Original Article

The Influence of Plasma Protein Glycation in Diabetes Mellitus Type 2 and its Complications

Effect of Glycation in Diabetic Complications

Zarrin Khaliq[1], Sajjad Ghani[2], Fariha Niaz[3] and Sidra Mushtaq[4]

ABSTRACT

Objective: The present project was planned to study effect of Glycation in diabetic complications.
Study Design: Correlational and experimental study
Place and Duration of Study: This study was conducted at the DHQ Faisalabad and Allied hospital Faisalabad from March 2018 to April 2019.
Materials and Methods: A total 100 patients were taken. They were grouped in 5, each having 20 patients. These groups were according to the complications, normal and normal diabetics. Blood samples of diabetic patients with and without complication were collected from D.H.Q and Allied Hospital Faisalabad. The information included age, sex, and duration, family history of diabetics, treatment status and diabetic control in relation to complications. (Neuropathy, nephropathy, foot ulcer, retinopathy) were taken by using a self-designed Performa. Specific techniques of spectrophotometer and software were used to analyze all the collected samples. The biochemical parameters were protein estimation, glucose estimation, determination of level of Glycation, determination of protein marker by SDS_PAGE analysis.
Results: We have found levels of glucose, protein and Glycation for both normal and diabetic patients. Correlational analysis between glucose, and Glycation level showed positive outcomes r=0.4199 for normal subjects and r=0.242 for glycated. Analysis between protein and Glycation level for normal individuals was r=0.4977.After SDS-PAGE Analysis of diabetic patient's blood with complications and without complications and normal revealed a difference of migrating bands with molecular weights between 20 and 30. One faster migrating band was missing in diabetic patients with Ischemic heart disease and two bands were of less intensity in with neuropathy and foot ulcer of age 64 years. While the diabetic patients without complications and normal showed no missing band. So, our results show that some protein is missing in diabetic complications.
Conclusion: Our results suggest that increased level of Glycation participate in long term complications.
Key Words: Diabetic complications, Glycation, plasma protein, glucose

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INTRODUCTION

Diabetes mellitus is a syndrome characterized via persistent hyperglycemia this is because of relative insulin deficiency, or resistance or both.

1. Department of Biochemistry, Abwa Medical College, Faisalabad.
2. Department of Biochemistry, Aziz Fatima Medical and Dental College, Faisalabad.
3. Department of Biochemistry, Sahiwal Medical College Sahiwal.
4. Department of Pharmacology, Independent Medical College, Faisalabad.

Correspondence: Dr. Sajjad Ghani, Senior Demonstrator, Department of Biochemistry, Aziz Fatima Medical and Dental College, Faisalabad.
Contact No: 0321-7801946
Email: dr.sajjadghani625@gmail.com

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It impacts extra than 30 million humans international. Diabetes is generally irreversible and, even though sufferers will have a reasonably everyday lifestyle, its overdue complications results in reduces existence expectancy and extensive uptake of heath resources. Macro-vascular disorder results in an increased incidence of coronary artery disease, peripheral vascular disease and stroke.[1] Micro-vascular damage reason diabetic retinopathy and nephropathy and contributes to diabetic neuropathy. Diseases result in lessens life expectancy and considerable uptake of health resources[2]. Almost 366 million people worldwide have diabetes and till 2030 it will affect 522 million people worldwide (Guariguata et al. 2011). WHO ranked Pakistan at 7th on diabetes prevalence list. Recent view on the occurrence has shown that about more than 4.7 million people suffering from diabetes.[3]

The continual hyperglycemia of diabetes is related to long time damage; disorder and failure of numerous organs, in particular the eyes, kidney, nerves, coronary heart and blood vessels, numerous pathogenic methods are concerned in the improvement of diabetes. These

variety from autoimmune destruction of β cells of the pancreas with consequent insulin deficiency to abnormalities the bring about resistance to insulin movement.

Diabetes can commonly be recognized on the idea of fasting or random blood glucose size. The GTT should be reserved for border line cases simplest. The main aim of diabetes management is to achieve the normal glycemic state long follow-up studied have shown good glucose control may prevent or delay the manifestation of complication, despite the long duration of the disease.

