A COMPARATIVE ANALYSIS OF BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN DIABETIC AND NON-DIABETIC ADULTS

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ABSTRACT

This study evaluated the biochemical and the hematological parameters in diabetic and non- diabetic patients. The measured biochemical parameters were fasting blood sugar, serum alanine aminotransferase (SGPT/ALT), total cholesterol, urea, creatinine and hematological parameters were hemoglobin, total white blood cell, neutrophil, lymphocyte,monocyte, eosinophil and ESR. There were 403 diabetic and 320 non-diabetic subjects included in this study and the study was carried out in BIRDEM (Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders) General Hospital). It was observed that the mean values of SGPT/ALT ($p<0.001$), total cholesterol ($p<0.001$) and creatinine ($p<0.001$) of biochemical parameters, and Monocyte ($p<0.001$), Eosinophil ($p<0.004$), ESR ($p<0.001$) of hematological parameters were significantly higher in diabetic patients than in the non-diabetic patients. In univariate analysis, all biochemical parameters and only four hematological parameters were found significantly associated with fasting blood sugar after adjusted with age and sex. The fasting blood sugar correlates highly with the other biochemical parameters but less or none with the hematological parameters. Our findings demonstrated that control of increased biochemical parameters and abnormal hematological levels in the early stage of diabetes mellitus may help the patients to raise quality of life.

KEY WORDS:

Diabetes mellitus, biochemical, hematological, hemoglobin and white blood cell count.

1. INTRODUCTION

Diabetes mellitus is an important endocrine health problem affecting major population in the world. In Bangladeshi population diabetes is being increased day by day. Bangladesh has the uncertain destination of being home to the huge number of people suffering from diabetes like in any other country. The World Health Organization (WHO) observed worldwide 170 million diabetic patients in 2002 and predicted the number to be 366 million or more in 2030(Bruke JP et al.,2004). Human being is taking dietary food in plant and animal sources and the main components of living cells are carbohydrate, fat and protein. However, due to the deficiency of insulin secretion disturbance of carbohydrate, fat and protein metabolism are occurred. (Prasad SK at al., 2009). Lack of Insulin leads to various metabolic complications in the human body with increases blood glucose, cholesterol, creatinine and transaminases enzyme level and decreases protein content. (Shanmugasundaram, K. R. at al., 1983).

Peripheral blood leukocytes produce polymorphonuclear cells, including monocytes as well as lymphocytes. Some previous studies showed that peripheral white blood cell(WBC) count might be associated with type-2 diabetes, coronary artery disease(CAD), stroke, micro and macro vascular complications (Oshita K et al., 2004; Tong PC et al., 2004 and Ford ES.,2002). Increased differential cell counts, including counts of eosinophils, neutrophils, and monocytes, also indicate the future incidence of coronary artery disease (CAD) (Madjid M at al., 2004; Prentice RL at al., 1982 & Olivares R at al., 1993). Fu-mei Chung et al, in 2005, showed that the white blood cells (WBC) might play a role in the development and progression of diabetic complications. However, there is no investigation concerning the differential leukocyte count in relation to diabetic nephropathy. In 2009 Anthony J. G. Hanley et al. in a large study of well-characterized non-diabetic subjects with risk factors for type 2 diabetes mellitus (DM) evaluate the associations of hematological parameters, including Hct, hemoglobin (Hgb), RBC and WBC with β-cell dysfunction and glucose concentrations (Anthony J. G. Hanley at al., 2009). Obesity is another major risk factor for diabetes, which is strongly suspected to develop diabetes. The major aim of this study was to observe if there was any relationship with the biochemical and hematological parameters in the newly diagnosed diabetes patients.

2. METHODS

2.1 Study subjects

This study, including all patients, diabetic and non-diabetic, was carried out during the period of January, 2014 to June,2014 in the department of clinical Biochemistry, Hematology and Pathology laboratory, BIRDEM(Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders)- a WHO collaborating center for prevention and control of diabetes, Dhaka, Bangladesh.

