for most non-pregnant adults with diabetes. However,

individualized goals should be determined in consultation with healthcare providers to optimize outcomes while considering the patient's overall health status**. (American Diabetes Association 2022)**

Limitations of using HbA1c as a glycemic marker

Using HbA1c as a tool for diabetes mellitus management has several limitations and challenges that need to be considered. HbA1c levels can be influenced by factors other than average blood glucose levels, such as erythrocyte lifespan, hemoglobin variants, and certain medical conditions (e.g., anemia, hemoglobinopathies).

These factors can lead to inaccurate HbA1c measurements and affect the interpretation of glycemic control. **(Little et al., 2013)**, It also does not any provide real-time information about glucose fluctuations. It cannot capture short-term glycemic variability or detect hypoglycemic or hyperglycemic episodes that may occur between HbA1c measurements. **(Sacks et al., 2013)**.Moreover the relationship between HbA1c and average blood glucose levels can vary among individuals. Factors such as age, race, ethnicity, and certain medical conditions can influence this relationship. Therefore, relying solely on HbA1c may not fully capture individual variations in glycemic control **(Weykamp et al., 2008)**. Certain medical conditions, such as chronic kidney disease and liver disease, can also affect HbA1c levels independently of glycemic control. In these cases, HbA1c may not accurately reflect the true average blood glucose levels. Despite immense international efforts, there is still a lack of standardization among different laboratory methods and equipment. This lack of standardization can lead to differences in reported HbA1c values, making it challenging to compare results from different laboratories **(Little et al., 2001)**.

HbA1c may also have limited applicability in certain populations, such as pregnant women, children, and individuals with hemoglobinopathies or conditions affecting erythrocyte turnover. Alternative measures, such as continuous glucose monitoring (CGM), may be more suitable for monitoring glycemic control in these populations. **(Chehregosha et al., 2019)**

It is important to consider these limitations and challenges when interpreting HbA1c results and making clinical decisions. Healthcare providers should integrate HbA1c measurements with other clinical information, such as SMBG, patient symptoms, and individual factors, to ensure comprehensive diabetes management.

In conclusion, the development of type 2 diabetes mellitus and the consequences linked to it have been linked to the buildup of protein carbonyls. The importance of protein carbonylation in T2DM has been clarified by a number of prior studies, emphasising the necessity for more research in this field. The study conducted by **Oriquat et al., (2013)** demonstrated a positive relationship between serum protein carbonyl content and glycated hemoglobin (HbA1c) levels in Egyptian T2DM patients, highlighting the potential role of protein carbonylation in glycemic control. Similarly, In T2DM patients with vascular problems, **Adeshara et al., (2017)** found a connection between plasma glycation, membrane modification, and oxidative stress, demonstrating the role of protein carbonylation in diabetic vascular injury.

Furthermore, the research conducted by **Pandey et al. in 2010** and **Tupe et al. in 2014** highlighted the existence of protein oxidation biomarkers and the presence of oxidative stress in T2DM patients, emphasising the need of looking at protein carbonylation as a potential indicator of oxidative damage. Protein carbonylation negatively affects diabetes-related difficulties, as **Bigagli et al. (2012)** found a link between lipid and protein oxidation products, levels of antioxidants, and vascular complications in poorly managed T2DM.

Furthermore**, Chawla et al. (2014)** clarified the significance of AGE-induced receptor expression in diabetic vascular problems, raising the possibility of a connection between protein carbonylation and the emergence of diabetes difficulties. The importance of protein carbonylation as a factor to the aetiology of T2DM difficulties was supported by **Liu et al.'s 2022** investigation of fluorescent advanced glycation end products and their connection with diabetes duration, HbA1c, and diabetic comorbidities. In addition, **Ye et al., (2016)** and **Indyk et al., (2021)** offered additional insights into the serum's advanced glycation end products and their receptors, highlighting their role in T2DM.

When taken in entirety, these findings offer convincing proof of the importance of protein carbonylation in T2DM and related problems. Our research intends to advance understanding of the function of protein carbonylation in T2DM pathogenesis by analysing the protein carbonyl content in diagnosed T2DM patients and control subjects.

AIM:

To find an association between protein carbonyl content and HbA1c in diagnosed Type 2 Diabetes Mellitus patients and control subjects

OBJECTIVES:

1. To determine the level of protein carbonyl content in diagnosed patients of Type 2 Diabetes Mellitus and control subjects.
2. To determine the level of HbA1c in diagnosed patients of Type 2 Diabetes Mellitus and control subjects.
3. To determine the correlation between level of protein carbonyl content and HbA1c of diagnosed Type 2 Diabetes Mellitus patients, if any

Total sixty subjects (thirty cases and thirty controls) were enrolled for this study. Results showed that Protein carbonyl content was remarkably increased in cases as compared to controls (p=0.004) (Fig.4) Similarly HbA1c levels were also elevated significantly in cases as compared to controls (p= 0.002)(Fig.5).