Erythrocytes are freely permeable to glucose. In cell, glucose attaches to the free ends of amino acids of hemoglobin molecules and this process called non-enzymatic glycation, caused glycosylated hemoglobin to be formed directly proportional to the blood glucose concentration. As the average erythrocyte life span is about 120 days, glycosylated hemoglobin levels give information on the mean average blood glucose level over the past 2 to 3 months. Glycosylation of serum proteins, mainly albumin, has also been used in diabetes monitoring. The half-life of albumin is 2 to 3 weeks and degree of albumin glycosylation hence provides an index of glycemia over a shorter period of time glycosylation of hemoglobin. One of the most widely used measurements of glycosylated serum proteins is fructosamine assay.[4]

The method of glycation has noteworthy impact on structures and functions of proteins. The analysis of proteins has always been a critical part of medical research and molecular prognosis. Proteins circulating in human blood are easily accessible and may examine at once to produce diagnostic data on disorder repute in sufferers[4]. Molecular weight determination of plasma from normal subjects in assessment from diabetic patients may additionally monitor huge and biologically essential difference in those proteins.

The main objective of the research was to increase a relationship between the blood glucose stage and plasma proteins glycation and protein awareness with diabetic complications like neuropathy, nephropathy, foot ulcer, and retinopathy.

MATERIALS AND METHODS

The main objective of the project was to develop a relationship between the blood glucose level and plasma proteins glycation and protein concentration with diabetic complications like neuropathy, nephropathy, foot ulcer, and retinopathy. To check the protein profile SDS_PAGE analysis was done. A total 100 patients were taken. They were grouped in 5, each having 20 patients. These groups were according to the complications, normal and normal diabetics. Blood samples of diabetic patients who were clinically diagnosed by physician were collected from D.H.Q. hospital Faisalabad and Allied Hospital Faisalabad. Blood of diabetics without complications and non-diabetics were also collected from above mentioned hospitals. 5 ml of blood sample from each of 100 patients were collected by using sterilize disposable syringes by venopuncture. The blood is transferred into tubes containing the anticoagulant like EDTA or heparin. These samples containing anticoagulants mixed gently by tapping then centrifuge at 3000 rpm. Plasma frictions were collected by using micro pipette, and then these samples were collected into autoclaved Eppendorf tubes and stored at -20 degree centigrade.

In this study we included socioeconomic and biochemical parameters. Basic informations of patients were collected by using a typical Performa containing all the information and history of patients. These informations included age, sex, and duration, family history of diabetics, treatment status and diabetic control in relation to complications. (Neuropathy, nephropathy, foot ulcer, and retinopathy).Specific techniques of spectrophotometer and software were used to analyze all the collected samples. The biochemical parameters were protein estimation, glucose estimation, determination of level of Glycation, determination of protein marker by SDS_PAGE analysis.

RESULTS

SDS-Page Analysis of plasma of diabetic patients with complications showed bands of less intensity in neuropathy, foot ulcer and retinopathy and a missing band in nephropathy.

In normal subjects, the glycation level ranged between 0.024 mole/mole of protein to 0.30 mole / mole of protein.

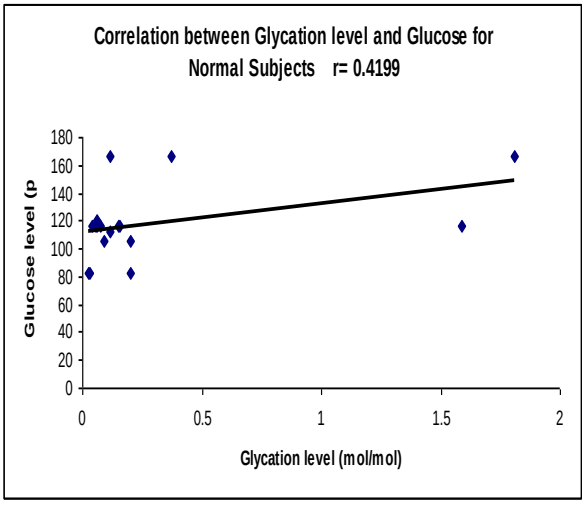


Figure No.1: correlation between glycation level and glucose for normal

The value of glycation level in diabetes patients ranged from 0.032 mole/mole to 1.96 mole/mole of protein. Glucose level in normal range from 83 mg/dl to 140 mg/dl. The glucose level in diabetics ranged from 133

