



Investigating the Relationship between Electrolyte Levels and ABO Blood Groups: Prospects for Customized Medicine

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Authors' contributions

This work was carried out in collaboration among all authors. The entire study procedure was conducted with the involvement of all authors. All authors read and approved the final manuscript.

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ABSTRACT

Human ABO blood type antigens exhibit alternative phenotypes and genetically derived glycoconjugate structures that are located on the red cell surface which play an active role in the cells' physiology and pathology. This study was carried out to evaluate the electrolytes, urea and creatinine of individuals with different ABO blood groups in Ekpoma. A total of one hundred (100) samples were used in this study. Electrolytes was estimated using ion selective electrode, urea was estimated using Berthelot method, while creatinine was estimated using Jaffe's method. The results were presented in tables as mean \pm standard deviation. Statistical analysis was done using one-way analysis of variance (ANOVA) and the student's t-test. Significant difference was accepted at $p < 0.05$. The results obtained showed that Sodium (Na) (mmol/L) was significantly higher ($p < 0.05$) in subjects with blood group O (136.72 ± 1.22) and blood group A (136.00 ± 1.36) when compared with blood group B (134.56 ± 2.56). Potassium (K) (mmol/L) was non-significantly higher ($p > 0.05$) in blood group A (3.62 ± 0.29), followed by blood group O (3.59 ± 0.18) and least in blood group B (3.51 ± 0.22). Bicarbonate (HCO_3) (mmol/L) was non-significantly higher ($p > 0.05$) in blood group A (21.17 ± 1.76), followed by blood group O (20.67 ± 1.09) and least in blood group B (20.52 ± 0.91). Chloride (Cl) (mmol/L) was non-significantly higher in blood group O (103.20 ± 1.81) followed by blood group A (102.10 ± 2.35) and least in blood group B (101.60 ± 3.58). Furthermore, Urea (mg/dl) was non-significantly higher ($p > 0.05$) in blood group O (23.20 ± 4.57), followed by blood group A (21.48 ± 4.15) and least in blood group B (20.76 ± 5.15). Similarly, Creatinine (mg/dl) was non-significantly higher in blood group O (0.65 ± 0.15), followed by blood group A (0.63 ± 0.12) and least in blood group B (0.62 ± 0.15). In conclusion, electrolytes, urea and creatinine were higher in blood group A and O compared with blood group B. With respect to gender, Na, HCO_3 , Cl, Urea and Creatinine were significantly higher in male subjects compared with female subjects. There was no significant difference in electrolytes, urea and creatinine of the subjects with respect to age ($p > 0.05$). Further studies should be carried out to understand the mechanism behind increase in electrolytes, urea and creatinine in certain blood group compared with others.

Keywords: Electrolyte; urea; creatinine; ABO; blood groups; ABO blood; bicarbonate.

1. INTRODUCTION

"The most essential blood groups in medical practice are ABO blood group systems. It was described by Landsteiner in 1900 and forms the major foundation of blood banking and modern transfusion medicine" [1]. "ABO blood groups are classified based on the presence or absence of A and B surface antigens into four types namely: A, B, AB and O. The frequency of these four major ABO blood groups differs across various ethnic, geographic and socioeconomic groups" [2]. "The variations of glycoprotein and glycolipids antigens present on red blood cells determine ABO blood groups. They were indicated by the expression of the carbohydrate antigens A and B on the erythrocyte membrane and blood plasma regular antibodies (anti-A, anti-B)" [3]. "These carbohydrate sugars are N-acetylgalactosamine for the A antigen and D-galactose for the B antigen while N-acetylgalactosamine and D-galactose for the blood type AB and absent for the phenotype O. The A, B and AB-related carbohydrate sugars are located on the H antigen and the unmodified H antigen explains the blood group O. The A and

B alleles encode a specific glycosyl-transferring enzyme" [4].

"Electrolytes are the positively and negatively charge molecules called ions that are found within the cells and extracellular fluid compartment of blood" [5]. "They play a role in conducting nervous impulses, contracting muscles, keeping the body hydrated and regulating body's pH levels. Disturbances in electrolytes may have a harmful effect on health and can even be fatal in rare cases. As a result, long periods of exercise or activity, particularly in the heat, can cause significant electrolyte loss" [6].

