

Haemostatic Status in Uraemics in UNTH, Enugu

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| Received: 11.03.2019 | Accepted: 23.03.2019 | Published: 30.03.2019

DOI: [10.21276/sjm.2019.4.3.7](https://doi.org/10.21276/sjm.2019.4.3.7)

Abstract

Uraemia is the clinical syndrome which occurs when there is a marked nitrogen retention due to renal failure. It had been suggested that urea level has an effect on coagulation and haematological profile. The haemostatic status was studied in Thirty known uraemic patients of the University of Nigeria Teaching Hospital Enugu. Fifteen non-uraemic people were used as controls. The mean result obtained are as follows:-Packed cell volume (PCV), $29 \pm 5.4\%$, Platelet count, $238 + 80.4 \times 10^9/L$, prothrombin time, $14.8 + 1.45$ seconds, Bleeding time $5.9 + 1.98$ minutes. For control subjects the following result were obtained: Packed cell volume $40.8 + 2.4\%$, Platelet count $267 \pm 48 \times 10^9/L$, Prothrombin time $12.9 + 0.93$ seconds and Bleeding time $2.43 + 0.52$ minutes. A statistical comparison of the above results between uraemic patients and control subjects showed that there were no significant difference in the platelet count ($P > 0.20$) and prothrombin time ($P > 0.1$) but showed significant difference in the packed cell volume and Bleeding time. A weakly positive correlation ($r = + 0.11$) was found to exist between prolongation of bleeding time and blood urea.

Keywords: Haemostatic status, uraemics, UNTH Enugu.

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INTRODUCTION

Urea is one of the end products of protein metabolism. A normal adult ingests 10 - 15g of nitrogen per day in dietary protein - on the average 1g nitrogen is derived from about 6g protein [1]. The nitrogen is then principally excreted in the urine as urea. Urea is formed in the liver from deaminated amino acids, most probably by way of the ornithine - arginine cycle [2].

Any excess urea in the circulation is eliminated from the blood stream by the kidneys and passed out in urine. In health, blood always contains some urea. The level of urea in the blood varies but the normal range is between 2.5 to 8.3 mmol per litre for a normal person on a full ordinary diet [2].

With a fall in protein intake, there is a fall in urea but a blood urea of over 8.3mmol/L is suggestive of impaired renal function. Uraemia is the clinical syndrome due to renal failure resulting from either disease of the kidneys themselves or from disorders or disease elsewhere in the body which induces the kidney dysfunction and which results in gross biochemical disturbance in the body including retention of urea and other nitrogenous substances in the blood.

Uraemia is also the name given to the clinical syndrome which occurs when there is a marked

nitrogen retention due to renal failure [1]. Acute renal failure is present when there is a rapid rise in blood urea or creatinine over a period of hours or a few days. It is classified as pre-renal (depletion of the extracellular fluid, ECF), Renal (acute tubular necrosis or acute glomerulonephritis) and post-renal (obstruction to urine flow). These cause acute uraemia [3].

Urea diffuses readily through body fluids so that the estimation can be carried through whatever body fluids are most readily available - whole blood, serum, plasma, CSF, Oedema fluid.

It has been suggested that urea level has an effect on coagulation and haematological profile - Bleeding time, prothrombin time, platelet count, packed cell volume and whole blood clotting time [1].

The bleeding time is one of the tests used to study patients thought to be suffering from blood clotting defects. In pathological conditions, bleeding time can be shortened within 12 hours after acute myocardial infarction [4] and also can be prolonged in thrombocytopenia, von willebrand's disease. Acute systemic lupus erythematosus, obstructive jaundice, cardiac bypass surgery, glycogen storage disease type I, disseminated intravascular coagulopathy, acute fibrinolysis, cyanotic heart disease and uraemia [5]. Therefore if all the above pathological conditions are

excluded in an uraemic patient, the prolongation of bleeding time can be said to be as a result of the increased blood urea.

The Prothrombin time, platelet count, whole blood clotting time and Haematocrit are other simple tests also used to investigate haemostatic status. Uraemia is quite prevalent in this part of the country [3] as there are various causes of high blood urea therefore the prevalence and type of haemostatic dysfunction in uraemics should be known.

