Research Article

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A Cross-sectional Study of Serum electrolytes, Calcium, and Magnesium Levels in Diabetic and Non-diabetic Subjects in Dhaka City, Bangladesh.

Md.Jahangir Alam[1]*, Most.Nur-E-Taj Mokarrama Mukti[2]; Bilkis sultana[1], Md. Anisuzzaman khan[1], Arnab Kumar Sarker[1] & Sunil Chandra Mallik[3]

1. Most. Nur-E-Taj Mokarrama Mukti, Department of Zoology, University of Rajshahi, Bangladesh. **E-mail:** muktialam156@gmail.com
2. Bilkis sultana, Junior Experimental officer, General laboratory. BRDEM General Hospital, Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Shabagh, Dhaka-1000. **E –mail:** bilkis78.bs@gmail.com
3. Md. Anisuzzaman khan, Experimental officer, General laboratory. BRDEM General Hospital, Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Shahbagh, Dhaka-1000, Bangladesh. **E-mail:** khanmd.zaman1968@gmail.com
4. Arnab Kumar Sarker, Senior Research officer, department of immunology. BRDEM General Hospital, Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Shahbagh, Dhaka-1000, Bangladesh. **E-mail:** sarkerarnab21f@gmail.com
5. Sunil Chandra Mallik, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh. **E-mail:** such.mallik@gmail.com

Corresponding author

Md. Jahangir Alam,
Principal Scientific Officer, Room# 321, General laboratory, BRDEM General Hospital, Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka-1000, Bangladesh.
Tele : +8801711069437, +8801886069437,
Email : jahangirmukti75@gmail.com

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ABSTRACT

The prevalence of diabetes is rising in Bangladesh, posing significant health challenges. This cross-sectional study conducted at BIRDEM General Hospital in Dhaka, Bangladesh, involved 1,020 subjects, including 752 with type-2 diabetes and 268 non-diabetic controls, aged 23 to 87 years. The study aimed to compare serum levels of magnesium, electrolytes (sodium, potassium, chloride, total carbon dioxide), calcium, amylase, and lipase between diabetic and non-diabetic subjects, and to explore correlations among these parameters. Diabetic subjects exhibited significantly higher mean levels of fasting blood sugar ($P < 0.01$), urea ($P < 0.01$), creatinine ($P < 0.01$), amylase ($P < 0.01$), and lipase ($P < 0.01$) compared to non-diabetic subjects. Conversely, sodium ($P < 0.01$), potassium ($P < 0.01$), chloride ($P < 0.01$), total carbon dioxide ($P < 0.05$), calcium ($P < 0.01$), magnesium ($P < 0.01$), and inorganic phosphate ($P < 0.01$) concentrations were significantly lower in diabetic subjects. Fasting blood sugar showed negative correlations with urea ($P < 0.01$) and inorganic phosphate ($P < 0.02$) in diabetic subjects. Multivariate analysis adjusted for age and sex revealed significant associations of fasting blood sugar with all parameters except urea, total carbon dioxide, and potassium. These findings underscore the importance of monitoring serum magnesium in type-2 diabetes and considering magnesium supplementation where necessary.

Keywords : *Electrolytes, Macrovascular complications, Normal glucose regulation, Diabetes mellitus.*

INTRODUCTION

Diabetes mellitus (DM) presents a significant public health challenge globally, including in the Bangladeshi population. It is a major health issue particularly in low and middle-income countries [1-5]. Projections indicate that by 2030, approximately 551.9 million people worldwide will be affected by DM. Bangladesh is expected to rank as the 8th highest country in terms of DM prevalence by that year [6].

Electrolytes such as sodium (Na+), potassium (K+), chloride (Cl-), total carbon dioxide (TCo2), inorganic phosphate (PO42-), calcium (Ca2+), and magnesium (Mg2+) are crucial components of serum [7]. The regulation of plasma glucose levels is closely associated with the serum concentrations of these electrolytes

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[8]. Magnesium plays a pivotal role in the synthesis of nucleic acids and proteins, intermediary metabolism, and has specific functions in organs like the neuromuscular and cardiovascular systems. It activates more than 300 enzymes in a human body and serves as a critical cofactor in many enzymatic reactions involved in carbohydrate metabolism [9]. Low levels of magnesium and other electrolyte imbalances have been identified as predictors of mortality in type 2 DM [10], and supplementation with oral magnesium has been shown to reduce fasting plasma glucose levels in DM patients [11].