"Sodium is the main extracellular cation. The plasma sodium level is a major factor in the control of water homeostasis and extracellular fluid volume" [7]. "An increase in plasma sodium normally results in three compensatory mechanisms coming into play, thirst prompts oral fluid intake, anti-diuretic hormone (ADH) secretion from the pituitary is increased, leading to renal water retention; there is a shift of water from intracellular to extracellular space. As the

total intake of sodium chloride is almost completely absorbed from the gastrointestinal tract with no active control, regulation of the retained body sodium is maintained by the kidneys, with the excess excreted in the urine and fine control carried out by tubular reabsorption" [8]. "After initial glomerular filtration some 60% of the filtered sodium is recovered in the proximal tubules together with bicarbonate. 25% is reabsorbed in the Loop of Henle of the renal tubule with chloride; the remainder is reabsorbed in the distal tubules where, with aldosterone governing its reabsorption, it competes with potassium and hydrogen ions" [7]. "Sodium is primarily responsible for maintaining osmotic pressure. Increased serum sodium is present in states of dehydration as a result of diarrhea or vomiting. Low sodium levels usually are as a result of too much water in the body. High levels of sodium can raise blood pressure and may indicate dehydration" [9].

"Potassium is the principal intracellular cation, 98% of which is maintained within the cells by the ATP dependent mechanism known as the sodium pump. Any sodium which diffuses into cells is actively excreted in exchange for potassium. In addition to its role in intracellular osmolality, potassium is essential for many enzymatic reactions, the regulation of heart muscle and for the transmission of nerve impulses. An important factor in the control of potassium cellular transport is the acid/base status" [10]. "In acidosis the flow of hydrogen ions into cells causes the outflow of an equivalent number of potassium ions. Dietary potassium intake is normally in excess of requirement and the surplus is excreted via the kidneys. Serum potassium is the most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure cause increased plasma potassium. Hyperkalaemia is the most significant and life-threatening complication of renal failure" [11].

"Urea is a waste product resulting from protein metabolism. It is made in the liver and carried via the blood to the kidneys where it is excreted" [12]. "Urea, also known as carbamide, is a nitrogen-containing chemical molecule that plays a significant role in animal nitrogen metabolism and is the predominant nitrogen-containing substance in mammalian urine" [13]. "When measured in the blood, urea is referred to as blood urea nitrogen (BUN). BUN is an indirect

and rough measurement of renal function that measures the amount of urea nitrogen in blood and is directly related to excretory function of kidney. An elevated BUN can indicate kidney dysfunction or poor blood circulation to the kidneys. Protein consumption, the body's ability to catabolize protein and appropriate urea excretion via the renal system all influence urea concentration" [14].

"Creatinine is created when creatine and phosphor-creatine are broken down and it can be used to assess renal function. The amino acids arginine, glycine and methionine are transaminated in the liver, pancreas and kidneys to produce creatine" [13]. "Because serum creatinine is an easily measurable indicator of muscle metabolism that is eliminated unchanged predominantly by the kidneys, mostly through glomerular filtration, but also by proximal tubular secretion, it is an essential indicator of renal health" [15]. "Elevated levels can indicate kidney dysfunction. Creatinine tests diagnose impaired renal function and measure the amount of creatinine phosphate in blood. Urea and creatinine are good indicators of a normal functioning kidney and increase in the serum are indications of kidney dysfunction" [12].

"ABO antigens or carbohydrate (N-acetylgalactosamine and D-galactose) are assumed to be situated on the arterial and venal renal vascular endothelium, peritubular and glomerular capillaries and the epithelial cells of the convoluted tubules and collecting ducts in the kidney" [4]. "In as much as the major focus of ABO blood group are on compatibility both for blood transfusion and organ transplantation however, various studies have made an effort to show associations between blood group types and some diseases including gastric cancer, duodenal ulcers, renal failure etc" [16]. "Attempts have been made to explore a possible association between blood groups and other diseases which generated such an association with various diseases including gastric cancer, salivary gland tumors, duodenal ulcer, colorectal cancer, thyroid disorders, ovarian tumors, small cell carcinoma of lung and coronary heart disease" [17]. Some researchers had indicated facts that these blood group antigens may serve as receptors for infectious disease agents and host inflammatory response [18,19].