In this study, people consisted of 723 subjects (Age limit 26- 65 years; and Sex matched) who were classified into two groups: Diabetic 403 subjects (male-202, female-201) and non-diabetic 320 subjects (male-164, female-156).Subjects with apparent hepatic, pulmonary and renal malignancies, immunologic, infectious disorders or hematologic diseases with WBC count >

15,000/mm^3 were excluded .Moreover, subjects who were on steroids, immune suppressants, hemodialysis, or erythropoietin therapy were also excluded. The subjects only were confirmed diabetic who had plasma glucose levels at fasting and 2 hours of OGTT/after breakfast > 7.0 mmol/L and >11.0 mmol/L respectfully.

3. LABORATORY EXAMINATIONS

Consent was taken from every subject and they were requested to fast overnight (10 to 12 hrs). Blood samples were collected by venepuncture from non-diabetic and diabetic subjects. The serum samples were separated after allowing them to clot by centrifugation at 2500 rpm for 10 minutes at room temperature and were stored at -20°C until tested. Blood samples were treated as follows: For SGPT/ALT, total cholesterol, urea & creatinine estimation 4 ml of whole blood was taken in a plain test tube and serum was separated by centrifugation at 2500 rpm. However, in case of glucose test, 2 ml of blood was taken into a separate test tube containing fluoride fluid and within one hour of sample collection, plasma was separated. For estimation of CBC, 2 ml of blood was taken in a separate test tube containing EDTA (Ethylene di-amino tetra acetic acid). Complete blood counts including WBC, differential count and hemoglobin were performed by automatic counting machine (Cell-Dyn Ruby, Abbott, USA; Sysmex 1800i, Japan). Plasma glucose and others biochemical parameters (SGPT/ALT, total cholesterol, urea & creatinine) were measured in the BIRDEM Biochemistry laboratory, Dhaka, by the following mentioned methods.

4. BIOCHEMICAL AND HEMATOLOGICAL INVESTIGATIONS

The levels of fasting blood sugar (FBS), total cholesterol, SGPT/ALT, urea and creatinine were measured by commercially available kits (Bio-Rad Laboratories, Richmond, USA; Randox laboratories Ltd., Antrim, UK; Merck, Germany; Sigma Chemicals Co, USA; Roche international Inc., USA; Jhonson & Jhonson Inc.,USA.) by Dade Behring, Hitachi-912 and Vitros-250 (Dry Chemistry) automated chemistry analyzers. The glucose test was done with the modified hexokinase-glucose-6-phosphate dehydrogenase method, presented as a general clinical laboratory method by Kunst, et al. (Kunst A at al., 1983). The normal value of BIRDEM laboratory SGPT/ALT was up to 40 U/L and urea (10-50) mg/dl, creatinine (0.67-1.2) mg/dl and total cholesterol <200 mg/dL and hematological parameters were WBC (4000-10,500)/cmm, haemoglobin male- (13-17) g/dl, female- (12-15) g/dl, ESR male<10 and female <20 mm in 1st hr (western green) and differential counts were neutrophil (40-70) %, lymphocyte (20-45) %, monocyte (2-8) % and eosionophil (1-5) %. All biochemical tests were done at 37°C.

5. STATISTICAL ANALYSIS

Statistical analysis was carried out with SPSS 17(Chicago, IL, USA) software package and p values less than 0.05 were considered as statistically significant.The study analyses the relationship between the fasting blood sugar with non-diabetic and diabetic subjects by Pearson correlation coefficients. To examine the relationships, multiple linear regression and partial correlations were used, after adjusting for covariates. The results were presented as mean ±SD.

