

Mean Corpuscular Volume (MCV): A Suitable Marker for the Diagnosis of Diabetic Nephropathy in Type 2 Diabetes Mellitus Patients

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Abstract

A cross sectional study was carried out to assess mean corpuscular volume (MCV) as a diagnostic marker of diabetic nephropathy in 122 type 2 diabetes mellitus patients and 61 non-diabetic participants at Kabwe Mine Hospital (KMH) in Zambia. A Structured questionnaire was used to capture age, sex, history of blood transfusion and cancer status of the participants. Urea, creatinine and urine albumin concentrations were measured and MCV determined. Renal profile tests (creatinine, urea and urine albumin) were used as a proxy marker for diabetic nephropathy in the patients. Patients with high renal profile tests (urea > 8.5 mmol/l, creatinine > 120 μ mol/l, urine albumin > 30mg/l) were considered to have diabetic nephropathy. The results revealed that creatinine concentration in the patients (750 \pm 4.0 μ mol/l) was significantly higher ($P=0.003$) than in the control group (250 \pm 2.1 μ mol/l). Similarly, the urea concentration in the patients (4.2 \pm 2.4 mmol/l) was significantly higher ($P=0.002$) than in the control group. However, the urine albumin concentration was similar ($P=0.168$) in the two groups. With regard to MCV, it was found to be higher ($P=0.001$) in the patients (150 \pm 7.0 fl) than in the control group (85 \pm 7.0 fl). MCV was found to have sensitivity of 93%, specificity of 96%, PPV 97%, NPV 91% and efficiency of 94% which were significant parameters to warrant the inclusion of MCV as one of the diagnostic markers of diabetic nephropathy. It is concluded that MCV is a reliable and suitable biomarker for detecting diabetic nephropathy in type 2 diabetes mellitus patients.

Keywords: diabetic nephropathy, type 2 diabetes mellitus, renal profile, mean corpuscular volume.

Introduction

Diabetes mellitus is a common metabolic disease affecting 180 million people worldwide in 2002. It was predicted to rise to 250 million in 2019 (23). Diabetes and its associated complications such as nephropathy, retinopathy and neuropathy have become a public health problem of considerable magnitude (18). Adults with diabetes have a 2- to 4-fold increased risk of renal complications compared to those without diabetes (5). Renal diseases account for up to 90% of premature excess mortality in diabetic patients (22). Because of the huge premature morbidity and mortality associated with this disease, prevention and interventions of these complications has become a key issue.

It has been established that diabetic nephropathy is the leading cause of morbidity and mortality in patients with diabetes mellitus (9). Starting in the second half of the 20th century, the prevalence of non-insulin-dependent (type 2) diabetes mellitus increased substantially in many populations and ethnic groups including African Americans, Native Americans, Mexican Americans and Pacific islanders. Around 3.2 million deaths every year are attributable to complications of diabetes and this translates into six deaths every minute. (6). In Zambia, an audit report conducted at Kabwe Mine Hospital (KMH) in the town of Kabwe in 2011, 2014 and 2017 indicated an increase in the number of diabetic nephropathy cases by more than 22% in patients with type 2 diabetes mellitus.

Pathogenesis of diabetic nephropathy (DN) involves damage to the tiniest blood vessels followed by increased concentration of blood urea, creatinine and urine albumin excretion. Diabetes causes unique changes in kidney structure, including Classic glomerulosclerosis which is characterized by increased glomerular basement membrane width, diffuse mesangial sclerosis, hyalinosis, microaneurysm, and

hyaline arteriosclerosis (12). Tubular and interstitial changes are also present (8). Areas of extreme mesangial expansion called Kimmelstiel-Wilson nodules have been observed in 40 –50% of patients developing proteinuria (10). Micro- and macro albuminuric patients with type 2 diabetes had more structural heterogeneity than patients with type 1 diabetes (13). Evaluated by electron microscopy, the severity of glomerular lesions is related to glomerular filtration rate and urea, albumin, electrolytes and to the duration of diabetes, degree of glycemic control and genetic factors (16).