2. MATERIALS AND METHODS

Population of Study: The population of this study comprised of one hundred (100) apparently

healthy individuals with different ABO blood group (A, B and O) in Ekpoma, Edo State, Nigeria. Apparently healthy individuals with different ABO blood groups in Ekpoma, Edo State who gave their consent were included in this study. Individuals with underlying health conditions, pregnant and lactating women, and those who did not give their consent were excluded from the study.

Sample Collection: Five millilitres (5.0mls) of blood sample was collected from fasting subjects via venipuncture and dispensed into a plain container (3ml) without any additive and EDTA containers used for ABO (2 ml). The sample was allowed to stand for one hour to clot. It was then centrifuged at 3000 g for 10 min in order to separate blood cells and suspended particles from serum. The serum was aliquoted and stored at -200c until required for analysis. The whole blood sample was dispensed into EDTA containers used for ABO blood group determination.

2.1 Sample Analysis

Sodium, Potassium, Chloride and Bicarbonate were analyzed using Ion-Selective Electrode (ISE).

Principle: An Ion-Selective Electrode (ISE) makes use of the unique properties of certain membrane materials to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution. The complete measurement system for a particular ion includes the ISE, a reference electrode, and electronic circuits to measure and process the EMF to give the test ion concentration. The sodium and potassium electrodes are based on neutral carriers and the chloride electrode is based on an ion exchanger. For determinations on the ISE Module(s), the sample is diluted 1:31 and a single 15 microliter sample is taken for the three assays.

Urea and Creatinine Analysis: Urea, Creatinine was estimated using Berthelot method [20].

ABO Blood Grouping: The ABO blood grouping was done using tile method.

Principle: ABO blood group testing depends upon testing the red cells with known anti-A and anti-B sera, the presence or absence of agglutination indicates the group of each sample. Thus, if agglutination occurs with neither serum,

red cell contains neither A or B agglutinin and blood group is O. Agglutination with only anti-A indicates A agglutinin on red cell. Agglutination with only anti-B indicates B agglutinin on red cell. Agglutination with anti-A and anti-B indicates A and B agglutinin on red cell.

Procedure (Tile Method): A drop of the anti-sera (A, B, AB and D) was placed on the white tile. A drop of blood was placed beside the anti-sera on the tile. It was mixed and rocked gently for 5 minutes. The control (known red cells) was treated same way as the test samples. It was then observed macroscopically for agglutination and result recorded.

2.2 Interpretation of Result

Blood group A: Agglutination with only Anti-A

Blood group B: Agglutination with only Anti-B

Blood group AB: Agglutination with both Anti-A and Anti-B

Blood group O: Agglutination with neither Anti-A nor Anti-B

Data Analysis: The results were presented using tables. Data was presented as mean \pm S.D (standard deviation). Comparison was made between subjects and control groups using one-way analysis of variance (ANOVA) and the student's t-test. Significant difference was accepted at $p < 0.05$.

3. RESULTS

3.1 Electrolytes, Urea and Creatinine of Subjects According to Blood Group

Table 1 showed the Electrolytes, Urea and Creatinine of Subjects according to Blood Group. The results obtained showed that Sodium (Na) (mmol/L) was significantly higher ($p < 0.05$) in subjects with blood group O (136.72 ± 1.22) and blood group A (136.00 ± 1.36) when compared with blood group B (134.56 ± 2.56). Potassium (K) (mmol/L) was non-significantly higher ($p > 0.05$) in blood group A (3.62 ± 0.29), followed by blood group O (3.59 ± 0.18) and least in blood group B (3.51 ± 0.22). Bicarbonate (HCO_3) (mmol/L) was non-significantly higher ($p > 0.05$) in blood group A (21.17 ± 1.76), followed by blood group O (20.67 ± 1.09) and least in blood group B (20.52 ± 0.91). Chloride (Cl^-) (mmol/L) was non-significantly higher in blood group O (103.20 ± 1.81) followed by blood group A

(102.10±2.35) and least in blood group B (101.60±3.58). Furthermore, Urea (mg/dl) was non-significantly higher (p>0.05) in blood group O (23.20±4.57), followed by blood group A (21.48±4.15) and least in blood group B (20.76±5.15). Similarly, Creatinine (mg/dl) was non-significantly higher in blood group O (0.65±0.15), followed by blood group A (0.63±0.12) and least in blood group B (0.62±0.15).