In Nigeria, the predisposing factors for uraemic/renal failure abound. Blood transfusion and Dialysis are almost always employed in the management which are not an unmixed blessing because they often present with complications.

OBJECTIVES

- To evaluate the relationship of bleeding time prothrombin time, platelet count, haematocrit and other aspects of haemostasis to blood urea in Uraemics in Nigeria.
- To evaluate the usefulness of simple laboratory tests in detecting bleeding risks of such patients.

MATERIALS AND METHODS

Subjects

The blood samples were collected from a total of forty-five male and female subjects of which thirty were on admission in the renal unit at the University of Nigeria Teaching Hospital, Enugu and 15 were normal subjects. Patients were screened for the creatinine and urea level to confirm that they had a high urea level at least 48 hours before the Bleeding time was carried out. Also, patients with non-renal disease, associated infection and those who have received blood transfusion within one month of the test, anti-platelet agent and anticoagulant therapy were excluded. Patients with family history of bleeding were also excluded.

Control population was drawn from students of medical laboratory sciences of University of Nigeria Teaching Hospital, Enugu and other students and staff in the hospital.

Sample Collection

Necessary precautions were taken on proper collection, separation and processing of blood samples. 5.0ml of blood was withdrawn without undue pressure on either the patient's arm or the plunger of the syringe. The blood was distributed 2.25mls into specially prepared bottles with sodium citrate anticoagulant (0.25mls of 32g/l trisodium citrate) for prothrombin time and 2mls into 0.01ml of 100g/l ethylene diamine tetra acetic acid (EDTA) tubes for haematocrit and platelet count. The speed of the centrifuge was increased to 2000RPM and spun for 15 minutes at room

temperature for collection of plasma for prothrombin time [6].

For creatinine and urea, 3 ails of blood was collected in a plain bottle. The same was also done on the 15 control samples from students and staff.

MATERIALS

- The composition of common reagents used is shown in the appendix.
- The following materials and reagents were used this work.

Brain Thrombolast [6]

The brain of the rabbit was stripped fresh immediately the rabbit was killed of its covering membranes, blood vessels and cerebellum. The brain was washed free from blood, it was emulsified in phenol saline and ground in mortar until it was non-granular. It was then incubated at 37°C for 30 minutes and later left at 4 °C for 24 hours. After 24 hours the brain extract was centrifuged at 700g for 30 minutes and the sediment discarded. The supernatant was stored at 4 °C for 2 weeks before standardization.

A serial dilution up to 1 in 8 of small quantity of the brain extract was made to find the dilution which would give the shortest time in quick's one stage test. Plasma pooled from 3 normal individuals was used for the test. The shortest time of 12 seconds was found at the dilution of 1 in 2. The remainder of the brain extract was diluted 1 in 2 and distributed in small quantities in test tubes and stored at 4 °C for subsequent use.

METHODS

Whole blood collected in trisodium citrate bottles was centrifuged immediately on getting to the laboratory. All the tests were performed within four hours of sample collection.

Creatinine [7]

Four tubes labeled high standard, low standard, test and blank were used as follows:

- 1ml serum was delivered into the tube labeled test.
- 0.5ml of working standard was delivered into the tube labeled low standard and 1.0ml to the tube labeled high standard.
- 1.0ml Distilled water was added to the tube labeled test, and 1.5ml to the tube labeled low standard, 1.0ml to the tube labeled high standard and 2.0ml to the tube labeled blank,
- To all the 4 tubes labeled test, low standard, high standard and blank, 1.0ml, of 5% sodium tungstate was added.
- Also, 1.0ml of 2/3N H₂S₀₄ was added to each of the 4 tubes dropwise.

The tubes were mixed thoroughly and allowed to stand for 10 minutes. They were then centrifuged at 500rpm for 5 minutes.

The following dilution to corresponding labeled tubes were made as follows:

- To all the 4 labeled tubes, 1.5ml of clear supernatant was added.
- 0.5ml of picric acid was added to all the 4 labeled tubes.
- 0.5ml of 0.75N NaOH was added to all the 4 tubes.

