JOURNAL OF SCIENTIFIC RESEARCH IN ALLIED SCIENCES
ISSN NO. 2455-5800
Contents available at www.jusres.com

Analysis of Biochemical Parameters and Insulin Resistance in Individuals with Type 2 Diabetes Mellitus

Saba Rana, Vikas Kumar, Ankit Kumar, Vipin Kumar Saini, Zehra Husaini, Girendra Kumar Gautam*[2]
[1]*Department of Bioscience, Shri Ram College, Muzaffarnagar*
[2]*Shri Ram College of Pharmacy, Muzaffarnagar*

ARTICLE INFO

Article History
Received: May 2022
Accepted: Sept 2022
Keywords:
Diabetes Mellitus, biochemical parameter, Haemoglobin

Corresponding Author
***Rana S.**

ABSTRACT

The research paper investigates the biochemical and insulin resistance profiles in Type 2 Diabetes Mellitus (T2DM) patients compared to healthy individuals. The study involved 200 subjects, with 100 in each group. Significant elevations were observed in fasting blood sugar (FBS), glycosylated haemoglobin (HbA1c), and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) in diabetic subjects compared to healthy controls. Dyslipidaemia markers such as triglycerides (TG), very low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol (LDL-C) were notably elevated, while high-density lipoprotein cholesterol (HDL-C) was diminished in diabetic subjects, indicating an increased cardiovascular risk. Insulin resistance was markedly higher in diabetic subjects, emphasizing its role in T2DM pathogenesis. These findings stress the importance of comprehensive management strategies targeting glycaemic and lipid control to mitigate complications in T2DM patients.

ORIGINAL RESEARCH ARTICLE

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) stands as a significant contributor to both mortality and morbidity worldwide (Yadav *et.al*., 2017). This condition often correlates with obesity, leading to insulin resistance (Henegar Jr. *et al.*, 2001; Chagnac *et.al*., 2003). T2DM primarily results in elevated levels of glycated proteins, triggering cytokine production associated with long-term complications such as heightened susceptibility to infections and impaired wound healing (Benfield *et.al.*, 2007), contributing to 5% of global annual deaths (Ram Vinod *et al.*, 2011). Over the past thirty years, the global prevalence of Diabetes Mellitus has more than doubled (Danaei *et.al.*, 2011), with an estimated 285 million individuals affected in 2010, 90% of whom had T2DM (Shaw *et al.*, 2001; Zimmet *et al.*, 2001). Projections suggest a rise to 439 million cases globally by 2030, representing 7.7% of the world's adult population aged 20-79 years (Shaw *et.al.*, 2010).

Diabetes Mellitus is characterized as a chronic metabolic disorder marked by chronic hyperglycaemia and disturbances in carbohydrate, fat, and protein metabolism, hindering the complete or partial utilization of glucose (World Health Organization, 1999). Immunological and inflammatory processes play pivotal roles in T2DM development and progression (Schwarz *et.al.*, 2009).

Insulin resistance is linked to alterations in lipid and lipoprotein metabolism, resulting in atherogenic

dyslipidaemia and potentially increasing the risk of cardiovascular disease (Grundy *et.al.*, 1997). Dyslipidaemia in obese individuals with T2DM and metabolic syndrome is characterized by elevated triglycerides, reduced HDL-C, increased apo B-100, non-HDL-C, and small dense LDL and HDL. Insulin sensitivity assessed by hyperinsulinemia euglycemic clamp technique strongly correlates with triglyceride concentration (Baldeweg *et.al.*, 2000). Lipodystrophy disorders, marked by severe insulin resistance and hypertriglyceridemia due to VLDL overproduction, represent extreme energy storage site loss (Gavrilova *et.al.*, 2000).

Endothelial cell dysfunction ensues from insulin resistance, reducing nitric oxide production and increasing procoagulant factor release, thereby promoting platelet aggregation. In an insulin-resistant state, the P13K pathway is affected while the MAP Kinase pathway remains intact, leading to insulin's mitogenic effects on endothelial cells and contributing to atherosclerosis (Wu *et.al.*, 2009; Wang *et al.*, 2003). Various disruptions in cellular signaling pathways, such as mitochondrial dysfunction and endoplasmic reticulum stress, have been associated with lipotoxicity. Mediators like reactive oxygen species, nitric oxide, ceramide, phosphatidylinositol-3-kinase, diacylglycerol, and leptin are implicated in promoting these lipotoxic effects and accelerating apoptosis rates (Wende *et.al.*, 2010).