The prevalence of DM in Bangladesh is escalating rapidly. Type 2 diabetes is characterized by insulin deficiency and chronic metabolic disease due to insulin resistance [12]. Hypomagnesemia is frequently observed in association with diabetes mellitus, with prevalence rates ranging from 25% to 39% [13].

In this study, we measured serum levels of magnesium, calcium, and other electrolytes in Bangladeshi subjects with diabetes to investigate their relationship with glucose levels.

METHODS

Study Subjects

This study was conducted at the Department of Clinical Biochemistry, Hematology & Pathology, BIRDEM (Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders) - a WHO collaborating center for prevention and control of diabetes, located in Dhaka, Bangladesh. The study spanned from January 2023 to June 2023.

A total of 1020 subjects aged between 23 to 87 years, matched for sex, were enrolled. They were categorized into two groups: diabetic (732 subjects; male: 412, female: 340) and non-diabetic (268 subjects; male: 120, female: 148). Subjects with evident pulmonary, hepatic, or renal malignancies, infectious diseases, or immunological disorders were excluded from the study. Additionally, individuals undergoing steroid therapy, immune suppressants, hemodialysis, or erythropoietin treatment during the study period were also excluded. All diabetic subjects had previously confirmed diabetes with fasting plasma glucose levels >7.0 mmol/L and plasma glucose at 2 hours of OGTT/ after breakfast >11.0 mmol/L.

Laboratory Examinations

Prior consent was obtained from each participant, who was instructed to fast overnight (10 to 12 hours). Blood samples were collected via venipuncture from both non-diabetic and diabetic subjects. The collected samples were allowed to clot, and serum was separated by centrifugation at 10,000 rpm for 15 minutes at room temperature. Serum samples were stored at -20°C until further analysis.

For the assessment of fasting blood sugar (FBS), urea, creatinine, sodium, potassium, chloride, total carbon dioxide, calcium, magnesium, phosphate, amylase, and lipase levels, 4 ml of blood was collected in a plain test tube and processed as described. Glucose estimation specifically utilized 1 ml of blood placed in a tube containing sodium fluoride, with plasma separated and analyzed promptly after collection.

Biochemical investigations were carried out at the BIRDEM Biochemistry laboratory, Dhaka, using enzymatic and Ion-Selective Electrode (ISE) methods. Commercially available kits from Bio-Rad Laboratories (Richmond, USA), Randox Laboratories Ltd. (Antrim, UK), Merck (Germany), Sigma Chemicals Co. (USA), Roche International Inc. (USA), Johnson & Johnson Inc. (USA), and Abbott (USA) were employed for the assays.

Analytical equipment such as Dimension EXL 200 (Siemens), Architect c8000 (Abbott), Beckman Coulter AU680, Advia 1800 (Siemens), and Vitros-250 (Dry Chemistry) automated analyzers were used for the measurements. The methodologies for each parameter followed standard clinical laboratory protocols.

Biochemical Investigation

Various biochemical parameters including fasting blood sugar (FBS), urea, creatinine, sodium, potassium, chloride, total carbon dioxide, calcium, magnesium, phosphate, amylase, and lipase were quantified using validated methods. Normal reference ranges according to the BIRDEM laboratory were applied for result interpretation.

Glucose measurement utilized the hexokinase-glucose-6-phosphate dehydrogenase method [14]. Urea was assessed via urease enzymatic hydrolysis [16], while creatinine was measured using the kinetic Jaffe reaction[17]. Sodium, potassium, and chloride were determined using ion-selective electrodes based on membrane potentials. Magnesium levels were analyzed through a modification of the methylthymol blue (MTB) complexometric procedure [18]. Phosphate estimation involved the reaction with ammonium molybdate to form a heteropolyacidcomplex[19]. Lipase activity was evaluated using enzymatic hydrolysis techniques.The rate of production of methylesorufin is measured by a bichromatic rate reaction. Amylase activity was measured by the hydrolysis of CNPG3 as the resulting increased absorbance is directly proportional to the α-amylase activity [15]. These biochemical analyses were performed at 37°C to maintain consistency and accuracy in results.