mg/dl to 283 mg/dl as in normal but their glucose *level* will be monitored during disease. These changes in glucose levels clearly indicates that glycation in diabetic patients increases the blood glucose level. The protein concentration in normal ranged from 6g/100 ml to 14g/100 ml. Protein concentration in diabetics was 5g/100 ml to 14g/ 100 ml. Correlation between glycation level and glucose in normal subjects showed a positive correlation (r= 0.4199), as shown in figure 3. Using the standard curve and the r intercept the dilutions for the obtained glucose samples were prepared in order to perform SDS-PAGE.

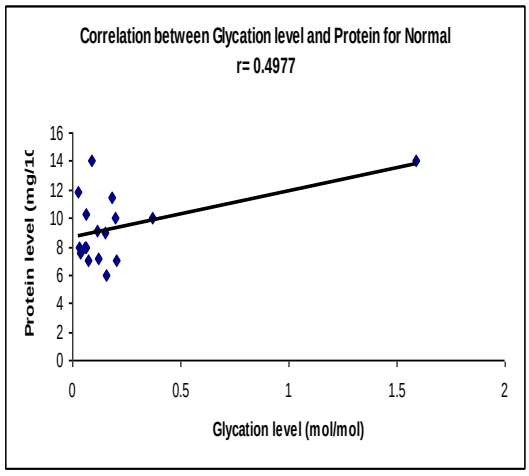


Figure No.2: Correlation between Glycation level and protein for normal

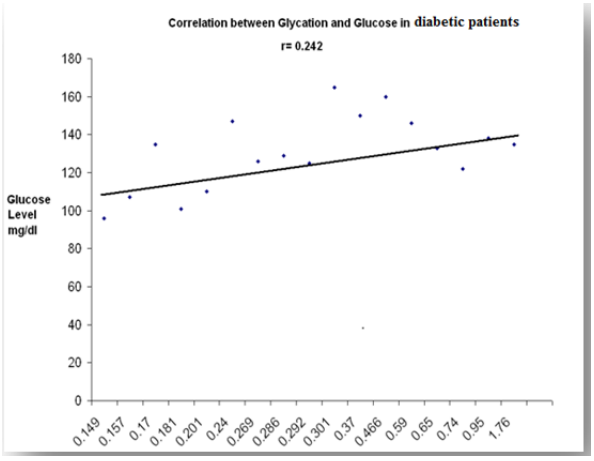


Figure No.3: correlation between glycation and glucose in diabetic patients

Considering the correlation between glycation level and proteins in normal subjects showed a positive correlation (r= 0.4977), as shown in figure. Using the standard curve and the r intercept the dilutions for the obtained protein samples were prepared in order to perform SDS-PAGE for the determination of molecular weight. Correlation between diabetes and glucose in diabetic patients also showed a positive result (r= 0.242).The standard curve was plotted by calculating the RF values of each standard protein against the log 10 of its molecular weight (Figure). The molecular weight of the unknown polypeptide or protein was determined by finding its RF value on the standard curve and reading the log10 molecular weight from the ordinate. The anti-log of this number was the molecular weight of the protein. Low (6,500 to 66,000) and High molecular weight protein markers (25000-127000 Daltons) from Sigma Chemicals and from life Technologies were used in this study.

