



DETERMINATION OF HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS AMONG TYPE-2 DIABETIC PATIENTS IN GARHWAL REGION OF UTTARAKHAND

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ABSTRACT

Introduction: The study was conducted to determine some of the haematological and biochemical parameters among patients suffering from type-2 diabetes mellitus by measuring haemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscle haemoglobin concentration (MCHC), Platelet count (PLT), white blood cell count (WBC), Neutrophil (NEUT), Lymphocyte (LYMPH), Monocyte (MONO), Eosinophil (EOSINO), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), serum Fasting Blood sugar (FBS), Urea, Creatinine and Cholesterol. **Materials and Methods:** This study was carried out in HNB Base hospital under VCSG Government Medical College and some private clinical centres at Srinagar (Garhwal region) of Uttarakhand. A total 107 type-2 diabetic patients, out of which 62 (58%) were males and 45 (42%) were females having age group 30-80 years and equal numbers of healthy volunteers. **Results:** MCH and WBC counts were found statistically insignificant in both groups while PLT, LYMPH and MONO were found statistically significant, while other parameters were highly significant. Activity of AST and ALT among type-2 diabetic revealed a statistically highly significant increase. FBS, Urea and Cholesterol were found highly significant in both whereas Creatinine was having a significant value in the present study. **Conclusion:** The study showed statistically significant difference in some haematological and biochemical parameters among type-2 diabetes mellitus as compared to normal subjects. Screening of primary biochemical and haematological parameters are of high importance in respect to diabetic patients.

KEYWORDS : Haematological, Biochemical, Type-2 Diabetic Patients.

Introduction

Diabetes mellitus is one of the commonest endocrinal disorder affecting a majority of the world's population and reaching to a wide-ranging proportions among many countries¹. Not only in India but also in many developed and developing countries, diabetic patients are increasing day by day which may be due to the process of shifting in lifestyle, increase consumption of fast foods and beverages containing high levels of sugars (e.g. different drinks and sodas), lack of exercise, unhealthy diet, obesity, and socioeconomic changes over the past four decades². Diabetes mellitus can be defined as a group of metabolic disorders that are characterized by several defects in the regulation of carbohydrate, fat or protein metabolism, or all of the above³. Diabetes mellitus is characterized by hyperglycaemia, glycosuria, hyperlipidaemia, and negative nitrogen balance. The level of hyperglycaemia in diabetes mellitus patients results mainly from defects in insulin secretion or function, or both that leads to metabolic deregulation which may be associated with secondary damage to multiple organ systems, especially in areas like nerves, eyes, blood vessels and kidneys. The prevalence of diabetes mellitus is intensifying worldwide⁴. In other words Diabetes mellitus

incorporates a heterogeneous group of disorders characterized by hyperglycaemia associated with multiple disorders including metabolic, cellular, and blood disturbances leading to vascular complications⁵. The number of people suffering from type 2 (T2) Diabetes Mellitus has been increasing due to the aging population, urbanization, and low physical activity. According to International Diabetes Federation (IDF) estimate of 2013, 382 million (8.3%) adults had diabetes worldwide. The number has been increasing by twofold over the past 20 years, and 80% of the people with diabetes particularly live in low and middle socio-economic countries⁶.

The relationship between some components of metabolic syndromes and leukocytes was noted in several epidemiological studies which showed association between white blood cell count (WBC) and diabetes mellitus⁷. Leukocyte plays an important role during inflammation and also play some roles in the development of diabetes. There are common findings of reduced haemoglobin concentrations in diabetic patients^{8,9}. The liver plays an important role in carbohydrate metabolism having capability to store glucose as glycogen and synthesize glucose from non-carbohydrate

sources¹⁰. Increased activities of liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are indicators of hepatocellular injury and also associated with insulin resistance, metabolic syndrome and type 2 diabetes¹¹⁻¹³. Diabetic patients may have induced dyslipidaemia basically characterized by elevated levels of total cholesterol, triglycerides, low levels of HDL and increased low-density lipoprotein (LDL)¹⁴ (Nakanishi et al, 2005). In the present study we aim to study various haematological and biochemical changes among Type 2 diabetes mellitus patients in hilly Garhwal region of the Himalayan state of Uttarakhand.