Statistical Analysis

Statistical analyses were performed using SPSS version 16 (Chicago, IL, USA). A significance level of $p < 0.05$ was considered statistically significant. The relationship between fasting blood sugar levels and variables among non-diabetic

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and diabetic subjects was initially explored using Pearson correlation coefficients. Multiple linear regression and partial correlations were subsequently utilized to investigate these relationships while adjusting for covariates. Descriptive statistics are presented as mean ± SD.

RESULTS

A total of 1020 subjects participated in the study, comprising 752 (73.72%) diabetic and 268 (26.28%) non-diabetic individuals. The mean ages of non-diabetic and diabetic subjects were 42.56 ± 9.17 and 48.72 ± 11.91 years, respectively, ranging from 23 to 87 years. The gender distribution showed 52.16% (532) male and 47.84% (488) female subjects.

Table 1. Gender and Group Distribution in Non-Diabetic and Diabetic Subjects.

Group	Gender	No.of Subjects (1020)	Percentage
Non-diabetic subjects	Male	120	11.77%
(N=268)	Female	148	14.51%
Diabetic Subjects	Male	412	40.39%
(N=752)	Female	340	33.33%

In this study, various biochemical parameters including urea, creatinine, sodium, potassium, chloride, total carbon dioxide, calcium, magnesium, phosphate, amylase, lipase, and serum fasting blood sugar were assessed in both diabetic and non-diabetic subjects. As shown in Table 2, the mean ± SD values of fasting blood sugar, urea, creatinine, amylase, and lipase were significantly higher in diabetic subjects compared to non-diabetic subjects ($p < 0.01$). Sodium, potassium, chloride, total carbon dioxide, calcium, magnesium, and phosphate concentrations were significantly lower in diabetic subjects compared to non-diabetic subjects ($p < 0.01$).

Table 2. Mean Values of Descriptive Characteristics and Biochemical Parameters of Non-Diabetic and Diabetic Subjects.

Biochemical Parameters	Non-Diabetic Subjects Mean ± SD	Diabetic Subjects Mean ± SD	p-value
No of subjects (Male+ Female)	268(120+148)	752(412+340)	
Age (years)	42.56± 9.17	48.72± 11.91	<0.01
Fasting Blood Sugar (mmol/l)	5.53± 0.72	9.99± 2.19	<0.01
S. Urea (mg/dl)	24.35± 5.13	25.98± 9.55	<0.01
S. Creatinne (mg/dl)	0.92± 0.17	1.15± 0.23	<0.01
S. Sodium (mmol/L)	140.34± 3.65	135.60± 7.00	<0.01
S.Potasium (mmol/L)	4.16± 0.43	4.13± 0.79	<0.01
S. Cloride (mmol/L)	100.07± 6.48	102.70± 4.16	<0.01
S. Total carbondioxide(mmol/L)	23.65± 4.13	22.80± 5.13	<0.05
S. Calcium (mg/dl)	8.56± 0.85	9.62± 0.64	<0.01
S. Magnasium (mg/dl)	0.98± 0.08	0.82± 0.11	<0.01
S. Phosphate (mg/dl)	3.98± 0.73	3.75± 1.16	<0.01
S. Amylase (U/L)	30.34± 9.56	50.05± 29.11	<0.01
S.Lipase (U/L)	21.19± 8.08	29.0± 14.22	<0.01

In univariate analysis, urea, potassium, and total carbon dioxide were not significantly associated with fasting blood sugar, while creatinine, sodium, chloride, calcium, magnesium, phosphate, amylase, and lipase showed significant associations ($p < 0.05$). After adjustment for age and sex, creatinine, sodium, potassium, chloride, calcium, magnesium, phosphate, amylase, and lipase remained independently associated with fasting blood sugar ($p < 0.05$).

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Table 3. Association between Fasting Blood Sugar (FBS) and Biochemical Parameters and Electrolytes in Non-Diabetic and Diabetic Subjects.