The mean corpuscular volume or mean cell volume (MCV) is a measure of the average volume of a red blood corpuscle (or red blood cell). MCV is part of a standard complete blood count. In patients with anemia, MCV measurement allows classification as either a microcytic anemia (MCV below normal range, i.e. < 80 FL), normocytic anemia (MCV within normal range 80-96 FL) or macrocytic anemia (MCV above normal range, i.e. > 96 FL). If red blood cells are too small or too large, it could be a sign of a blood disorder such as anemia, a vitamin deficiency, or other medical condition. It has been known for some time that elevated MCV predicts poor outcome in both diseased and normal populations (14).

Recently, elevated mean corpuscular volume (MCV) was identified and proposed to be of diagnostic importance in diabetic nephropathy (DN) (19). MCV was reported to be a risk marker of morbidity and mortality for renal and cardiovascular diseases in various study populations (10) but no study has been done to determine its diagnostic importance in type 2 diabetic mellitus patients with DN.

The aim of this study was to assess the suitability of MCV as a diagnostic marker for diabetic nephropathy in type 2 diabetes mellitus patients.

Methods

The study was conducted at Kabwe Mine Hospital (KMH), a second level Hospital situated in the Central Province of Zambia and a referral Centre for the central province with the bed capacity of 300. The study involved indigenous Zambian adults with diabetes type 2 attending diabetes clinic at Kabwe Mine Hospital out-patient department (OPD) from December 2018 to May 2019. The total number of participants recruited in this study was 183. This included 122 type 2 diabetes mellitus patients and 61 non-diabetic healthy individuals as controls. Purposive sampling method was used to enroll study participants.

Inclusion and exclusion criteria

This study was limited to male and female adults above the age of 18 years. Participants who had a history of having been transfused within 2 to 3 weeks, cancer, or pregnant, were excluded from the study.

Collection of qualitative data

Information relating to diabetes type2 and nephropathy was obtained from the prospective participants using a questionnaire. Structured interview schedule with close-ended questions was used to collect qualitative data. The interview schedule captured demographic variables, knowledge on common diabetes symptoms and factors associated with these symptoms. The questionnaire was administered in the simple English language and translated into local language (Bemba) for those who did not understand English.

Collection of blood and urine samples

Venous Blood amounting to 4ml was collected from each eligible patient using the evacuated blood collection system through vein puncture. Blood was then immediately dispensed into an Ethylene Diamine tetra Acetic acid (EDTA) and sodium fluoride containers in quantities of 2ml in each container respectively. Tube inversion was done 10 times to ensure proper mixing of the anticoagulant (EDTA and sodium fluoride) with blood to prevent clotting. After blood collection, each patient was provided with a graduated urine container and asked to submit 4ml of urine for an on-spot albuminuria check using the multistrix, the rapid albumin diagnostic method and results were entered on the information sheet that was provided.

Blood specimens were stored in the refrigerator at the temperature of 2 degrees Celsius until the time of specimen analysis. At the time of testing, samples for MCV, Glucose, Creatinine and Urea determination were removed from the refrigerator and brought to room temperature (22 – 25 degrees Celsius).

Mean corpuscular volume (MCV) determination

Blood samples were put on the blood mixer for 15 minutes to ensure thorough mixing of the blood and the anticoagulant while carrying out quality control checks on ABX Micros 60 hematology Analyzer before running the sample and obtaining MCV results in FL. ABX Micros 60 uses Miniclean and Minilyse reagents manufactured by Roche Company Limited (Germany). Red blood cells pass through a Micro-aperture that generates electronic pulses, as the cells pass through it. These pulses are grouped according to size, volume, threshold and calculated to form a Histogram (Distribution curve). This distribution curve is then used to calculate the volume of the Red blood cells in femtolitres (fl). MCV levels were grouped into low MCV level (< 80fl), normal (80 – 90 fl) and high MCV level (> 96 fl).

Glucose estimation

Two (2) ml of venous blood was collected in sodium fluoride containers. The samples were then refrigerated at 2 degrees Celsius during which period the plasma was separated from whole blood. Samples were then analyzed using COBAS INTEGRA 400+ Analyzer in batches of 30. Glucose levels were grouped into low glucose level (< 3.3 mmol/l), normal (3.3-7.8 mmol/l) and high glucose level (>11.0 mmol/l).