Materials and Methods

Cross-sectional study was done in Type -2 diabetic patients with long time history of suffering from diabetes. This present study was carried out in HNB Base Hospital and few private clinical centres, at Srinagar in Garhwal region of Uttarakhand during January to December, 2018. A total 107 type-2 diabetic patients, out of which 62 (58%) were males and 45 (42%) were females with the age group 30-80 years and equal numbers of healthy volunteers with 48 (45%) males and 59 (55%) females having the age group 22-84 years were included in the study. The Institutional ethical committee clearance was obtained.

For the purpose of the study 10 ml of venous blood were drawn from each volunteer using a disposable plastic syringe. Haematological analysis were done using 2 ml of blood collected in tubes containing Ethylene diamine tetra-acidic acid (EDTA) for the assessment of haematological changes like haemoglobin (Hb), red blood corpuscles (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscle haemoglobin concentration (MCHC), Platelet count (PLT), white blood cell count (WBC) and Neutrophil (NEUT), Lymphocyte (LYMPH), Monocyte (MONO) and Eosinophil (EOSINO) as studied by Sood in 1996¹⁵ and Al-Ali et al. in 2016¹⁶.

Biochemical analysis were done using 8ml of the blood, poured in test tube container and then centrifuged after clotted. Serum was kept at 20°C in sterile condition till used for the assessment of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Fasting blood sugar (FBS), serum Urea (as revealed by Will and Savory in 1981)¹⁷, Creatinine and Cholesterol (showed by Kind and King in 1954)¹⁸, Reitman and Frankel in 1957¹⁹ and Meitattini in 1978²⁰).

Statistical analysis: Collected data were entered into Microsoft Excel and analysed using SPSS of windows version-21 (Trial). Descriptive statistical measures such as percentage and Student t-test were applied to identify the relationship between variables and to know statistically significant ($p < 0.05$).

Results

The values of haematological parameter in type-2 diabetic patients and healthy volunteers were shown in Table-1. MCH and WBC counts were found statistically insignificant ($p > 0.05$) in both diabetic patients and healthy volunteers while PLT, Lymph and Mono were found statistically significant ($p < 0.05$), remaining all others parameters were found statistically highly significant ($p < 0.001$).

Activity of serum marker enzymes (AST and ALT) in type-2 diabetic and healthy volunteers were shown in table-2. Results revealed a statistically highly significant increase ($p < 0.001$) in both parameters AST and ALT in diabetic patients as compared to the healthy volunteers.

The biochemical parameters FBS, Urea, Creatinine and Cholesterol values (Mean \pm SD) of both groups were shown in Table-3. FBS, Urea and Cholesterol were found statistically

highly significant ($p < 0.001$) in both type-2 diabetic patients and healthy volunteers while Creatinine was found statistically significant ($p < 0.05$) in present study.

Discussion

The results showed a decrease in the mean values of haemoglobin concentration (Hb), red blood cell (RBC) count, Packed cell volume (PCV), mean corpuscular volume (MCV) Neutrophil (NEUT) count among the type-2 diabetic patients against the healthy volunteers whereas mean value of mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets counts (PLT), white blood corpuscles (WBC), lymphocytes (LYMPH), monocytes (MONO) and eosinophil (EOSINO) count increases among type-2 diabetic patients in contrary to the healthy volunteers. Whereas, other studies have shown that several morphological changes in RBCs are significantly present among diabetic patients. Such changes include anisocytosis, poikilocytosis and hypochromia a direct impact on erythrocyte (RBC) functions that may contribute upon patient's complex pathology also as revealed by Zafer Saad Al Shehri²¹ in 2017 and Neam et al.²² in 2015. But some previous studies also demonstrated that in uncontrolled type-2 diabetic patients, erythrocytes persist throughout their life span in the hyperglycaemic condition and consequently subjected to a series of changes which in turn affect their flow properties and functions (Sing et al. 2009)²³. Anaemia is a common feature in patients with diabetes due to the high burden of chronic kidney disease (Thomas 2007) with glycosylation of red blood cells membrane protein (Oyedemi et al., 2011)²⁴. The study results demonstrated that levels of WBCs and differential leukocyte counts were slightly increased in type-2 diabetic patients compared to controls. A study by Vozarova et al. in 2002²⁵ also demonstrated the relationship between WBC and diabetes mellitus as a result of increased inflammatory mediators. Inflammatory agents, insulin and human blood components form a critical signal for any abnormalities, resulting in invasion mainly by foreign agents and/or inflammation (Ohshita et al. 2004)²⁶. These latter factors can induce in defense mechanisms and mainly contribute in changes of blood parameters levels, including mean white blood cell count, mean platelet count, and phagocyte percentage (Weyer et al. 2000)²⁷. Moreover, the study also showed an increase in mean values of platelet count in diabetic patients compared to control group. This increase may be a reflection of platelet activation which are demonstrated through some previous studies that platelets have large and variable sizes as observed in numerous clinical conditions, such as haemorrhages and myeloproliferative disorders. The increase might also designate microvascular dysfunction (Beyan et al. 2006)²⁸. Our results also agreed with other results which had shown that patients with diabetes mellitus show altered platelet function, such as high platelet counts and high mean platelet volume (MPV). It has also been reported that diabetic patients are likely to experience an increased risk for vascular disease (Papanas 2004)²⁹.