MATERIALS AND METHODS

The blood sampling procedure involved collecting samples from subjects in plain vials, followed by incubation at 37℃ for 30 minutes. After clot removal, the remaining sample was centrifuged at 3000 rpm for 10 to 20 minutes to separate the serum. The supernatant, containing the serum, was then transferred to clean and dry test tubes for analysis of fasting blood glucose, lipid profile, HbA1C, creatinine, and urea. Additionally, a separate procedure was conducted for the preparation of hemolysate. Blood samples collected in sodium citrate vials were centrifuged at 3000 rpm for 15 minutes to obtain plasma. Packed Cell Volume (PCV) was determined, and cells were washed thrice with saline before lysing with distilled water. After centrifugation, the chloroform layer was collected, resulting in a clear hemolysate solution utilized for glycosylated haemoglobin estimation. The study, conducted at the Department of Biochemistry, LLRM Medical College, Meerut, included 150 individuals: 100 healthy and 100 controlled Type 2 diabetic individuals under oral hypoglycemic drug treatment. Age-matched healthy controls were selected, and written consent was obtained from all participants. Clinical history and previous investigations were compiled in a proforma, and ethical measures were strictly adhered to throughout the study. Blood samples, comprising 5ml, were withdrawn from the antecubital vein following overnight fasting and collected in plain, fluoride, and EDTA vacutainers for biochemical and immunological investigations.

The Estimation of Serum Glucose was carried out using the Enzymatic-colorimetric GOD-POD method, where glucose oxidase (GOD) catalysis the oxidation of glucose to gluconic acid and hydrogen peroxide (H_2O_2). The released hydrogen peroxide reacts with phenol and 4-Amino antipyrine (4-AAP) in the presence of peroxidase, forming a colored quinonimine dye. The absorbance of this colored dye was measured at 505 nm, directly correlating with the glucose concentration in the sample.

For the Estimation of Glycosylated Haemoglobin, Hemolysate (0.3 ml) was added to a mixture of 0.18 M HCL in acetone (8 ml), and after standing for 15 minutes, centrifugation was performed. The precipitated globin was washed thrice with HCL-acetone mixture and acetone alone to remove free glucose. The dissolved globin was treated with Tris HCL buffer, followed by the addition of phenol and conc. H_2SO_4. The color developed was measured at 480 nm, with color intensity directly proportional to the globin concentration in the range of 5-30 µg of bound hexose.

Additionally, the procedure for Estimation of HDL Cholesterol involved the separation of HDL cholesterol from serum or plasma using a precipitating reagent. The clear supernatant obtained was used for HDL cholesterol estimation, following incubation and subsequent measurement of absorbance at 505 nm. Calculation of LDL-Cholesterol and VLDL Cholesterol was performed using Friedwald's Formula (1972), where LDL-c was calculated as (TC-HDL-c-VLDL-c), and VLDL-c as (TG/5). The reference values for LDL-c were up to 190 mg/dl and for VLDL-c were 14-31.8 mg/dl.

Insulin Resistance was determined using the homeostasis model assessment (HOMA) method, employing the equation: IR = (Fasting plasma insulin micro units/L) x (Plasma fasting glucose mmol/L)/22.5. It is recommended to establish individual normal and abnormal values for the assays conducted.

RESULTS AND DISCUSSION

Showing the comparative changes between biochemical parameters (Figure 1) and insulin resistance (HOMA-IR) in group 1 healthy male and group 2 diabetic male (Figure 2).Compares biochemical parameters in females (Figure 3) and insulin resistance (HOMA-IR) between healthy (Group 1) and diabetic (Group 2) females (Figure 4). Significant increases were observed in FBS, HbA1c, and HOMA-IR ($p<0.001$) in diabetic females. TC, TG, LDL-C, and VLDL-C were also significantly elevated ($p<0.01$), while HDL-C decreased significantly ($p<0.01$) in diabetic females (n=48 and 50, respectively).