The test Principle uses an enzymatic reference method with hexokinase which catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by NADP⁺ to form NADPH.

Urea estimation

Two (2ml) of venous blood was collected in sodium fluoride containers, the samples were immediately centrifuged at 1500g for 15 minutes and plasma separated and stored at 2 degrees Celsius until time of analysis. Samples were analyzed in batches of 30 using the Cobas Integra 400+ fully automated Chemistry analyzer. Urea levels were classified as normal if less or equal to 8.5 mmol/l and raised if greater than 8.5 mmol/l.

The principle of Urea determination involves a kinetic test with urease and glutamate dehydrogenase. Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD for each mole of urea hydrolyzed.

Creatinine estimation

Two (2 ml) of venous blood was collected in sodium fluoride containers, and the samples were immediately centrifuged at 1500g for 15 minutes, and plasma was separated and stored at 2 degrees Celsius until the time of analysis. Samples were analyzed in batches of 30.

The cassette COBAS INTEGRA Creatinine plus ver.2 (CREP2) contains an in vitro diagnostic reagent system intended for use on COBAS INTEGRA systems for the quantitative determination of the creatinine concentration in serum, plasma, and urine. Reference range for creatinine is 63-120µmol/l. All values above 120µmol/l were considered to be high.

Albumin estimation

Four (4ml) of urine specimen was collected in a plain container. The dip stick coated with the reagent was then dipped into urine and the results were obtained from the protein pad as indicated below.

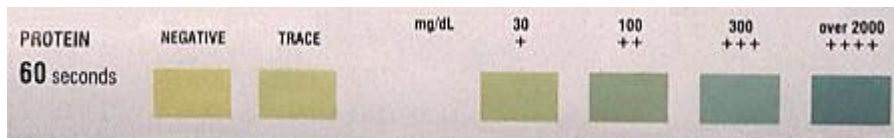


Figure 1. Determination of albumin in Urine (Ziadeh and Wolf, 2008)

The Principle of the test is that the protein pad on the multi reagent dipstick (Multistix[®], illustrated above) is based on the "protein error of pH indicator dyes". The test is dependent on the ability of amino groups in the proteins to bind to and alter the color of acid-base indicators.

Ethical considerations

Ethics approval was obtained from the University of Zambia Biomedical Research Ethics Committee (Assurance No. FWA00000339, IRB0000112 of IOR0000776). Written permission was obtained from Kabwe Mine Hospital. The information sheet about the study was given to patients, translated in Bemba which is the local language or read out to them in cases where they could not read. Patients were informed about the study and given an option to decide if they did not want to take part in the study.

The purpose of the study was explained to all the study participants and those that declined to participate in the study were not forced, but were assured of their protected privileges and other benefits such as being managed by clinicians as per standard treatment and guidelines. The respondents were interviewed individually in a private room and only 4mls of blood and urine specimens were collected from them.

Privacy and confidentiality were maintained by using codes for the patients instead of names in the report and data was kept in a locked cabinet and keys kept by the researcher. The respondents were thus assured of utmost confidentiality. Patient's consent to be included in the study was obtained. Patient's comfort and dignity during and after the procedure was paramount. The well-being and prompt definitive management of the patient was first before the research.

Data management and statistics

Raw data and results from patients were edited for consistency and legibility on a daily basis. For qualitative data, the close ended responses were pre-coded before the interview to ensure easy entry and analysis of data using statistical package for social science (SPSS) Computer Software and center for evidence-based Medicine (CEBM) calculator. Hematological and chemistry data was entered on the data sheet and was used for analysis. Results obtained from the analyzing machines were tabulated in the data sheets. All the parameters were normally distributed and hence reported as the mean +/- standard deviation. The significance of the differences between patients and controls for normally distributed parameters were determined using the independent samples T-test for continuous variables and the Chi-square test for categorical variables. Risk factors and patient attributes associated with diabetic nephropathy in diabetes type 2 were determined by logistic regression analysis. Odds ratios and their 95% confidence intervals were reported.

Sensitivity, specificity and positive predictive values for MCV as a marker for diabetic nephropathy were calculated from a 2 by 2 table computed in CEBM statistics calculator. P-Values of less than 5% were taken as significant.