Dependent variable	Independent Variable	
	Fasting Blood Sugar	
	Unadjusted	Adjusted
S. Urea β-Coefficient (95% CI) p-value	8.448 (0.015 to 0.047) 0.138	8.465 (0.012 to 0.041) 0.188
S. Creatinine β-Coefficient (95% CI) p-value	5.187 (3.326 to 0.294) <0.01	5.407 (3.132 to 0.283) <0.001
S. Sodium β-Coefficient (95% CI) p-value	22.464 (-0.100 to -0.241) <0.01	22.526 (-0.100 to -0.234) <0.001
S. Potassium β-Coefficient (95% CI) p-value	9.429 (-0.146 to -0.038) 0.225	10.192 (-0.342 to -0.091) <0.04
S. Chloride β-Coefficient (95% CI) p-value	15.089 (-0.063 to -0.137) < 0.01	14.748 (-0.059 to -0.130) < 0.01
Total Cabondioxide β-Coefficient (95% CI) p-value	8.576 (0.011 to 0.019) 0.537	8.354 (0.018 to 0.033) 0.289
S. Calcium β-Coefficient (95% CI) p-value	16.865 (- 0.914 to - 0.325) < 0.01	17.306 (- 0.959 to -0.325) < 0.001
S. Magnasium β-Coefficient (95% CI) p-value	17.487 (- 9.999 to -0.468) 0.01	16.940 (- 9.382 to -0.451) <0.001
S. Phosphate β-Coefficient (95% CI) p-value	9.322 (- 0.130 to 0.051) 0.05	9.684 (- 0.236 to -0.089) 0.04
S. Amylase β-Coefficient (95% CI) p-value	7.918 (0.020 to 0.198) 0.01	7.664 (0.025 to 0.225) <0.01
S. Lipase β-Coefficient (95% CI) p-value	8.002 (0.030 to 0.148) 0.01	7.645 (0.042 to 0.192) <0.01

*p-values were from multivariate linear regression, adjusted for age and sex. β - Standard regression coefficient.

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Table 4. Correlation between Fasting Blood Sugar and Other Parameters Among Non-Diabetic and Diabetic Subjects.

Name of the tests	Non diabetic		Diabetic	
	r	p-value	r	p-value
Age (years)	-0.018	0.773	0.040	0.269
S. Urea (mg/dl)	0.113	0.029	-0.157	<0.001
S. Creatinine (mg/dl)	-0.147	0.016	0.070	0.050
S. Sodium (mmol/L)	0.044	0.475	0.141	<0.01
S. Potasium (mmol/L)	0.007	0.903	0.004	0.917
S. Chloride (mmol/L)	0.179	<0.01	0.155	<0.01
S. Total Carbondioxide (mmol/L)	0.074	0.230	0.060	0.098
S. Calcium (mg/dl)	0.047	0.201	-0.076	0.213
S. Inorganic Phosphate (mg/dl)	-0.086	0.160	-0.111	<0.02
S. Magnasium (mg/dl)	0.035	0.564	-0.038	0.304
S. Amylase (U/L)	-0.075	0.002	0.004	0.922
S. Lipase (U/L)	-0.075	0.221	0.176	<0.01

Pearson's correlation coefficients (r) were used to assess the correlations. In non-diabetic subjects, fasting blood sugar was positively correlated with urea ($p = 0.029$) and chloride ($p = 0.011$), while it was negatively correlated with creatinine ($p = 0.016$) and several other parameters. In diabetic subjects, fasting blood sugar was positively correlated with urea, sodium, chloride, and lipase (all $p < 0.05$), and had no significant correlation with age, potassium, total carbon dioxide, calcium, magnesium, or amylase.

DISCUSSION

Disturbances in water and electrolyte balance are common in diabetic subjects due to factors such as insulin deficiency, hyperglycemia, and hyperketonemia [20]. Our study confirmed significant reductions in serum Mg^{2+}, Na^{+}, and K^{+} levels among Bangladeshi diabetic subjects, as observed in Table 2. Conversely, we noted elevated serum Ca^{2+} and Cl^{-} levels in individuals with diabetes mellitus (DM). These findings are consistent with prior research highlighting lower serum magnesium levels in type 2 diabetes [21, 22, 23].

Elevated levels of urea and creatinine in diabetic subjects typically indicate impaired kidney function. Our study observed moderate increases in serum urea and creatinine among diabetic patients, aligning with findings by Sugam Shrestha et al. in 2008 [24].