Table.1 Demographics and biochemical parameters in control subjects and cases.

Parameters	Cases(n=30)	Control(n=30	p-value	Significance

BMI	28.23±2.94	29.50±2.42	0.074	Not Significant
AGE	49.03±7.91	47.9±8.22	0.588	Not Significant
PCC	0.65±0.103	1.48±0.389	**0.004**	Significant
HbA1c	5.37±0.27	8.20±2.033	**0.002**	Significant

14

n=number of samples, $p < 0.05$ considered statistically significant

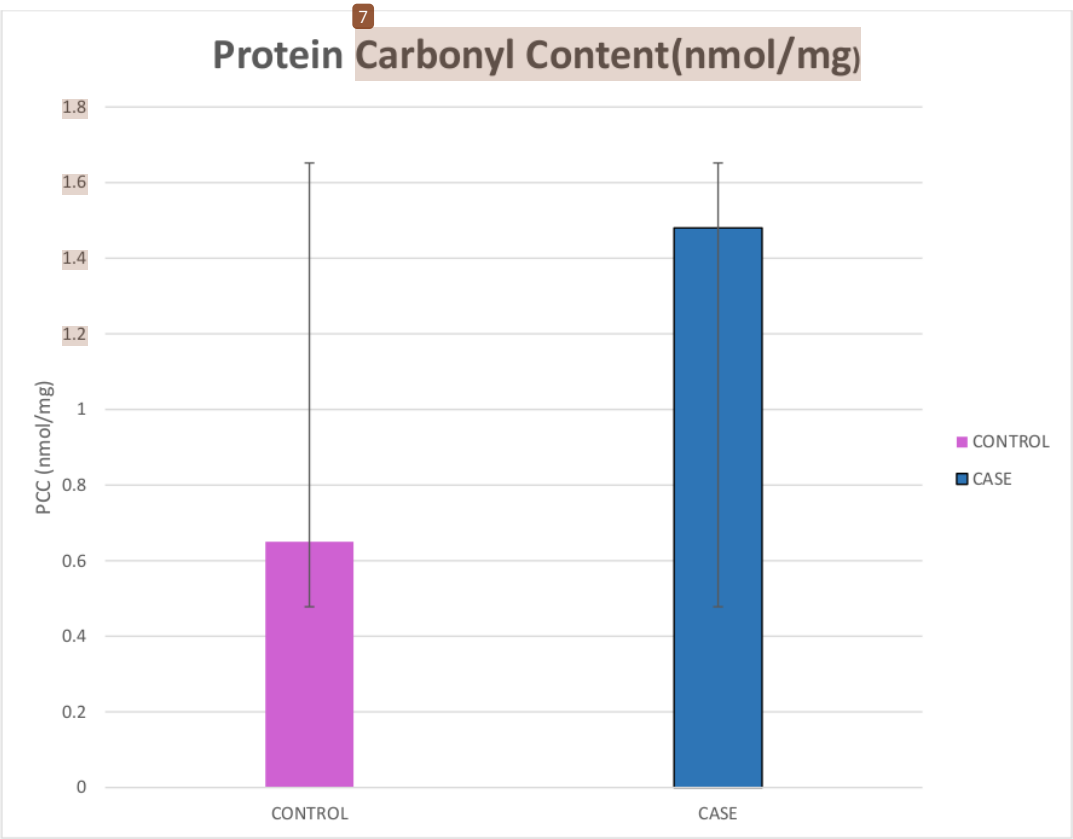


Fig.4 Comparison of Protein Carbonyl Content between Control and Cases

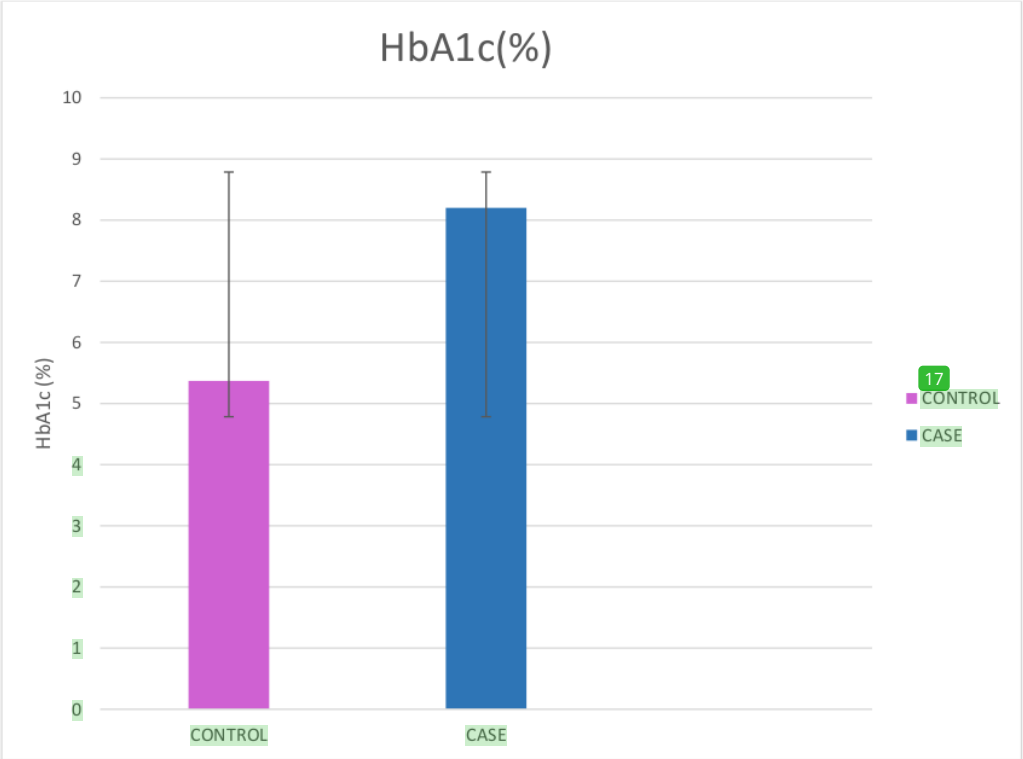


Fig. 5 Comparison of HbA1c between Control and Cases

Table 2 **Karl Pearson's Correlation Coefficient among the study parameters in cases.**

Correlation is Significant at the 0.01 level (2-tailed)

	BMI	**PCC**	**HbA1c**
BMI	1	**.0535**	**.560**
Sig. 2 tailed		.002	.001
n	30	30	30
PCC	**.535**	1	**.897**
Sig. 2 tailed	.002		<.001
n	30	30	30
HbA1c	**.560**	**.897**	1
Sig. 2 tailed	.001	<.001	
n	30	30	30

RESULT

This study was designed to check for an association, if any between Protein carbonyl content and HbA1c in Type 2 diabetes mellitus patients and controls.

The parameters estimated were:

1) Protein Carbonyl Content (nmol/mg)
2) HbA1c (%)

The parameters levels were compared with control levels, who are apparently healthy individuals.

The study observations were as follows-:

1) The level of HbA1c (%) was significantly increased in cases (p=0.002) as compared to control subjects.
2) The level of Protein Carbonyl Content (nmol/mg) was significantly increased in cases (p=0.004) as compared to control subjects.
3) There was a significant positive correlation between Protein Carbonyl content and HbA1c among cases.

DISCUSSION