3.2 Electrolytes, Urea and Creatinine of Subjects with Respect to Gender

Table 2 showed the Electrolytes, Urea and Creatinine of Subjects with respect to gender. The results obtained showed that Na (mmol/L) was significantly higher in male subjects (136.69±1.38) compared with female subjects (135.52±2.23), K (mmol/L) was insignificantly higher (p>0.05) in male subjects (3.64±0.19) compared with female subjects (3.60±0.30), HCO₃ (mmol/L) was significantly higher (p<0.05) in male subjects (21.07±1.38) compared with female subjects (20.38±1.03), Cl⁻ (mmol/L) was significantly higher (p<0.05) in male subjects (103.24±1.94) compared with female subjects (101.21±2.93), Urea (mg/dl) was significantly higher (p<0.05) in males subjects (24.93±4.27) compared with female subjects (19.02±4.48) and Creatinine (mg/dl) was significantly higher (p<0.05) in male subjects (0.72±0.14) compared with female subjects (0.57±0.12).

3.3 Electrolytes, Urea and Creatinine of Subjects According to Age

Table 3 showed the Electrolytes, Urea and Creatinine of Subjects according to age. The results obtained showed that Na (mmol/L) of subjects in age group 15-20 years, 21-25 years and 26-30 years was 135.01±1.31, 135.61±2.50 and 136.72±1.22, K (mmol/L) was 3.48±0.22, 3.52±0.23 and 3.59±0.18, HCO₃ (mmol/L) was

20.22±1.71, 20.52±0.94 and 20.67±1.09, Cl⁻ (mmol/L) was 101.36±2.30, 101.66±2.51 and 102.20±1.81, Urea (mg/dl) was 22.81±4.45, 21.81±4.36 and 22.20±4.57, while Creatinine (mg/dl) was 0.60±0.11, 0.62±0.14 and 0.65±0.15 respectively. There was no significant difference in electrolytes, urea and creatinine of the subjects with respect to age (p>0.05).

3.4 Correlation between Weight, Electrolytes, Urea and Creatinine of Subjects

Table 4 showed the correlation between Electrolytes, Urea and Creatinine of Subjects. The results obtained showed that sodium had significant positive correlation with potassium (r = 0.198, p=0.048), Chloride (r = 0.428, p =0.000) and Urea (r = 0.277, p=0.005) respectively. Similarly, bicarbonate had significant positive correlation with urea (r = 0.228, p=0.023), while chloride positively correlated with urea (r =0.333, p=0.001).

4. DISCUSSION

“Human ABO blood type antigens exhibit alternative phenotypes and genetically derived glycoconjugate structures that are located on the red cell surface which play an active role in the cells' physiology and pathology. Associations between the blood type and disease have been studied since the early 1900s when researchers determined that antibodies and antigens are inherited. However, due to lack of antigens of some blood groups, there have been some contentious issues with the association between the ABO blood group and vulnerability to certain infectious and noninfectious diseases” [21]. This study was carried out to evaluate the electrolytes, urea and creatinine of individuals with different ABO blood groups in Ekpoma, Edo State.

Table 1. Electrolytes, urea and creatinine of subjects according to blood group

Parameters	Group A Mean ± SD (n = 30)	Group B Mean ± SD (n = 25)	Group O Mean ± SD (n = 45)	F-value	p-value
Na (mmol/L)	136.00±1.36 ^a	134.56±2.56 ^b	136.72±1.22 ^c	3.766	0.001
K (mmol/L)	3.62±0.29 ^a	3.51±0.22 ^a	3.59±0.18 ^a	1.089	0.283
HCO ₃ (mmol/L)	21.17±1.76 ^a	20.52±0.91 ^a	20.67±1.09 ^a	1.890	0.071
Cl ⁻ (mmol/L)	102.10±2.35 ^a	101.60±3.58 ^a	103.20±1.81 ^a	1.683	0.105
Urea (mg/dl)	21.48±4.15 ^a	20.76±5.15 ^a	23.20±4.57 ^a	1.746	0.094
Creatinine (mg/dl)	0.63±0.12 ^a	0.62±0.15 ^a	0.65±0.15 ^a	0.799	0.432