They were mixed well and allowed to stand for 15 minutes. The extinction was measured in the colorimeter using blue green (500nm) filter against the blank.

$$\text{Calculation - } \frac{\text{Test}}{\text{Standard}} \times \frac{\text{concentration of the standard}}{I}$$

Creatinine reacts with picric acid in alkaline medium to form a red yellow coloured complex (Creatinine picrate). This is referred to as Jaffe reaction.

Normal values for serum creatinine - 44.2-194.5 $\mu\text{mol/L}$ [7]

Urea [7]

Under strong acidic conditions and heat, diacetyl monoxime is hydrolysed (by acid) to diacetyl and monoxime. The diacetyl couples with urea to form a red colour from yellow. 2mls of mixed acid reagent was added in a tube. Into the same tube, 2mls of mixed colour reagent was also added to the same tube. 0.02mls of serum was added and mixed. The tube was then corked with cotton wool and heated in boiling water bath for 10 minutes and cooled immediately. The optical density was read colorimetrically at 520nm.

Platelet Count [8]

Blood was diluted in diluting fluid (1% ammonium oxalate) that causes lysis of erythrocyte but has no effect on platelets, which was counted in improved Neubauer chamber. A 1 in 20 dilution of EDTA blood was made by adding 0.38ml of diluting fluid (see appendix) and 0.02ml of EDTA blood sample. The suspension was mixed by inversion for 10 minutes. The improved Neubauer chamber was charged and filled with the suspension using a glass capillary. The Neubauer counting chamber was placed in a moist petri dish for 20 minutes to give time for platelets to settle. The number of platelets which appeared as small refractile bodies in five squares in the central ruled area of haemocytometer was counted. The counting was done with x 10 eye- piece of the microscope.

Bleeding Time [6]

- A sphygmomanometer cuff was placed around the patients upper arm and the pressure of the cuff was raised to 40mmHg.
- The pronator surface of the forearm was sterilized with alcohol (70%) and 2 separate punctures were

made in quick succession 5cm apart with a sterile disposable lancet to a depth of 2.5mm. Care is taken to avoid superficial veins.

- Filter paper is used to blot off gently but completely at 15 seconds intervals the blood exuding from each puncture.

The bleeding time of the 2 punctures was taken. The result from the puncture that bleed? longest is taken as the bleeding time. Normal range is 2-7 minutes.

Prothrombin Time [6]

A potent preparation of human or rabbit brain emulsion was added to citrated plasma. The mixture was then recalcified and the clotting time estimated. 0.1ml of plasma and brain suspension were delivered into the bottom of test tube placed in a water-bath at 37°C, the contents of the test tubes were mixed very well and left at 37°C for 2 minutes. After 2 minutes, 0.1ml of 2.55 mmol/L calcium chloride was blown into the test tube and a stop watch started simultaneously. The tube was continuously tilted until the first sign of clot was seen and the stopwatch stopped. The development of the clot marked the end point of the test. The time was recorded immediately the clot was seen [6]. Normal range is between 10-16 seconds [6].

Packed Cell Volume [6]

Capillary tubes 75mm in length and having an internal diameter of about 1mm are required. They can be obtained plain or coated inside with 21U of heparin. The latter type is suitable for the direct collection of capillary blood. Plain tubes are used for anticoagulated venous blood.

Allow the blood to enter the tube by capillary, leaving at least 13mm unfilled. Then seal the tube by heating the dry end of the tube rapidly in a fine flame from a bunsen burner, combined with rotation. It is centrifuged at 120Gg for 5 minutes and the PCV is then measured using a reading device [6]. Normal range is 0.45L/L for men and 0.41L/L for women.

RESULTS

A comparison of haemostatic parameters between the controls and the patients is given in Table-1. The creatinine and urea level were significant between the controls and the patients ($P < 0.05$) in both cases. The mean urea level in the patients was 19.4 ± 8.6 $\mu\text{mol/L}$ whereas in the controls, it was 4.9 ± 0.5 $\mu\text{mol/L}$. The mean creatinine level in patients was 951 ± 46.3 mmol/L while in the controls it was 105 ± 53.4 mmol/L . The bleeding time prolongation was significant in Uraemics compared to controls ($P < 0.05$) with a mean bleeding time of 5.9 ± 1.98 minutes whereas in the controls it was 2.43 ± 0.53 minutes. The packed cell volume in Uraemics and controls also showed a significant difference ($P < 0.05$) with a mean of $29 \pm 5.4\%$ in Uraemics and $40.8 \pm 2.4\%$ in controls.