The aim of the present study was to compare the oxidative stress indicators HbA1c and protein carbonyl concentration in individuals with type 2 diabetes mellitus and apparently healthy controls. Our study's results demostrated a strong positive correlation between protein carbonyl concentration and HbA1cwhich was statistically significant, suggesting a possible connection between glycemic management and oxidative damage in T2DM. These results align with previous studies conducted in the field.

Dayanand et al., (2012) demonstrated the validity of carbonyl protein content as a reliable marker of increased oxidative damage in type 2 diabetes. Their findings support the hypothesis that the pathophysiology of T2DM is associated with increased protein carbonyl content, a sign of increased oxidative stress. Similar to this, **Sharma et al., (2021)** demonstrated the importance of oxidative stress in T2DM by discovering a significant correlation between protein carbonyl content and glycated haemoglobin.

Mahmoud et al., (2013) examined the activity of glucose-6-phosphate dehydrogenase and protein modification due to oxidative stress in T2DM patients, highlighting the significance of oxidative stress in the disease. Their findings provide more evidence for the significance of protein the carbonylation process as a biological indicator of oxidative injury in T2DM.

The results of our investigation have significant importance for ongoing research and clinical practice. Firstly, our results support the potential utility of protein carbonyl content as a biomarker for assessing oxidative stress levels in T2DM patients. Monitoring protein carbonyl levels may aid in evaluating disease progression, assessing treatment efficacy, and identifying high risk individuals for developing diabetes-related complications.