*Values with different superscript in a row is significant at p<0.05

Keys: Na – Sodium; K – Potassium; HCO₃ – Bicarbonate; Cl⁻ – Chloride; SD – Standard deviation; n - Number

Table 2. Electrolytes, urea and creatinine of subjects with respect to gender

Parameters	Male Mean \pm SD (n = 42)	Female Mean \pm SD (n = 58)	t-value	p-value
Na (mmol/L)	136.69 \pm 1.38	135.52 \pm 2.23	3.027	0.004
K (mmol/L)	3.64 \pm 0.19	3.60 \pm 0.30	0.900	0.373
HCO ₃ (mmol/L)	21.07 \pm 1.38	20.38 \pm 1.03	2.627	0.012
Cl ⁻ (mmol/L)	103.24 \pm 1.94	101.21 \pm 2.93	4.291	0.000
Urea (mg/dl)	24.93 \pm 4.27	19.02 \pm 4.48	6.841	0.000
Creatinine (mg/dl)	0.72 \pm 0.14	0.57 \pm 0.12	4.868	0.000

Keys: Na – Sodium; K – Potassium; HCO₃ – Bicarbonate; Cl⁻ – Chloride; SD – Standard deviation; n - Number

Table 3. Electrolytes, urea and creatinine of subjects according to age

Parameters	15–20 years Mean \pm SD (n = 43)	21–25 years Mean \pm SD (n = 35)	26–30 years Mean \pm SD (n = 22)	F-value	p-value
Na (mmol/L)	135.01 \pm 1.31 ^a	135.61 \pm 2.50 ^a	136.72 \pm 1.22 ^a	0.876	0.236
K (mmol/L)	3.48 \pm 0.22 ^a	3.52 \pm 0.23 ^a	3.59 \pm 0.18 ^a	0.685	0.364
HCO ₃ (mmol/L)	20.22 \pm 1.71 ^a	20.52 \pm 0.94 ^a	20.67 \pm 1.09 ^a	1.009	0.125
Cl ⁻ (mmol/L)	101.36 \pm 2.30 ^a	101.66 \pm 2.51 ^a	102.20 \pm 1.81 ^a	0.455	0.312
Urea (mg/dl)	22.81 \pm 4.45 ^a	21.81 \pm 4.36 ^a	22.20 \pm 4.57 ^a	0.868	0.291
Creatinine (mg/dl)	0.60 \pm 0.11 ^a	0.62 \pm 0.14 ^a	0.65 \pm 0.15 ^a	0.841	0.410

*Values with different superscript in a row is significant at p<0.05

Keys: Na – Sodium; K – Potassium; HCO₃ – Bicarbonate; Cl⁻ – Chloride; SD – Standard deviation; n - Number

Table 4. Correlation between weight, electrolytes, urea and creatinine of subjects

		Na	K	HCO ₃	Cl ⁻	Urea	Creatinine
Na	Pearson Correlation	1	0.198 [*]	0.114	0.428 ^{**}	0.277 ^{**}	0.110
	Sig. (2-tailed)		0.048	0.258	0.000	0.005	0.274
	N	100	100	100	100	100	100
K	Pearson Correlation	0.198 [*]	1	-0.040	0.111	0.092	0.176
	Sig. (2-tailed)	0.048		0.694	0.270	0.360	0.081
	N	100	100	100	100	100	100
HCO ₃	Pearson Correlation	0.114	-0.040	1	0.064	0.228 [*]	0.113
	Sig. (2-tailed)	0.258	0.694		0.527	0.023	0.264
	N	100	100	100	100	100	100
Cl	Pearson Correlation	0.428 ^{**}	0.111	0.064	1	0.333 ^{**}	0.129
	Sig. (2-tailed)	0.000	0.270	0.527		0.001	0.201
	N	100	100	100	100	100	100
Urea	Pearson Correlation	0.277 ^{**}	0.092	0.228 [*]	0.333 ^{**}	1	0.567 ^{**}
	Sig. (2-tailed)	0.005	0.360	0.023	0.001		0.000
	N	100	100	100	100	100	100
Creatinine	Pearson Correlation	0.110	0.176	0.113	0.129	0.567 ^{**}	1
	Sig. (2-tailed)	0.274	0.081	0.264	0.201	0.000	
	N	100	100	100	100	100	100