The mean differences of ALT and AST were found significantly increased through liver function tests (LFT) in patients of type-2 diabetes as compared to healthy control group. Idris et al. in 2011³⁰ also found significant increase in liver function tests (ALT and AST) in type-2 diabetic patients. Clore et al. in 1992³¹ had suggested that increase in liver enzymes levels in patients of diabetes mellitus resulted mainly from influence of insulin on liver and muscle tissue whereas Vazarova et al. (2002)³² proposed that a raised ALT reflects fatty changes in the abnormal liver morphology that antedates the development of type-2 diabetes. The mean differences of Fasting Blood sugar (FBS), Urea, Creatinine and Cholesterol were statistically significant compare to normal which resembles to the findings of Lal et al. 2009³³. The biochemical

changes may be because patients with long-term diabetes have repression of glycolytic enzymes and depression of gluconeogenic enzymes that indorses gluconeogenesis in liver which further contributes to hyperglycaemia (Wun et al. 2008)34.

Conclusion

Type-2 diabetes mellitus has become a serious public health problem worldwide. Primary biochemical and haematological changes in type-2 diabetic patients which mainly lead to the development of long-term complications and deprived quality of life or death. So, it is highly important to follow up and carefully screen the biochemical and haematological parameters in case of diabetic patients.

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Table 1: Some Haematological Parameters in Diabetic Patients and Healthy Volunteers Group

Parameters	Patients (n=107)		Health Volunteers (n=107)		t-test value	p value
	Mean	S.D	Mean	S.D		
Hb (g/dl)	12.5	2.0	13.2	1.7	2.76	<0.00
RBC(millions/mm ³)	4.3	0.5	4.7	0.6	5.30	<0.001
PCV	36.5	5.1	39.5	5.2	4.26	<0.00
MCV(fL)	83.4	8.9	83.9	5.3	3.49	<0.00
MCH(pg)	28.8	3.6	28.2	2.5	1.41	>0.05
MCHC(gm/dl)	34.3	1.4	33.2	0.9	13.05	<0.001
PLT(lack/mm ³)	2.5	1.1	2.2	0.9	2.18	<0.05
WBC(cell/mm ³)	8259.8	2717.9	7973.2	2720.6	0.77	>0.05
NEUT(%)	63.3	10.2	67.4	7.6	3.33	<0.00
LYMPH(%)	31.7	9.1	28.5	7.0	2.88	<0.05
MONO(%)	2.6	1.6	2.1	1.1	2.66	<0.05
EOSINO(%)	2.8	2.1	2.0	1.0	3.55	<0.00

Table 2: Some Serum Enzymes in Diabetic Patients and Healthy Volunteers Group

Parameters	Patients (n=107)		Health Volunteers (n=107)		t-test value	p value
	Mean	S.D	Mean	S.D		
ALT(U/L)	40.3	28.5	30.5	13.2	3.22	<0.001
AST(U/L)	46.0	37.8	29.7	11.2	4.27	<0.001

Table 3: Some Biochemical Parameters in Diabetic Patients and Healthy Volunteers Group