96.6% of the Uraemics studied had reduced PCV. This is shown in a pie chart. However, in the platelet count of the Uraemics, ($238 \pm 80.4 \times 10^9/L$) there was no significant difference from the controls ($P > 0.2$). In the prothrombin time in Uraemics (15.1 ± 3.2 Seconds) there was no significant difference between the Uraemics and the controls ($P > 0.1$). There was no

significant difference between the urea levels in male and female Uraemics patients ($P > 0.1$) with a mean urea of 17.0 ± 6.6 Umol/L in females and 21.8 ± 9.3 Umol/L in males. A weak positive correlation ($r = +0.11$) was found to exist between prolongation of bleeding time and blood urea.

Table-1: Haemostatic data of uraemic patients and controls

Subjects	Urea level (umol/L)	Creatinine level (Mmol/L)	PCV (%)	Platelet count ($\times 10^9/L$)	Prothrombin time (seconds)	Bleeding time (Minutes)
Patients n = 30	19,4+8.6	951+463	29+5.4	238+80.4	15.1+3,2	5.9+1.98
Controls n= 15	4.9+2.5	105+53,4	4Q.JT+2.4	2-6T+48	12.9+0.93	2.43+0.52
Remark	P <0.05	P <QX)5	P<0.05	P >0.20	P >0. 10	P <0,05