6. RESULTS

A total of 723 non-diabetic and diabetic subjects (mean age, 41.49± 8.12 years; male/female, 164/156 and 43.92±9.27 years; male/female, 202/201) participated in this study. The biochemical parameters' mean value presented in table-1 was higher than that of non-diabetic subjects. The hematologic parameters were as follows: mean of hemoglobin concentration of non-diabetic and diabetic subjects were 13.26 ± 1.30 and 12.76 ± 1.49 g/dl; mean of total WBC count, 6739.06± 1936.09 and 9540.75± 2646.55 (/cmm); and mean of ESR, 18.13 ± 8.19 and 34.74 ± 27.67 (mm in1st hr) respectively. But Lymphocyte count (p=0.065) was not significant than non-diabetic subjects. Diabetic subjects had low monocyte and hemoglobin concentration but elevated in other parameters.

Table 1: Mean values of descriptive characteristics and biochemical and hematological parameters of the non-diabetic and diabetic subjects.

Name of Biochemical and Hematological parameters	Non-Diabetic Mean ± SD	Diabetic Mean ± SD	p-value
No. of subjects (male/female)	320 (164/156)	403 (202/201)	
Age (Years)	41.49 ± 8.12	43.92 ± 9.27	0.033
Fasting blood sugar (mmol/L)	5.64 ± 1.50	9.61 ± 4.50	<0.001
SGPT/ ALT (U/L)	27.39 ± 8.83	34.92 ± 28.62	<0.001
Total Cholesterol (mg/dl)	171.03 ± 31.42	184.56 ± 40.66	<0.001
Urea (mg/dl)	21.78 ± 5.63	23.76 ± 7.60	0.040
Creatinine (mg/dl)	0.99 ± 0.19	1.03 ± 0.33	<0.001
Hemoglobin (H7gb) (g/dl)	13.26 ± 1.30	12.76 ± 1.49	0.008
Total WBC count (/cmm)	6739.06 ± 1936.09	9540.75 ± 2646.55	0.001
Neutrophil (%)	61.20 ± 7.64	57.56 ± 9.35	0.020
Lymphocyte (%)	29.96 ± 7.26	33.60 ± 8.65	0.065
Monocyte (%)	6.58 ± 2.34	4.92 ± 1.52	<0.001
Eosinophil (%)	2.09 ± 1.55	3.95 ± 2.79	<0.001
ESR (mm in 1^{st} hr)	18.13 ± 8.19	34.74 ± 27.67	<0.001

Data presented are Mean ± Standard Deviation, *P*-value obtained from Independent-Samples "t" test.

The Pearson's correlations (*r*) among biochemical parameters are shown in table-2. Non- diabetic patients' fasting blood sugar showed no significant correlations with ALT, total cholesterol, urea and creatinine; whereas, fasting blood sugar showed very high correlations with SGPT/ALT (p=0.048), total cholesterol (p=0.020), urea (p=0.036) and creatinine (p=0.050) and were observed more frequent and significant in diabetic than non-diabetic subjects (table-2). On the other hand non-diabetic subjects, fasting blood sugar showed high correlation with lymphocytes. In diabetic subjects, fasting blood sugar was found to be correlated with hemoglobin (p=0.018),

total WBC count (p=0.020) and ESR (p=0.016) but neutrophil (p=0.120), lymphocyte (p=0.204), monocyte (p=0.146) and eosinophil (p=0.602) had no significance in diabetic subjects.

Table 2: Pearson's Correlation between fasting blood sugar with biochemical and hematological marker among non-diabetic and diabetic subjects

Name of Biochemical & Hematological markers	Non-Diabetic subject		Diabetic subject	
	r	*p*-value	*r*	*p*-value
Age	0.102	0.069	-0.050	0.315
SGPT/GPT	0.015	0.796	0.099	0.048
Total Cholesterol	0.054	0.340	0.116	0.020
Urea	0.019	0.729	0.105	0.036
Creatinine	0.036	0.523	0.095	0.050
Hemoglobin	-0.061	0.275	0.180	0.018
Total WBC Count	0.099	0.421	0.040	0.020
Neutrophil	0.098	0.080	0.078	0.120
Lymphocyte	-0.123	0.028	-0.064	0.204
Monocyte	0.019	0.732	-0.073	0.146
Eosinophil	-0.004	0.943	-0.026	0.602
ESR	0.043	0.441	0.155	0.016

r- Pearson's correlation coefficient, coefficient correlation is signifiant at the level 0.005