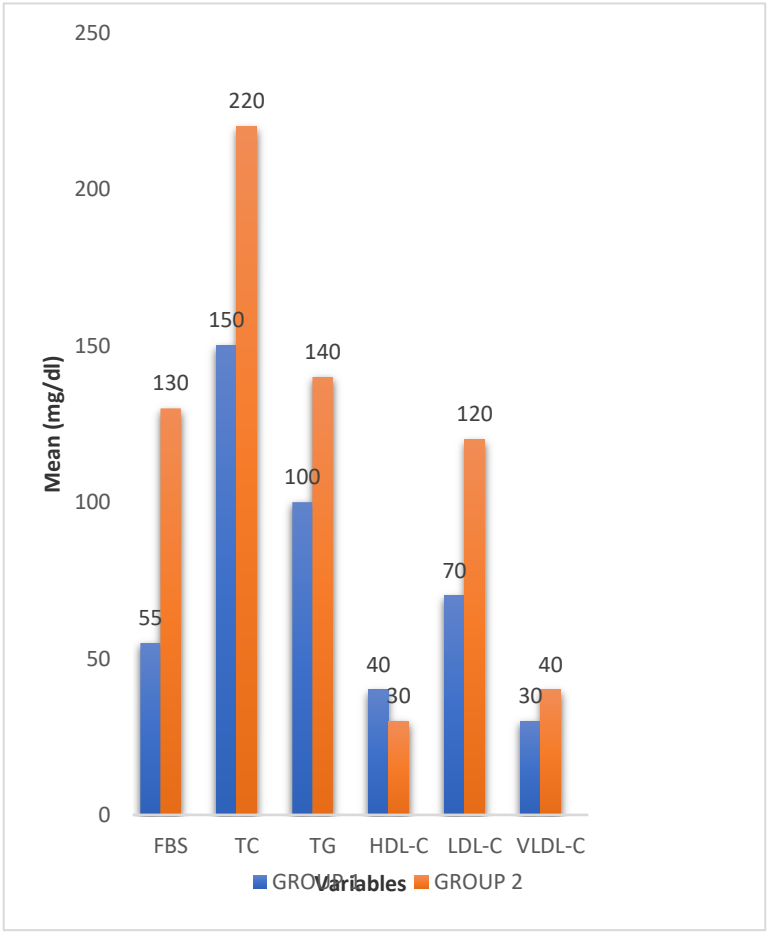

Figure 1: Showing comparative changes of biochemical parameters in Group 1 and Group 2 Male Subjects.

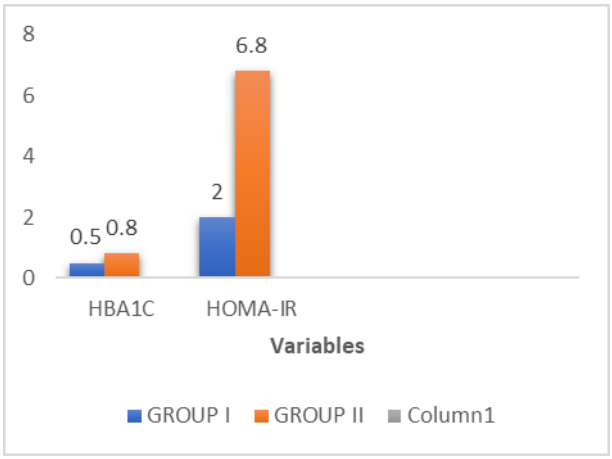

Figure 2: Showing comparative genomics changes of HBA1c & HOMA-IR in Group 1 and Group 2 Male Subjects.

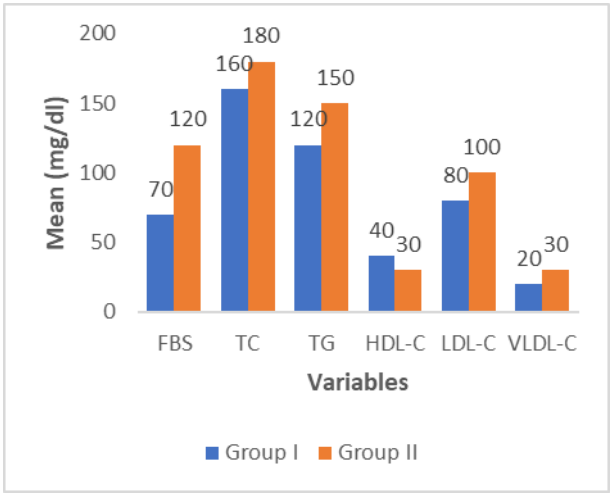

Figure 3: Showing comparative changes of biochemical parameters in Group 1 and Group 2 Female Subjects.