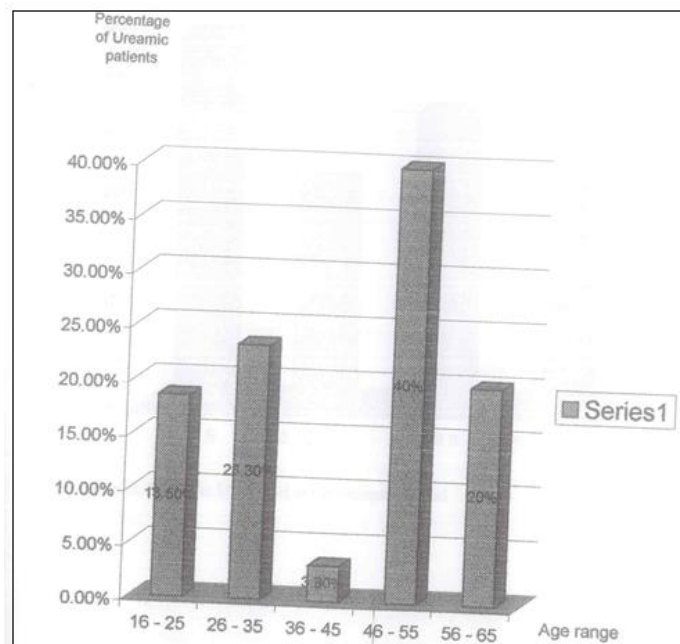


Fig-1: The Age Distribution of Uraemic Patients Studied

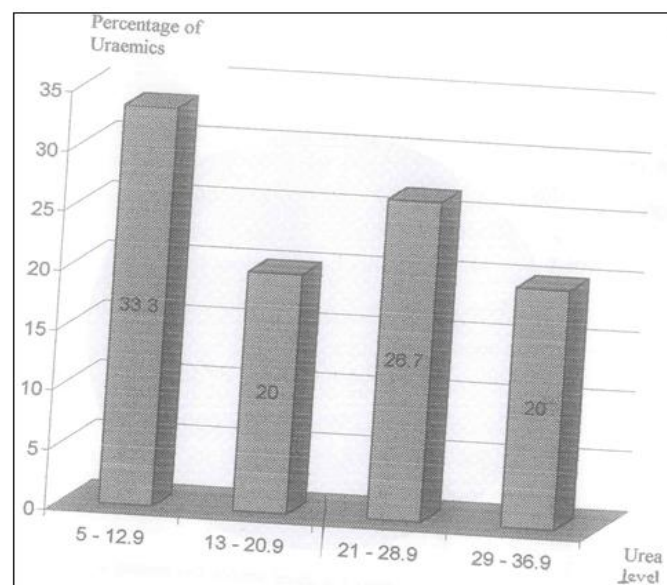


Fig-2: The Urea level in the patients studied

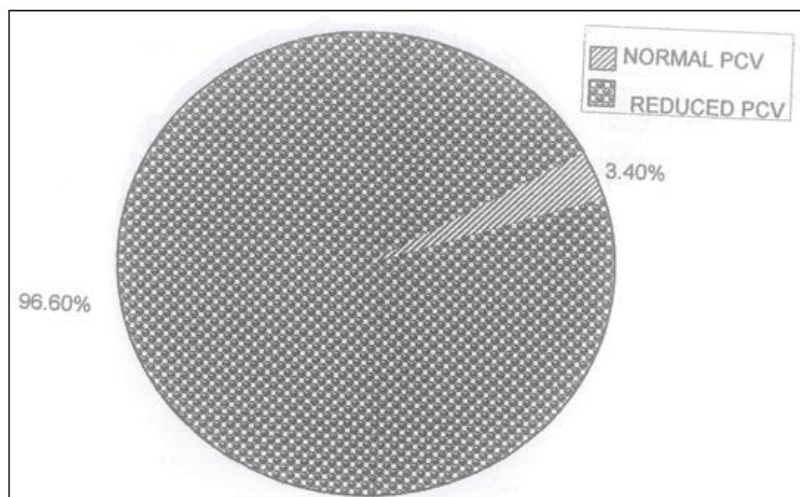


Fig-3: The packed cell volume levels in Uraemic patient

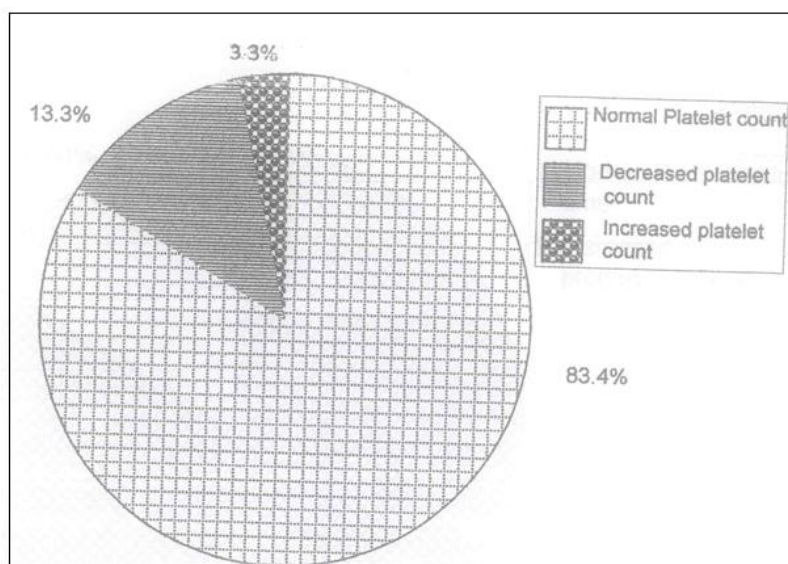


Fig-4: The platelet count levels in Uraemic patients

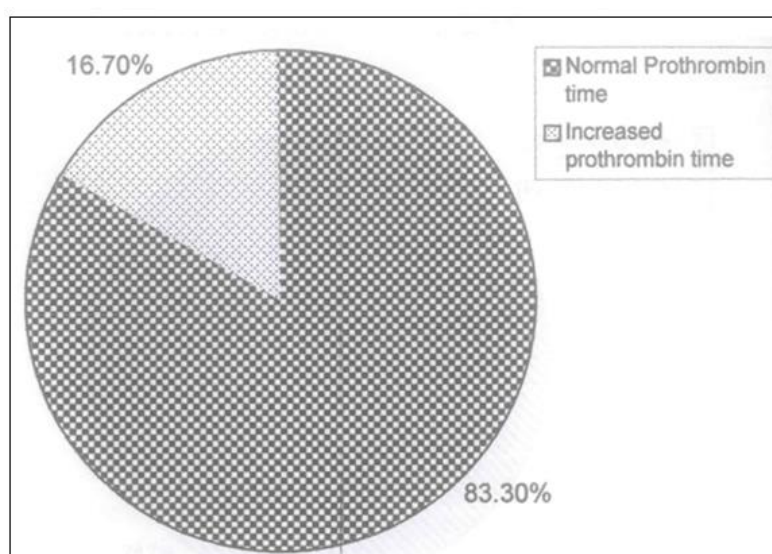