In univariate analysis, haemoglobin, neutrophil and lymphocyte were not significantly associated with fasting blood sugar whereas all biochemical parameters SGPT/ALT, total cholesterol, urea and creatinine had significant odds ratios (OR) of 4.384 (0.161 to 0.900), 4.737 (0.174 to 1.620), 2.703 (0.100 to 0.170), 2.999 (0.111 to 0.008); hematological parameters WBC count, monocyte, eosinophil and ESR had significant odds ratios (OR) of 7.990 (0.286 to 194.420), - 6.138 (- 0.223 to- 0.117), 4.411 (0.162 to 0.101), 6.044 (0.220 to 1.257) respectively (Table-2). After adjustment for age and sex, SGPT/ALT, total cholesterol, urea, creatinine, WBC (total count), monocyte, eosinophil and ESR the odds ratios (OR) were 4.379 (0.158 to 0.910), 4.725 (0.171 to 1.548), 2.701 (0.098 to 0.167), 2.984 (0.108 to 0.007), 7.936 (0.278 to 194.387), - 6.955 (- 0.222 to - 0.116), 4.399 (0.158 to 0.098), 5.988 (0.219 to 1.255) respectively and independently associated with fasting blood sugar. When the model was adjusted for age, and sex, the association of SGPT/ALT, total cholesterol, urea, creatinine, total WBC count, monocyte, eosinophil and ESR with fasting blood sugar remained significant ($P<0.001$, $P<0.001$, $p=0.008$, $p=0.004$, $P<0.001$, $p<0.001$, $p<0.01$ and $p<0.01$ respectively).

Table 3: Association between fasting blood sugar with biochemical and hematological parameters effects on newly diagnosed of diabetic and non-diabetic subjects.

Dependent variable	**Independent variable**	
	FBS	
	Unadjusted	**Adjusted**
SGPT/ALT β-Coefficient (95% CI) *p*-value	4.384 (0.161 to 0.900) < 0.001	4.379 (0.158 to 0.910) <0.001
Total Cholesterol β-Coefficient (95% CI) *p*-value	4.737 (0.174 to 1.620) < 0.001	4.725 (0.171 to 1.548) <0.001
Urea β-Coefficient (95% CI) *p*-value	2.703 (0.100 to 0.170) 0.007	2.701 (0.098 to 0.167) 0.008
Creatinine β-Coefficient (95% CI) *p*-value	2.999 (0.111 to 0.008) 0.003	2.984 (0.108 to 0.007) 0.004
Hemoglobin β-Coefficient (95% CI) *p*-value	- 1.096 (-0.041 to -0.015) 0.274	- 1.095 (-0.040 to -0.013) 0.269
Total WBC Count β-Coefficient (95% CI) *p*-value	7.990 (0.286 to 194.420) < 0.001	7.936 (0.278 to 194.387) <0.001
Neutrophil β-Coefficient (95% CI) *p*-value	- 0.963 (- 0.036 to - 0.079) 0.336	- 0.955 (- 0.035 to -0.077) 0.340
Lymphocyte β-Coefficient (95% CI) *p*-value	1.290 (0.048 to 0.099) 0.197	1.287 (0.046 to 0.097) 0.240

Monocyte β-Coefficient (95% CI) *p*-value	- 6.138 (- 0.223 to- 0.117) <0.001	- 6.955 (- 0.222 to -0.116) <0.001
Eosinophil β-Coefficient (95% CI) *p*-value	4.411 (0.162 to 0.101) <0.001	4.399 (0.158 to 0.098) <0.01
ESR β-Coefficient (95% CI) *p*-value	6.044 (0.220 to 1.257) <0.001	5.988 (0.219 to 1.255) <0.01

P-values were from Multivariate linear regression. When adjusted for age and sex. B= Standard regression coefficient.