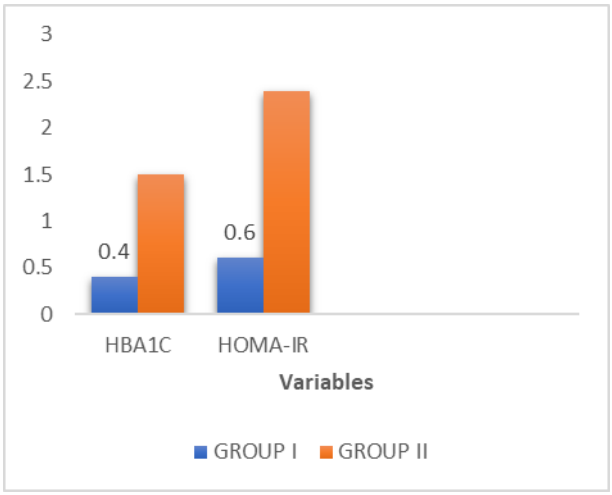

Figure 4: Showing comparative genomics changes of HBA1c & HOMA-IR in Group 1 and Group 2 Female Subjects**.**

CONCLUSION

The study, involving 200 subjects divided into 100 healthy individuals (Group 1) and 100 controlled Type 2 Diabetes Mellitus (T2DM) patients (Group 2), delved into the biochemical and insulin resistance profiles.

Notably, Group 2 exhibited significant elevations ($p<0.001$) in fasting blood sugar (FBS), glycosylated haemoglobin (HbA1c), and HOMA-IR compared to Group 1, corroborating earlier research findings (Bastard *et al.*, 2006; Al-Dhar MHS et al., 2010; Sheetz *et al*., 2002). Dyslipidaemia markers like triglycerides (TG), very low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol (LDL-C) were notably elevated in diabetic subjects, while high-density lipoprotein cholesterol (HDL-C) was diminished, signifying an augmented cardiovascular risk profile (Yach *et al*., 2004; Samatha *et al*., 2012). Moreover, insulin resistance was markedly higher in diabetic subjects, underscoring its pivotal role in the pathogenesis of T2DM (Fernandez-Real *et al*., 2000; Cao *et al*., 2008). These findings underscore the necessity for comprehensive management strategies targeting glycemic and lipid control to mitigate the risk of complications in T2DM patients.

REFERENCES

- Barbagallo, C., Danaei, G., Finucane, M., Lu, Y., Singh, G., Cowan, M., & Ezzati, M. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2· 7 million participants. *The Lancet*, *378*(9785), 31-40.
- Cao, Y. L., Hu, C. Z., Meng, X., & Zhang, J. (2008). Expression of TNF-α protein in omental and subcutaneous adipose tissue in obesity. *Diabetes research and clinical practice*, *79*(2),
- Cerf, M. E. (2013). Beta cell dysfunction and insulin resistance. *Frontiers in endocrinology*, *4*, 37.
- Chagnac, A., Weinstein, T., Herman, M., Hirsh, J., Gafter, U., & Ori, Y. (2003). The effects of weight loss on renal function in patients with severe obesity. *Journal of the American Society of Nephrology*, *14*(6), 1480-1486.
- Chan, J. C., Malik, V., Jia, W., Kadowaki, T., Yajnik, C. S., Yoon, K. H., & Hu, F. B. (2009). Diabetes in Asia: epidemiology, risk factors, and pathophysiology. *Jama*, *301*(20), 2129-2140.
- Danaei, G., Finucane, M. M., Lu, Y., Singh, G. M., Cowan, M. J., Paciorek, C. J., & Ezzati, M. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2· 7 million participants. *The lancet*, *378*(9785), 31-40.
- Dimas, A. S., Lagou, V., Barker, A., Knowles, J. W., Mägi, R., Hivert, M. F., ... & Magic Investigators. (2014). Impact of type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. *Diabetes*, *63*(6), 2158-2171.
- Dyck, P. J., Kratz, K. M., Karnes, J. L., Litchy, W. J., Klein, R., Pach, J. M., ... & Melton, L. 3. (1993). The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. *Neurology*, *43*(4), 817-817.
- Fernández-Real, J. M., Broch, M., Vendrell, J., Gutiérrez, C., Casamitjana, R., Pugeat, M., ... & Ricart, W. (2000). Interleukin-6 gene polymorphism and insulin sensitivity. *Diabetes*, *49*(3), 517-520.
- Henegar, J. R., Bigler, S. A., Henegar, L. K., Tyagi, S. C., & Hall, J. E. (2001). Functional and structural changes in the kidney in the early stages of obesity. *Journal of the American Society of Nephrology*, *12*(6), 1211-1217.
- Jun, H. S., & Yoon, J. W. (2004). A new look at viruses in type 1 diabetes. *ILAR journal*, *45*(3), 349-374.
- Kohei, K. A. K. U. (2010). Pathophysiology of type 2 diabetes and its treatment policy. *JMAJ*, *53*(1), 41-46.