Results

A total number of 183 type 2 diabetes mellitus patients and controls aged 18 to 75 years participated in this study. The majority (37%) of participants were in the age range of 35-55 years. The mean age was 49 years.

Mean corpuscular volume

Figure 3 shows that type 2 diabetes mellitus patients with diabetic nephropathy had higher ($P=0.004$) MCV than control participants. Figure 4 indicates that the mean MCV for female type 2 diabetes mellitus patients (130 ± 5 fL) was significantly higher ($P=0.002$) than male participants (80 ± 5 fL). Similarly, overall, the mean MCV for type 2 diabetes mellitus patients (150 ± 7.0 fL) was significantly higher ($P=0.001$) than the control participants (85 ± 7.0 fL).

Creatinine, urea and urine albumin concentration

Table 1 shows that the mean creatinine concentration for type 2 diabetes mellitus patients (750 ± 4.0 $\mu\text{mol/l}$) was significantly higher ($P=0.003$) than control participants (250 ± 2.1 $\mu\text{mol/l}$). The mean urea concentration for type 2 diabetes mellitus patients (4.2 ± 2.4 mmol/l) was significantly higher ($P=0.002$) than control participants (2.2 ± 1.5 mmol/l). The mean urine albumin concentration for type 2 diabetes mellitus patients (12.4 ± 3.3 mg/l) and that for control participants (12.2 ± 2.9 mg/l) were not significantly different ($P=0.168$).

Tables 2 and 3 illustrate the cross tabulations of renal profiles (Creatinine, Urea and Urine albumin) results in comparison to the MCV test results. Table 2 shows that 59% of the participants had diabetic nephropathy (Creatinine $>120 \mu\text{mol/l}$, Urea $>8.3 \text{mmol/l}$, Urine albumin $>30 \text{mg/l}$). Out of these patients, results from sixty-seven (55%) participants were true positive, implying that both the renal profile tests and MCV were abnormal. Two percent of the results were false positive test results with the MCV test but the patients did not have diabetic nephropathy because the renal profile results were low (Creatinine $<120 \mu\text{mol/l}$, Urea $< 8.3 \text{mmol/l}$, Urine albumin $< 30 \text{mg/l}$). Results from five (4%) participants were false negative, implying that MCV test results were negative but the participants had diabetic nephropathy in the actual fact. MCV results from forty-eight (39%) participants were true negative because both renal profile tests and MCV results were low, implying that patients did not have DN. MCV had good Sensitivity (93%), Specificity (96%), PPV (97%), NPV (91%) and efficiency (94%) respectively.

Discussion

This study revealed that patients aged 35 years and above were at risk of developing DN than those who were below 35 years old. These results were consistent with the study done by Tsuboi et al., (2013) who found that the risk group was above 65 years. However, the reduction in the risk age group of DN observed in this study may be due to a number of factors including HIV, poor glycemic control, lack of exercises and poor knowledge on the complications of diabetes mellitus to some extent. On the other hand, the risk of DN with increasing age could be attributed to changes that occur in the glomerular basement membrane as a result of aging tilting the scale to DN in older patients.

This study reveals that diabetic nephropathy in type 2 diabetes mellitus patients was related to the level of MCV among type 2 diabetes mellitus patients. Respondents with diabetic nephropathy had a higher proportion of MCV 84 (69%) than control participants 18 (29%). The difference was significant $X^2 = 96.93$, $p = 0.004$. This result correlates with Al-Najjar (2009) who found an association of 28%, this shows that there is a significant correlation between MCV and diabetic nephropathy among type 2 diabetes mellitus patients in Zambia as compared to the general population. Diabetic nephropathy may influence erythropoiesis, circulatory half-life and deformability of erythrocytes, promoting anisocytosis and thus elevating MCV levels (21). High level of MCV was also associated with the metabolic syndrome (17), leading to postulate a possible effect of an underlying diabetic nephropathy state (which typically occurred in diabetes and metabolic syndrome) on increased destruction of erythrocytes. Similar finding was previously reported by Acosta *et al.* (2010), where they reported shortened half-life of red blood cells in diabetes mellitus. However, the Relationship between MCV and diabetic complications has not been investigated (11). So, the present study was done to study the relation between MCV and diabetic nephropathy in patients with type 2 diabetes mellitus and its possible use as a diagnostic marker for Diabetic nephropathy.