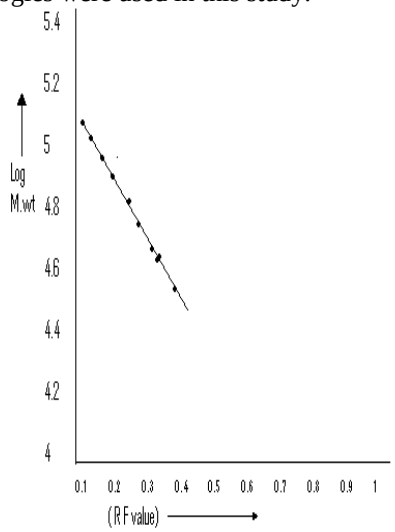
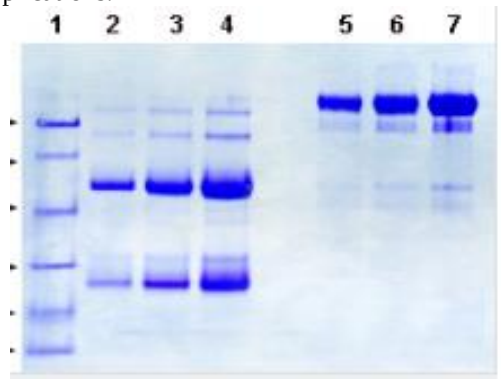
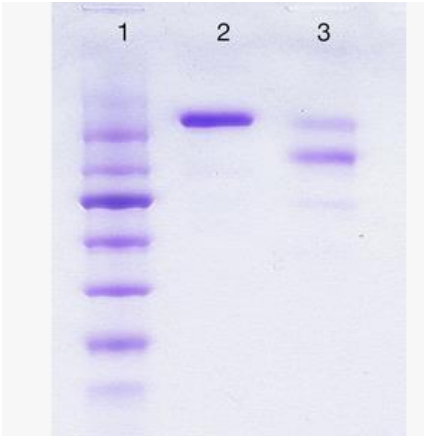


Figure No.4: Analysis

After SDS-PAGE Analysis of diabetic patient's blood with complications and without complications and normal revealed a difference of migrating bands with molecular weights between 20 and 30. One faster migrating band was missing in diabetic patients with Ischemic heart disease and two bands were of less intensity in with neuropathy and foot ulcer of age 64 years. While the diabetic patients without complications and normal showed no missing band. So, our results show that some protein is missing in diabetic complications.



Lane 1: Protein marker; Lane 2: Diabetic with nephropathy
Lane 3: diabetic with neuropathy; Lane4: Normal
Lane 5: Diabetic ischemic heart disease
Lane 6: Diabetic with foot ulcer;
Lane 7: Neuropathy with foot ulcer



Lane 1: protein marker
Lane 2: diabetic with complications
Lane 3: normal

DISCUSSION

The present study was designed to study non-enzymatic glycation which is one of the primary modification factors that contribute to various alterations of intrinsic protein structure and functions.[5] Blood is a complex mixture of different biological molecules including proteins. The analysis of proteins has always been an important part of medical research and molecular diagnosis. Proteins circulating in human blood are readily accessible and can analyze directly to produce diagnostic information on disease status in patients[6]. So blood sample was taken from diabetic and non-diabetic patients. To study the status of glycation in controlled and diabetic patients was the first objective of the study. For it level of glycation was measured for further proceedings, result showed glycation level ranged from 0.047 mole/mole of protein to 0.448 mole/mole of protein. The value of glycation level in diabetes patients ranged from 0.032 mole/mole to 1.96 mole/mole of protein.[6] The results can be compared with[7] who checked the glycation level from 0.2 mole / mole to 1.13 mole/mole of protein. Our results can be compared with who reported the increase level of glycosylated proteins in diabetics as compared to normal subjects[8]. Glucose level in normal range from 83 mg/dl to 140 mg/dl. Our results can be compared with[9] who reported glucose range from 45mg to 130 mg/dl in normal. The protein concentration in normal ranged from 6g/100 ml to 14g/100 ml. Results can be compared with[10], who reported 6.5 g/100ml to 7.55g/100ml.Protein concentration in diabetics was 5g/100 ml to 14g/ 100 ml. Our results can be compared with[11] who reported that protein level of diabetic patients was mostly like normal healthy persons. Our results are not compared to[12] who reported that malnutrition caused low proteins level as 3.5 g/100ml. Correlation between glycation level and glucose in normal subjects showed a positive correlation (r= 0.4199), Glycation level and protein in normal subjects also showed a positive correlation (r=0.4977*). Correlation between glycation level and glucose in patients of diabetes showed a positive correlation.(r= 0.242*). After SDS-PAGE Analysis of diabetic patient's blood with complications and without complications and normal revealed a difference of migrating bands with molecular weights between 20 and 30. One faster migrating band was missing in diabetic patients with Ischemic heart disease and two bands were of less intensity in with neuropathy and foot ulcer of age 64 years. While the diabetic patients without complications and normal showed no missing band. So, our results show that some protein is missing in diabetic complications. Our results can be compared with[13], who observed the fast migrating bands of molecular weights between 20 and 40kDa in normal subjects but not in diabetic patients. This difference of range of molecular weights may be due to age difference. Increase mol.wts of human proteins for diabetic subjects is also revealed through the work of[14]. The difference in molecular weights of protein among diabetic and non-diabetic is due to complications.