Amylase and lipase measurements are crucial in diagnosing pancreatitis and other diseases. In our study, diabetic subjects exhibited elevated serum amylase and lipase levels, which positively correlated with fasting blood sugar. This observation supports findings by Majharul Haque et al., who noted an inverse relationship between these enzymes and plasma glucose levels in DM patients [25].

Diabetic ketoacidosis often leads to excessive phosphate loss through osmotic diuresis. Treatment with insulin and fluids may exacerbate this deficiency, resulting in a significant decrease in plasma phosphate levels due to intracellular shifts [26], as observed in our study among diabetic subjects compared to non-diabetic counterparts.

We identified a negative correlation between serum magnesium levels and fasting blood sugar ($P < 0.001$), consistent with Karim et al. [27] and Mishra S et al. [12], who reported similar findings in type 2 diabetes.

Physiologically, Na^{+} reabsorption primarily occurs in the proximal tubules of the kidneys. In diabetes, hyperglycemia-induced osmotic diuresis can artificially lower plasma sodium concentrations. Calcium (Ca^{2+}) reabsorption, on the other hand, is regulated independently of Na^{+}, influenced by factors like calcitonin, parathyroid hormone, and vitamin D [28]. These factors, alongside energy metabolism and glucose regulation, contribute to diabetic macrovascular complications, particularly cardiovascular diseases, a leading cause of mortality in diabetic patients.

Our study, being cross-sectional with a specific sample size, suggests the need for longitudinal studies to validate these findings. While we did not analyze common risk factors like obesity and hypertension, factors such as dietary habits and medications could indirectly influence electrolyte regulation and impact diabetic complications.

CONCLUSION

Our findings indicate significant alterations in electrolyte levels, particularly lower Na^{+} and Mg^{2+} and higher Ca^{2+} levels in diabetic subjects compared to non-diabetic controls. Hypomagnesemia, as observed in type-2 diabetes, correlates strongly with glycemic control and may pose additional risks for uncontrolled diabetes and its complications. In

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such perspective, routine measurement of magnesium in type-2 DM and itssupplementation may be recommended where necessary. Addressing electrolyte imbalances could potentially mitigate the risk of various diabetic complications.

REFERENCES

1. Islam SMS, Lechner A, Ferrari U, Froeschl G, Niessen LW, Seissler J et al. "Social and economic impact of diabetics in Bangladesh: Protocol for a case–control Study".BMC Public Health, 2013, 13(1):1217

2. Islam FMA, Chakrabarti R, Dirani M, Islam MT, Ormsby G, Wahab M et al. "Knowledge, attitudes and practice of diabetes in rural Bangladesh: the Bangladesh Population Based Diabetes and Eye Study (BPDES)". PLoS One, 2014a, 9(10):110368

3. Islam SM, Lechner A, Ferrari U, Froeschl G, Alam DS, Holle R et al. "Mobile phone intervention for increasing adherence to treatment for type 2 diabetes in an urban area of Bangladesh: protocol for a randomized controlled trial" BMC Health Serv Res 2014b, 14(1):586

4. Islam SMS, Purnat TD, Phuong NTA, Mwingira U, Schacht K, Fröschl G. "Non-communicable diseases (NCDs) in developing countries" a symposium report. Global Health, 2014c, 10(1):81

5. Islam SMS, Alam DS, Wahiduzzaman M,Niessen LW, Froeschl G, Ferrari U et al. "Clinical characteristics and complications of patients with type 2 diabetes attending an urban hospital in Bangladesh". Diabetes MetabolSyndr Clin Res Rev 2015, 9(1):7–13

6. WhitingDR,GuariguataL,WeilC,ShawJ."IDFdiabetesatlas: global estimates of the prevalence of diabetes for 2011 and 2030" Diabetes Res Clin Pract 2011, 94(3):311–321

7. Hall JE, Guyton AC: "Textbook of medical physiology" St Louis, Mo: Elsevier Saunders; 2006, 228.

8. De Fronzo RA, Goldberg M, Agus ZS: "The effects of glucose and insulin on renal electrolyte transport' J Clin Invest 1976, 58(1):83–90.

9. ALLISON C. TATEN, MD: "Epigastric pain with elevated serum calcium, lipase and amylase" BUMC PROCEEDINGS 2000, 13:419–420

10. Haglin L, Tornkvist B, Backman L: "Prediction of all-cause mortality in a patient population with hypertension and type 2 DM by using traditional risk factors and serum-phosphate,-calcium and-magnesium" Acta Diabetol. 2007, 44(3):138–143.