Fig-5: The Prothrombin time chart in Uraemic patients

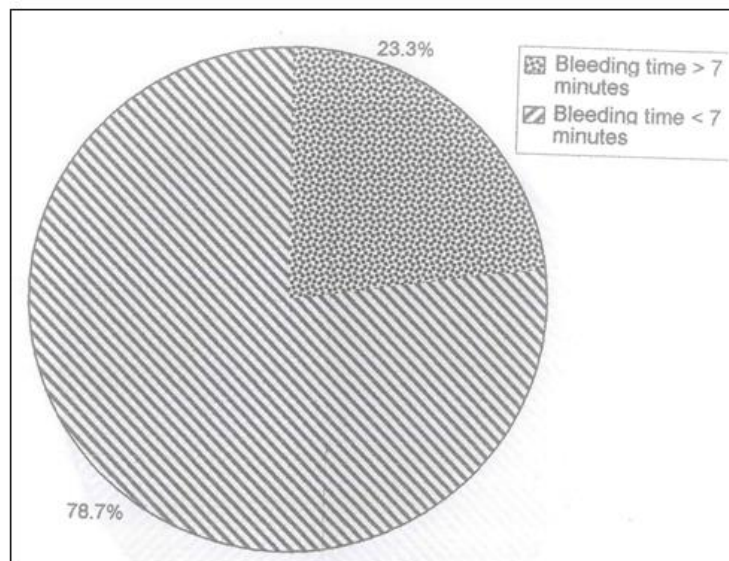


Fig-6: The Bleeding time chart in Uraemic patients

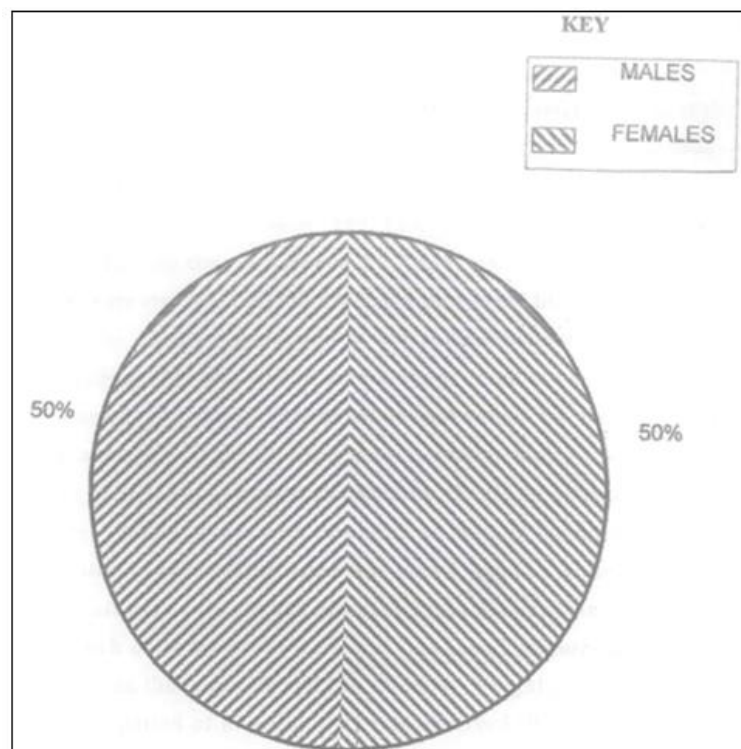


Fig-7: Prevalence of Uraemia between Males and Females

DISCUSSION

Thirty Uraemics were assayed for urea level, creatinine, prothrombin time, packed cell volume and platelet count in this work and the values were compared with that of fifteen normal patients.