- King, G. L. (2008). The role of inflammatory cytokines in diabetes and its complications. *Journal of periodontology*, *79*, 1527-1534.
- Kumar, R., Saha, P., Kumar, Y., Sahana, S., Dubey, A., & Prakash, O. (2020). A Review on Diabetes Mellitus: Type1 & Type2. *World Journal of Pharmacy and Pharmaceutical Sciences*, *9*(10), 838-850.
- Ma, C. (2003). Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation*, *108*, 1527-1532.
- McCarthy, M. I. (2010). Genomics, type 2 diabetes, and obesity. *New England Journal of Medicine*, *363*(24), 2339-2350.
- Mohan, V., & Pradeepa, R. (2009). Epidemilogy of diabetes in different Regions of India. *Health administrator*, *22*(1-2), 1-18.
- Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice*, *87*(1), 4-14.
- Sheetz, M. J., & King, G. L. (2002). Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *Jama*, *288*(20), 2579-2588.
- Siddiqui, A. A., Siddiqui, S. A., Ahmad, S., Siddiqui, S., Ahsan, I., & Sahu, K. (2013). Diabetes: Mechanism, pathophysiology and management-A review. *Int J Drug Dev Res*, *5*(2), 1-23.
- Smith, L., Burnet, S., & McNeil, J. (2003). Musculoskeletal manifestations of diabetes mellitus. *British journal of sports medicine*, *37*(1), 30.
- Suji, G., & Sivakami, S. (2004). Glucose, glycation and aging. Biogerontology5: 365–373.
- Vinod Mahato, R., Gyawali, P., Raut, P. P., Regmi, P., Singh, K. P., Pandeya, D. R.,& Gyawali, P. (2011). Association between glycaemic control and serum lipid profile in type 2 diabetic patients: Glycated haemoglobin as a dual biomarker. *Biomedical Research (0970-938X)*, *22*(3).
- Vlassara, H., & Palace, M. R. (2002). Diabetes and advanced glycation endproducts. *Journal of internal medicine*, *251*(2), 87-101.
- Wang, C. C. L., Gurevich, I., &Draznin, B. (2003). Insulin affects vascular smooth muscle cell phenotype and migration via distinct signaling pathways. *Diabetes*, *52*(10), 2562-2569.
- Wu, G., & Meininger, C. J. (2009). Nitric oxide and vascular insulin resistance. *Biofactors*, *35*(1), 21-27.
- Yach, D., Hawkes, C., Gould, C. L., & Hofman, K. J. (2004). The global burden of chronic diseases: overcoming impediments to prevention and control. *Jama*, *291*(21), 2616-2622.
- Yadav, D., Kochar, B., Mathur, A., &Chougle, A. (2017). Prevalence of microalbuminuria in type 2 diabetes mellitus: a hospital-based study. *Int. J. Res.-Granthaalayah*, *5*(12), 217-222.
- Yoon, J. W. (1995). A new look at viruses in type 1 diabetes. *Diabetes/metabolism reviews*, *11*(2), 83-107.
- Zheng, Y., Ley, S. H., & Hu, F. B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature reviews endocrinology*, *14*(2), 88-98.
- Zimmet, P., Alberti, K. G. M. M., & Shaw, J. (2001). Global and societal implications of the diabetes epidemic. *Nature*, *414*(6865), 782-787.
- Zimmet, P. Z., Tuomi, T., Mackay, I. R., Rowley, M. J., Knowles, W., Cohen, M., & Lang, D. A. (1994). Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabetic medicine*, *11*(3), 299-303.