11. Song Y, He K, Levitan E, Manson J, Liu S., "Effects of oral magnesiumsupplementationonglycaemiccontrolinType 2 diabetes: a meta-analysis of randomized double-blind controlled trials". Diabetic med 2006, 23(10):1050–1056.

12. Mishra S, Padmanaban P, Deepti GN, Sarkar G, Sumathi S, Toora BD., "Serum Magnesium and Dyslipidemia in Type-2 Diabetes Mellitus". Biomedical Res. 2012, 23(2):295-300.

13. Rude RK." Postgrad Med" 1992, 92: 217–24.

14. Kunst A, Drager B, Ziegenhorn J., "UV methods with hexokinase and glucose-6-phosphate dehydrogenasen, Methods of enzymatic Analysis", Vol. VI, Bergmeyer, HY, Ed, Verlag chemie Deerfield, FL., 1983, 163-172.

15. Wallenfels, K., et al., "Carbohydrate Research" 1978, 61:359

16. Burtis CA, Ashwood ER, Tietz., "Fundamentals of Clinical Chemistry, 5th ed. Philladelphia' W.B Saunders, 2001, 23-25, 419-420.

17. Tietz NW, Pruden EL, Siggaard-Anderson O. Eletrolyte, In: Burtis Ca, AshwoodER, editors., "TietzTexbook of Clinical Chemistry, 2nd ed. Philadelphia" PA: WB Saunders, 1994, 1354-1374.

18. Burtis CA. Ashwood ER. editors. Tietz. "Texbook of clinical chemistry, 2nd ed.Philladelphia". PA: WB Saunders, 1994, 1909-1910.

19. Lott JA, Patel ST, Sawhney AK Kazmierczak SC, Love JE., "Assays of serum lipase: analytical and clinical consideration". Clin Chem, 1986, 32/7: 1290-1302.

20. Kitabchi, A.E.; Umpierre, G.E.; Murphy, M.B.; Kriesberg, R.A., "Hyperglycemic crisis in adult patients with diabetes: A consensus statement from the American diabetes association". Diabetes Care, 2006, 29, 2739–2748.

21. Supriya, Mohanty S, Pinnelli VB, Murgod R, Raghavendra DS., "Evaluation of serum copper, magnesium and glycated haemoglobin in type 2 diabetes mellitus" Asian J Pharm Clin Res. 2013, 6:188-90.

22. Wang, S.; Hou, X.H.; Liu, Y.; Lu, H.J.; Wei, L.; Bao, Y.Q.;

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Jia, W.P., "Serum electrolyte levels in relation to macrovascular complications in Chinese patients with diabetes mellitus" Cardiovasc. Diabetol. 2013, 12.

23. DeFronzo RA, Goldberg M, Agus ZS., "The effects of glucose and insulin on renal electrolyte transport" J Clin Invest. 1976, 58(1):83–90.

24. Sugam Shrestha1, Prajwal Gyawali2, Rojeet Shrestha2, Bibek Poudel2, Manoj Sigdel2,Prashant Regmi2, Manoranjan Shrestha2, Binod Kumar yadav2*. "Serum Urea and Creatinine in Diabetic and non-diabetic Subjects" JNAMLS. 2008, 9:11-12.

25. Majharul Haque1*, Golam Azam2*, Debashis Kumar Sarkar3, Anisur Rahman4. "Serum Lipase Amylase Ratio in Predicting Aetiology, Severity and Outcome of Acute Pancreatitis in a Tertiary Care Hospital". Bangladesh Crit Care J September. 2017, 5 (2): 88-92.

26. Jay Wook Lee, M.D., "Fluid and Electrolyte Disturbances in Critically Ill Patients". Electrolyte Blood Press. 2010, 8:72-81.

27. Karim R, Nargis, KA Begum, SS Subhan, Uddin MN: "Bangladesh J Med Biochem", 2014, 7:4-8.

28. Guyton, A.; Hall, J., "Textbook of Medical Physiology; Elsevier Saunders: Philadelphia", PA, USA. 2014, pp. 348–381.