*Significant at p>0.05; **Significant at p>0.01; ***Significant at p>0.001

The results obtained showed that Sodium and Chloride (mmol/L) higher in blood group O and A subjects compared with blood group B subjects, however only sodium was significant (p<0.05). This may be associated with increased risks of skin cancer and non-hodgkin's lymphoma in blood group O patients. On the other hand,

Potassium and Bicarbonate (mmol/L) was non-significantly higher (p>0.05) in blood group A, followed by blood group O and least in blood group B. ABO blood group has been observed to link with many diseases [21]. A previous study indicates that blood groups A and O were most commonly associated with renal failure while the

AB blood group was least associated. Another separate study observed A and O blood group antigen subtypes were involved in the progression of immune-mediated Immunoglobulin A nephropathy [4]. However, these were the contrast of finding where the B blood group was the one associated with acute renal failure. Asma et al. [22] reported that people suffering from kidney disease was directly related to imbalance in electrolytes which not only cause kidney disease but also caused certain endocrine gland disease. It is noteworthy to state that though all individuals of different ABO blood groups had values of these electrolytes within normal range; the higher levels observed in blood group A relative to blood group O and B may be indicative of potential risk factor or exposure to associated kidney disorders [23].

In this study, Urea and Creatinine (mg/dl) were non-significantly higher ($p>0.05$) in blood group O, followed by blood group A and least in blood group B. This finding is similar to Samar et al. (2016), who found no significant association between renal function test and blood groups of patients, except the level of potassium. In contrast to this study, Alhawary et al. [24] reported that the level of BUN was the highest among B blood group and the lowest level of BUN was among renal patients with AB blood group. Creatinine level was at highest level among renal patients with B blood group, and the lowest level was among renal patients with O blood group. Similarly, Amin et al. [25] study showed high urea in B blood group and lowest in AB blood group, while creatinine was higher in blood group A and lowest in AB blood group. However, the difference was not statistically significant.

“Elevated creatinine level signifies impaired kidney function or kidney diseases. As the kidney become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction of failure of the kidneys. It is for this reason that standard blood test routinely check the amount of creatinine in the blood” [26]. “Blood urea level is another indicator of kidney function. Urea is also a metabolic by product which can build up if kidney function is impaired. The blood urea ratio can actually give a more information about kidney function and its possible underlying cause compared with creatinine level alone blood urea also increases with

dehydration. Blood urea concentrations can be increased by numerous factors linked to prerenal causes or renal/postrenal causes” [27].

“It was reiterated that D-galactose is present in the red blood cell of group B antigen. D-galactose metabolism occurs in the kidney and liver. It was observed in recent studies that treatment with D-galactose resulted in to increase in oxidative damages of kidney and liver damage thereby leading the rise in Creatinine and Blood Urea Nitrogen levels, increase the severity of the acute renal failure, impaired renal and liver function” [28,29]. “Free radicals released by oxidative damage attack essential cell constituents and also induce lipid peroxidation, damage the membranes of cells and organelles in the liver and kidney, cause the swelling and necrosis of hepatocytes and nephrocytes and ultimately result in liver and kidney injury” [30,8]. It can therefore be inferred that D-galactose on the red cell of group B is responsible for the strong association with acute renal failure.

5. CONCLUSION

In conclusion, electrolytes (Na, K, HCO₃ and Cl), urea and creatinine were higher in blood group A and O compared with blood group B. With respect to gender, Na, HCO₃, Cl, Urea and Creatinine were significantly higher in male subjects compared with female subjects, while K was non-significantly higher in male subjects compared with female subjects. There was no significant difference in electrolytes, urea and creatinine of the subjects with respect to age ($p>0.05$). In terms of correlation, sodium had significant positive correlation with potassium, Chloride and Urea respectively, while bicarbonate and chloride had significant positive correlation with urea. Further studies should be carried out to understand the mechanism behind increase in electrolytes, urea and creatinine in certain blood group compared with others.

CONSENT

Informed consent was also obtained from each subject who participated in the study before the collection of blood sample.

ETHICAL APPROVAL

Ethical approval for the collection of sample was obtained from the Ethics and Review Committee, Ambrose Alli University, Ekpoma, Edo State.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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