Parameters	Patients (n=107)		Health Volunteers (n=107)		t test value	p value
	Mean	S.D	Mean	S.D		
FBS(mg/dl)	198.0	82.1	94.7	12.3	12.87	<0.001
UREA(mg/dl)	39.6	29.5	24.9	9.2	4.92	<0.0
CREATININ E(mg/dl)	1.2	1.1	0.9	0.2	2.77	<0.05
CHOLESTEROL(mg/dl)	203.8	39.0	166.9	20.5	8.87	<0.001

References

- Guariguata DR, Whiting DR, Hambleton I, Beagley J, Linnenkamp U & Shaw JE, Whiting I, Hambleton I, Beagley U, Linnenkamp and J E Shaw (2014). "Global estimates of diabetes prevalence for 2013 and projections for 2035 [View at Publisher · View at Google Scholar · View at Scopus]. Diabetes

- Research and Clinical Practice, 103(2), 137-149. <https://doi.org/10.1016/j.diabetes.2013.11.002>
- Zahid N (2015). Burden of Diabetes Mellitus in Saudi Arabia. *Int J Health Sci (Qassim)*, Jul; 9(3). V-VI.
- Guyton A C & Hall J E (2002). *Medical Physiology* (10th ed., pp. 797-801). London: W.B. Saunders.
- Aslant M, Orhan D D, Orhan N, Sezik E, & Yesilada E (2006). In vivo Antidiabetic and Antioxidant Potential of *Helichrysum Plicatum* ssp. In Streptozotocin induced diabetic rats. *Journal of Ethnopharmacology*, 109, 54-59. <https://doi.org/10.1016/j.jep.2006.07.001>
- Elalamy I, Chakroun T, Gerotziakas GT et al. Circulating platelet-leukocyte aggregates: a marker of microvascular injury in diabetic patients. *Thromb Res*, 2008; 121(6): 843-848.
- Rizvi A, Sanders MB. Assessment and monitoring of glycemic control in primary diabetes care: monitoring techniques, record keeping, tests of average glycaemia, and point of care evaluation. *J Am Acad Nurse Pract*. 2006; 18: 11-21.
- Barzilay JL, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, Tracy RP (2001). The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. *Diabetes*, October; 50(10): 2384-9. <https://doi.org/10.2337/diabetes.50.10.2384>
- Chung FM, Tsai J C R, Chang D M, Shin S J, & Lee Y J & the Fu-mei Chung. (2005). Peripheral Total and Differential Leukocyte Count in Diabetic Nephropathy. *Diabetes Care*, 28(7), 1710-1717. <https://doi.org/10.2337/diabetes.28.7.1710>
- Thomas M, MacIsaac R, Tsalamandris C, (2003). Unrecognized anemia in patients with diabetes: A cross sectional survey. *Diabetes Care*, 26(4), 1164-1169. <https://doi.org/10.2337/diabetes.26.4.1164>
- Levinthal G N & Tavill A J (1999). Liver disease and diabetes mellitus. *Clinical Diabetes*, 17, 73.
- Marchesini, G., Brizi, M., & Bianchi, G. (2001). Nonalcoholic fatty liver diseases. A feature of metabolic syndrome. *Diabetes*, 50(8), 1844-1850. <https://doi.org/10.2337/diabetes.50.8.1844>
- Sattar N O, Scherbakova I, Ford O R, Eilly D S, Stanley E A, Forrest P W, Cobbeand J (2004). Shepherd. Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. *Diabetes*, 53(11), 2855-2860. <https://doi.org/10.2337/diabetes.53.11.2855>
- Wannamethee S G, Shaper A G, Lennon L, & Whincup P H (2005). Hepatic enzymes the metabolic syndrome and the risk of type 2 diabetes in old men. *Diabetes Care*, 28(12), 2913-291. <https://doi.org/10.2337/diabetes.28.12.2913>
- Nakanishi N T S & Wada M (2005). Association between fasting glucose and C-reactive protein in a Japanese population: The Minoh study. *Diabetes Res. Clinics and Practice*, 69, 88-98.
- Sood R 1996. *Hematology for students and practitioners*. 4th ed., Jaypee brothers, New Delhi, India.
- Al-Ali Z A J (2016). Some hematological and biochemical parameters in type 2 diabetic patients Missan/Iraq Int. J. Adv. Res. Biol. Sci. (2016); 3(4): 30-34.
- Will MR and J Savory (1981). *Biochemistry of renal failure*. Am. Clin. Lab. Sci., 11: 292-299.