According to Dacie and Lewis [6] the normal range for bleeding time is between 2 – 7 minutes. Out of the 30 patients studied, only 23.3% had a prolonged bleeding time greater than 7 minutes and in the control patients, the bleeding time was within normal range in all but according to Bruce Evatt [9], 95% of all normal values are shorter than 4 minutes and if the bleeding

time is greater than 3-4 minutes, the possibility of a mild abnormality should be considered. This finding agrees with this work as most of the normal controls had bleeding times less than 4 minutes while most of the uraemic patients had bleeding times greater than 4 minutes though quite a number were still less than 7 minutes so that between the controls and the patients, a highly significant prolongation of bleeding time was observed ($P < 0.05$). This finding then agrees with the observation made by Jaiyesimi *et al.*, [10].

The platelet count assessed in the patients were not significantly different from that of the normal

controls ($P > 0.05$). This result is in parity with the findings of Eknoyan *et al.*, [11] and Jaiyesimi *et al.*, [10] which reported that the platelet counts were normal in Uraemics and controls. However, this disagrees with the work by Horowitz *et al.*, [12] in which they reported that Uraemics were frequently thrombocytopenic. This thrombocytopenia and other platelet dysfunction in renal failure maybe due to accumulation of toxic metabolites such as urea, guanidosuccinic acid and phenolic acids [10]. In such cases, there could be both qualitative and quantitative platelet defect [13]. The packed cell volume assayed in the patients was not within the same range as the normal controls used ($P < 0.05$). This then agrees with the earlier works carried out by Eknoyan *et al.*, [11] and Jaiyesimi *et al.*, [10] here the packed cell volume was considerably lower in the patients than in the controls they used.

According to Dacie and Lewis (1994) the prothrombin time range is between 11 - 16 seconds and most of the patients and controls (15.1 ± 3.2 and 12.9 ± 0.93 respectively) fell within this range in this work. This is in parity with the works of Eknoyan *et al.*, [11] and Jaiyesimi *et al.*, [10]. In the study, $P > 0.1$ which shows no significant difference.

The relationship of bleeding time to blood urea in Uraemics was analysed by co-efficient correlation. A weak positive correlation ($r = + 0.11$) between prolongation of bleeding time and blood urea was present which agreed with the work by Jaiyesimi *et al.*, [10] in which there was a highly significant correlation ($r = + 0.778$). Though the correlation in the work by Jaiyesimi *et al.*, was considerably higher, the difference could be as a result of environmental difference in the progression of the disease and in the techniques used.

Shortening of bleeding time in Uraemics may result from dialytic therapy which most of the patients in the teaching hospital are subjected to.

Though not haemostatic parameters, the urea and creatinine levels are assayed in both Uraemics and controls as a means of assessing their suitability for the study. There was a significant difference between the patients and the controls in both urea and creatinine levels ($P < 0.05$). Infact for all the patients used, their urea and creatinine levels exceeded the normal levels whereas all controls used fell within the normal range.

Choice of methods depends on many factors but mostly on the accuracy and availability of such methods. Though Dacie and Lewis [6] reported that the automated method for estimating packed cell volume could be fraught with less error, the micro-haematocrit method was used in this study due to the availability of the materials and equipment.

Kuse *et al.*, [14] reported that ammonium oxalate which lyses erythrocytes produced higher and

more accurate platelet count than does brilliant cresyl blue which leaves the erythrocytes intact. The ammonium oxalate method was used in this study. Though standard microscopy is mostly used, phase contrast microscopy is recommended since it permits better visualisation of platelet [15]. The standard microscopy was used because of the unavailability of the phase contract microscopy.

Dacie and Lewis [6] reported that automated full blood counters produce platelet counts with a precision which is much superior to that of manual platelet counts. The manual method was chosen for this study because it is the available method. The one-stage prothrombin time of Quick's was used for this work using locally prepared brain thromboplastin. The commercially prepared reagents e.g plasmaseann was not used because of its exorbitant price. However, the brain thromboplastin used was standardised and gave a time of 12 seconds at the dilution of 1 in 2. The result was expressed in seconds and also as a ratio.