7. DISCUSSION

It is found that diabetes mellitus may be associated with revised biochemical and hematological parameters. But this phenomenon has been observed mainly in newly diagnosed Type-1 and Type-2 diabetic patients. In this study, a substantial number of young diabetic patients do not show typical characteristics of either Type-1 or Type-2 diabetes mellitus. Many studies have previously been performed at BIRDEM on newly diagnosed diabetic and alteration of their work has been found. In compared to non-diabetic subjects the enzymes levels were found markedly elevated in diabetic patients. Everhart JE (Everhart JE, 1995) more frequently observed elevated SGPT/ALT levels in diabetic than in the studied general population. This study also showed that 22.5% diabetic subjects had elevated SGPT/ALT enzymes. In this cross sectional study of 403 patients newly diagnosed with diabetes total cholesterol were found independently associated with the early stage of diabetes. It is noticeable that, 33% had abnormal total cholesterol levels. It is known that an increased cholesterol level leads to serious pathological condition. Umesh CS et al suggested that hyperlipidaemia is linked with diabetic control. In this study there is also an increase in serum concentration of TC (Umesh CS at al., 2005). In diabetes a major component of the metabolic syndromes are altered lipid and lipoprotein metabolism (Ferrannini E at al., 1991; Ford ES at al., 2002; Park Y-W at al., 2002 & Cleeman JI at al., 1998). Specifically, non-diabetic subjects with the metabolic syndrome manifest high serum triglycerides and low HDL-C levels, whereas cholesterol levels tend to be normal (Ferrannini E at al., 1991; Ford ES at al., 2002; Park Y-W at al., 2002 & Cleeman JI at al., 1998). This study had shown 67% serum cholesterol levels were normal and 33% were abnormal in diabetic and (79% normal & 21% abnormal) non-diabetes subjects. For this reason, if diabetes subjects are long time suffering from hyperlipidaemia then cardiovascular risk factors and macrovascular complications increases to an alarming level. Basit et al (Basit A et al, 2005) demonstrated an association hypertension and hypertriglyceridemia with poor glycemic control.

Kwame Osel et al observed that a multiple syndrome and other defects existed before the development of diabetes and hypertension in these high-risk subjects (Kwame Osel at al., 2003). In diabetes subjects increased urea and creatinine level is seen when there is damage to the kidney or the kidney is not functioning properly. Moderate increased level of serum urea and creatinine in our studied diabetes subjects is consisted with the observation of Sugam Shrestha et al 2008. Presence of higher hemoglobin level in non-diabetic subjects compare to that of diabetic subjects can be attributed as a constitutional feature. In this study of Bangladeshi adult population, determination of leukocyte count was significantly associated with diabetes after adjustment of data for age and sex. These results are consistent with the theory that inflammation has a role in the etiology of diabetes (Teresa A. Hillier at al., 2001). However, the erythrocyte sedimentation rate (ESR) was significantly associated with diabetes mellitus incidence after data were adjusted for age and sex. Glycosylated hemoglobin levels would have needed to be measured to evaluate the level of control of diabetes. Our data demonstrated that hematological parameters is not uncommon and may be associated with diabetes mellitus in the Bangladeshi population. Limitation of our study were that it was a cross sectional observational study and was carried out in only one institution. The sample size was rather small to draw inference regarding the whole population. As total lipid profile, BMI, habitual and nutritional status were not included in the study these could not be compared. Other socio-demographic and biophysical risk factors may be important to be investigated in order to prevent newly diagnosed diabetes

8. CONCLUSION

The p-values of different biochemical and hematological parameters of newly diagnosed diabetic subjects were significantly higher than those of non-diabetic subjects. Diabetic subjects had lower hemoglobin and neutrophils level in compare to that of non-diabetic subjects. Our findings demonstrated that decreasing of neutrophil and hemoglobin levels in the early stage of diabetes may help patients improve their health and reduce their morbidity rate. A cholesterol abnormality which is common in diabetic subjects is an important etiological factor for atherosclerosis and coronary heart disease. A significant correlation between them was established by our study.

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