In addition, our study highlights the need for further investigations with larger sample sizes and diverse geographical populations. The generalizability of the results is constrained by the study's limited sample size, as was acknowledged. Future studies should aim to include a larger and more diverse cohort to validate the observed correlations and provide a comprehensive understanding of the relationship between HbA1c and protein carbonyl content in T2DM.

Our study's findings, which show a significant positive association of HbA1c with protein carbonyl concentration in T2DM patients, raise the possibility that oxidative stress plays a role in the development of the condition. The results confirm the significance of evaluating the extent of carbonylation in proteins as a sign of excessive oxidative damage and the possible application of this biomarker in the treatment of T2DM. However, the shortcomings of our

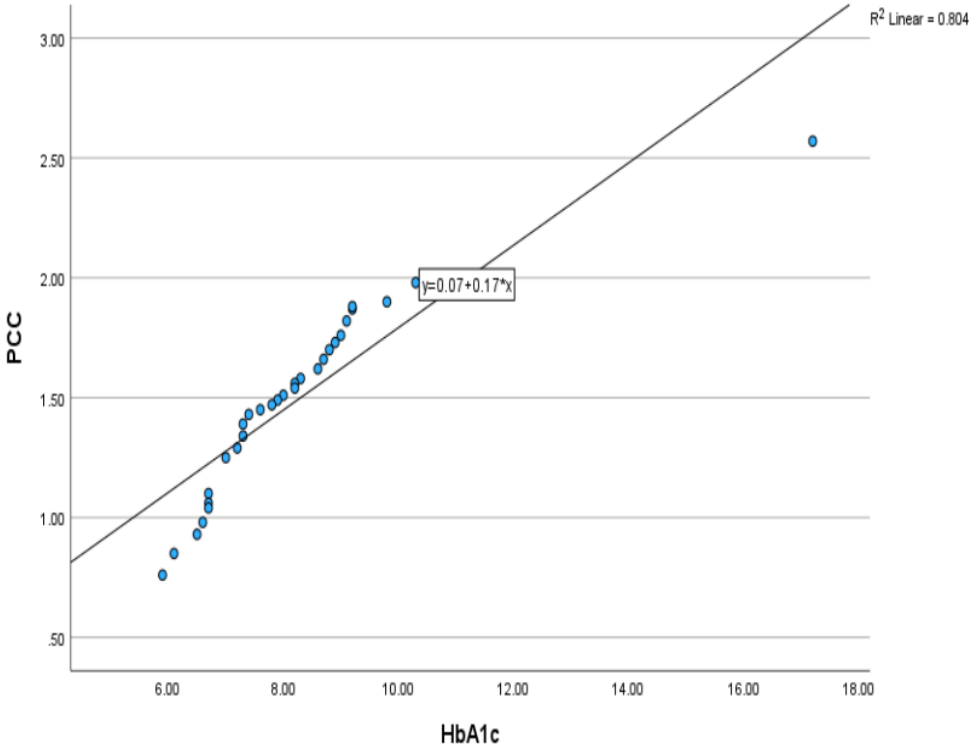
investigation, such as the small sample size and geographical restrictions previously noted, call for additional research to confirm our findings and offer more thorough insights into the function of protein carbonylation in T2DM.

SUMMARY

The goal of the study was to examine the carbonyl protein content and percentage of HbA1c in type 2 diabetic mellitus patients and control individuals. The study used a manual Levine method to detect protein carbonyl levels, with a total of 30 cases and 30 controls. The results showed a significant positive link between HbA1c and protein carbonyl concentration, suggesting that glycemic management and oxidative damage in T2DM may be related. The study was constrained, nonetheless, by the small sample size and constrained geographic distribution. It is necessary to conduct a more thorough research with a bigger sample size and a broader location.

CONCLUSION

The results of the current investigation validates the alternate hypothesis that increased oxidative stress may play a role in the pathogenesis of T2DM by showing a positive connection between HbA1c and protein carbonyl concentration. The findings support earlier research, highlighting the importance of protein carbonylation as a biomarker of oxidative damage in T2DM. Screening protein carbonyl concentrations may have consequences for determining those at risk of problems, tracking the effectiveness of treatment, and gauging the development of the disease. Future research should, however, address the study's shortcomings, including the small number of participants and constrained geographic scope. To confirm the observed associations and offer a thorough knowledge of the link between HbA1c and protein carbonyl concentration in T2DM, larger and more diverse cohorts are required.



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