Two methods are available for bleeding time measurement, the template method and Ivy's method. The Ivy's method was used because the materials were available.

The problems encountered in the course of this study is that many of the Uraemics on admission at the teaching hospital Enugu had to be excluded from the study as quite a number of them had non-renal diseases, other infections and those who had received blood transfusion within 1 month of the study, anti-platelet agent and anticoagulant therapy were excluded, this of course reduced the number of patients suitable for the study and at any point in time, there were quite a few indeed.

From the findings in this work, blood urea per se was not a reliable predictor of prolonged bleeding as in some cases, the blood urea was quite high whereas the bleeding time was not prolonged and vice versa. Evaluation of bleeding time in Uraemics could help to reduce the attendant morbidity and mortality as a useful screening test for bleeding risk as well as in assessing the efficacy of therapeutic modalities to treatment of bleeding in uraemia prior to operative procedures.

CONCLUSION

Conclusion can be drawn from the findings in this work that in uraemic patients, there is a deviation from the normal in 2 of the haemostatic parameters studied, that is in the packed cell volume and bleeding time whereas in the other 2 parameters (Platelet count and prothrombin time) there was no significant difference with controls.

REFERENCE

1. Baron, A., Kaufman, A., & Stauber, K. A. (1969). Effects Of Instructions And

- Reinforcement- Feedback On Human Operant Behavior Maintained By Fixed- Interval Reinforcement 1. *Journal of the Experimental Analysis of Behavior*, 12(5), 701-712.
2. Baker, F. J., & Silverton, K. E. (1985). Introduction to medical laboratory Technology. Butterworth & Co. London 6th Edition, 158-160.
3. Ndukauba, P. I. (1996). Artificial kidney and clinical problems of uraemia Fasmen communications Okigwe, Nigeria, 64-68.
4. Milner, B., Petrides, M., & Smith, M. L. (1985). Frontal lobes and the temporal organization of memory. *Human neurobiology*, 4(3), 137-142.
5. Eastman, R. D., & Slade, R. R. (1990). Clinical haematology Butterworth and Heinemann, 7th Edition, 140-143.
6. Dacie, J. V., & Lewis, S. M. (1994). Investigation of bleeding tendency. Practical haematology. Churchill Livingstone, London, 8th Edition, 317-350.
7. Gray, C. H., Howarth, P. J. N., & Rinsler, M. G. (1985). Clinical Chemical pathology 10th Edition, 288-301.
8. Lynch, M. L., & Raphael, S. S. (1983) Haemostasis, principles and practice. Lynch's medical laboratory, Technology: W. B. Saunders company 4th Edition, 74-53.
9. Evatt, B. L., Veltkamp, J. J., Lewis, S. M., & McArthur, J. (1986) Fundamental diagnostic laboratory haematology: The Bleeding Disorders. U.S. Department of health services, Atlanta Georgia and World health organisation, Geneva.
10. Jaiyesimi, I. O., & AEA, M. (1990). Usefulness of the bleeding time to predict the risk of clinical bleeding in patients with uraemia. *Central african journal of medicine*, 36(5), 132-135.
11. Eknoyan, G., Wacksman, S. J., Glueck, H. I., & Will, J. J. (1969). Platelet function in renal failure. *New England Journal of Medicine*, 280(13), 677-681.
12. Horowitz, H. I., Cohen, B. D., Martinez, P., & Papayouanou, M. F. (1967). Defective ADP-induced platelet factor 3 activation in uremia. *Blood*, 30(3), 331-340.
13. Tschugguel, W., Blaicher, A., Kramer, L., Felfernig, M., Jüngling, G., Kurz, M., ... & Zimpfer, M. (1995). Platelet disorders in uraemia before and after haemodialysis under the influence of low dose aspirin. *Thrombosis research*, 80(3), 225-233.
14. Giles, C. (1981). The platelet count and mean platelet volume. *British journal of haematology*, 48(1), 31-37.
15. Bain, M. B., Finn, J. T., & Booke, H. E. (1985). A quantitative method for sampling riverine microhabitats by electrofishing. *North American Journal of Fisheries Management*, 5(3B), 489-493.