- Kind P R N and E J King (1954). *Journal of clinical pathology*; 7: 322, cited by Wootton L.D. P and Freeman H (1982); *Microanalysis in medical biochemistry*, 6th ed.; Churchill Livingstone.
- Reitman S and S Frankel (1957). A colorimetric method for determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase. *Am. J. Clin. Path.*, 28:56.
- Meattini F (1978). The 4-hydroxy benzoate/4-amino phenazone chromogenic system. *Clin. Chem.*, 24: 2161-2165.
- Zafar Saad Al Shehri (2017). The relationship between some biochemical and hematological changes in type 2 diabetes mellitus; *Biomedical Research and Therapy*; 4(11): 1760-1774.
- Neam u MC1, Cr i oi u S, Avramescu ET, Margin DM, B c noi u MV, Turneanu D, D nciulescu Miulescu . (2015). The prevalence of the red cell morphology changes in patients with type 2 diabetes mellitus. *Romanian Journal of Morphology and Embryology*; 56(1): 183-189.
- Sing M, & Shin S (2009). Changes in erythrocyte aggregation and deformability in diabetes mellitus: A brief review. *Indian Journal of Experimental Biology*, 47(1), 7-15.
- Oyedemi S O, Yakubu M T & Afolayan A J (2011). Antidiabetic activities of aqueous leaves extract of *Leonotis leonurus* in streptozotocin induced diabetic rats. *Journal of Medicinal Plants Research*; 5: 119-125.
- Vozarova B, Weyer C, Lindsay R S, Pratley R E, Bogardus C, & Tataranni P A (2002). High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes; *Diabetes*; 51(2): 455-461. <https://doi.org/10.2337/diabetes.51.2.455>
- Ohshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, Kohno N (2004). Elevated white blood cell count in subjects with impaired glucose tolerance. *Diabetes Care*; 27(2): 491-496. <https://doi.org/10.2337/diabetes.27.2.491>
- Weyer C, Hanson K, Bogardus C, & Pratley R E (2000). Long-term changes in insulin action and insulin secretion associated with weight gain, loss, regain and maintenance of body weight. *Diabetologia*; 43(1): 36-46. <https://doi.org/10.1007/s001250050005>
- Beyan C, Kaptan K, & Ifran A (2006). Platelet count, mean platelet volume, platelet distribution width, and plateletcrit do not correlate with optical platelet aggregation responses in healthy volunteers. *Journal of Thrombosis and Thrombolysis*, 22(3), 161-164. <https://doi.org/10.1007/s11239-006-9014-7>
- Papanas N, Symeonidis G, Maltezos E, Mavridis G, Karavageli F, Vosnakidis T & Lakasas G (2004). Lakasas, Mean platelet volume in patients with type 2 diabetes mellitus. *Platelets*, 15(8), 475-478. <https://doi.org/10.1080/0953710042000267707>
- Idris A, K F H Mekky, B E E Abdalla and K A Ali (2011). Liver function tests in type 2 Sudanese diabetic patients. *Inter. J. Nut. Met.*, 3:17-21.
- Clore J N, E P Post, D J Baily, J F Nester and W G Blackard (1992). Evidence for increased liver glycogen in patients with Non-insulin dependent diabetes

- mellitus after a 3-day fast . J.Clin .Endocrine Metabolism; 74 : 660-666 .
32. Vazarova B, N Stefan, R S Lindsay, A Saremi, R E Pratley, C Bagardus and P A Tataranni (2002). High alanine aminotransferase is associated with decrease hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 51:1189-1895.
 33. Lal S S, Y Sukla, A Singh and E A Andriyas (2009). Hyperuricemia, high serum urea and hypoproteinemia are the risk factor for diabetes; Asia J. Med. Sci.,1:33-34.
 34. Wun Y T, C S Chan and C S Lui (2008). Hyperuricemia in type 2 diabetes mellitus in Bangladesh; Med. Res. Counce. Bull.; 4: 84-89.