CONCLUSION

Diabetes mellitus is a disease caused by either insulin deficiency or nonfunctioning of hormone insulin. The insulin deficiency results in prolonged exposure to hyperglycemia which is primary factor for diabetic complication. Therefore, present project was planned to analyze relationship between protein concentration and Glycation and diabetic complications. Hence our results suggest that increased level of Glycation participate in long term complications.

Author's Contribution:

Concept & Design of Study:	Zarrin Khaliq
Drafting:	Sajjad Ghani, Fariha Niaz
Data Analysis:	Sidra Mushtaq
Revisiting Critically:	Zarrin Khaliq, Sajjad Ghani
Final Approval of version:	Zarrin Khaliq

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Arasteh A, Farahi S, Habibi-Rezaei M, Moosavi-Movahedi AA. Glycated albumin: an overview of the in vitro models of an in vivo potential disease marker. J Diabetes Metabolic Disorders 2014;13: 49.
2. Basit A, Hydrie MZ, Hakeem R, Ahmedani MY, Masood Q. Frequency of chronic complications of type 2 diabetes. J Coll Physicians Surg Pak 2004;14(2):79-83.

3. Dunn EJ, Ariëns RA, Grant PJ. The influence of type 2 diabetes on fibrin structure and function. Diabetologia 2005;48(6):1198-206.
4. Fremeau RT, Burman J, Qureshi T, Tran CH, Proctor J, Johnson J, et al. The identification of vesicular glutamate transporter 3 suggests novel modes of signaling by glutamate. Proceedings of the National Academy Sci 2002;99: 14488-14493.
5. Fukuda H, Yamada K, Shoji T, Takahashi M, Tsuchizawa T, Watanabe T, et al. Four-wave mixing in silicon wire waveguides. Optics Express 2005;13: 4629-4637.
6. Ginsberg BH. Apparatus and method for monitoring blood glucose levels including convenient display of blood glucose value average and constituent values: Google Patents 2009.
7. Guariguata L, Whiting D, Weil C, Unwin N. The International Diabetes Federation diabetes atlas methodology for estimating global and national prevalence of diabetes in adults. Diabetes research and Clin Prac 2011;94: 322-332.
8. Humeny A, Kislinger T, Becker CM, Pischetsrieder M. Qualitative determination of specific protein glycation products by matrix-assisted laser desorption/ionization mass spectrometry peptide mapping. J Agricultural and Food Chem 2002; 50(7):2153-60.
9. Kaloudi O, Basta G, Perfetto F, Bartoli F, Del Rosso A, Miniati I. Circulating levels of Nε-(carboxymethyl) lysine are increased in systemic sclerosis. Rheumatol 2006;46:412-416.
10. Kopping J, Biyani K, Connor E, Hecker S, Lees I, Huynh G, et al. Crosslinked polyvinylamine, polyallylamine, and polyethyleneimine for use as bile acid sequestrants. United States patent US 2015;(9):181,364.
11. Krinsley JS. Effect of an intensive glucose management protocol on the mortality of critically ill adult patients. In Mayo Clinic Proceedings 2004; 79(8):992-1000,
12. Makino H, Haneda M, Babazono T, Moriya T, Ito S, Iwamoto Y, et al. Prevention of transition from incipient to overt nephropathy with telmisartan in patients with type 2 diabetes. Diabetes Care 2007; 30: 1577-1578.
13. Ozougwu J, Obimba K, Belonwu C, Unakalamba C. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol 2013;4: 46-57.
14. Raghav A, Ahmad J. Glycated serum albumin: a potential disease marker and an intermediate index of diabetes control. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2014;8(4):245-51.