The mean MCV in female patients with DN ($130 \pm 5 \text{Fl}$) was significantly higher than in the male patients ($80 \pm 5 \text{Fl}$), P – Value 0.002. The differences revealed in the levels of MCV between males and females correlate very well with results obtained by Patel et al (2010) among Mexican adult patients with DN in which MCV was high in females than males. The main reason why females with diabetic nephropathy tend to have high MCV than males with the same condition in type 2 diabetes mellitus is still unclear. This study reveals that the proportion of participants who had high MCV differed significantly among different age groups in type 2 diabetes mellitus patients. The proportion of type 2 diabetes mellitus patients who had higher MCV was in the age range of 35 – 55 years 91 (74%). Results obtained in this study corresponds to those obtained by Quinn et al., (2008) who reported a correlation between old age and diabetic nephropathy. These results were also consistent with the study done by

Tsuboi et al., (2013) who found that ≥ 65 years was the most vulnerable age group. Old age tends to decrease resistance in both the afferent and efferent arterioles of the glomerulus. These early hemodynamic changes facilitate albumin leakage from the glomerular capillaries and overproduction of mesangial cell matrix, as well as thickening of the glomerular basement membrane and injury to podocytes resulting in diabetic nephropathy and consequent raise in MCV levels (11). The peak incidence of diabetic nephropathy (40 years) observed in this study, could be associated with the occurrence of early complications which lead to early death as indicated by the decline in the study participants above the age of 55 years.

It was further observed that MCV of patients with diabetic nephropathy (150 ± 7.0 fL) was significantly higher in patients with diabetic nephropathy than in the control participants (85 ± 7 fL), P – Value 0.001. These results are consistent with Jannuzzi et al., (2010) who found a correlation between MCV and diabetic nephropathy in type 2 diabetes mellitus patients. The results obtained are also similar to those of Patel et al., (2009) who found a significant association between high MCV and high mortality rate in patients with various renal complications, but in this study, the association observed was specifically between high MCV and diabetic nephropathy. Mean corpuscular volume (MCV) is considered as a prognostic marker which may reflect an underlying renal process (Lippi et al., 2009). It is a measure of volume of red cells in a blood sample, which is calculated by both impedance and flow cytometric analyzers as part of the routine complete blood count. MCV is effectively a free test which, is reported alongside a complete blood count without extra cost and has good prognostic value (14).

The reason why MCV is considered to be a predictor in a wide range of conditions is unclear, it was shown to be a strong predictor of mortality in general population of adults aged 45 years and more (14). High MCV values were associated with deaths from cardiovascular disease (CVD), cancer, and other causes, however the effect was stronger for CVD Patel et al (2010). It was reported that MCV is a strong predictor of mortality in many other conditions such as obesity, malignancies, and chronic kidney disease Patel et al (2010).

The motivation for this study was due to the mounting evidence that raised MCV values in some cases could reflect diabetic nephropathy which is potentially associated with increased risk of death mainly from cardiovascular causes (19). The actual cause of high MCV in diabetic nephropathy is not very clear though Ferruci and his colleagues suggested that it could partly be due to the disruption in the release of the hormone erythropoietin which is produced by the Kidneys and plays a major role in the regulation and normal production of the red blood cells from which the MCV is derived. Felker et al., (2008) observed that there was a significant high level of MCV in type 2 diabetes mellitus patients with diabetic nephropathy, especially in those patients with long term diabetes and chronic complications. This is consistent with the results of this study. Cook et al., (2008) found that DN detected by high MCV was independently associated with cardiovascular complications and suggested that high MCV could be considered as a risk marker for cardiovascular diseases.

The mean urea concentration in patients with DN (4.2 ± 2.4) was significantly higher than in the controls (2.2 ± 1.5), P – Value 0.02. These results accord that of Ziyader et al., (2008) who found increased urea level in a study done on African Americans with DN. Urea is the major end product of protein nitrogen metabolism (19). It is synthesized by the urea cycle in the liver from ammonia which is produced by amino acid deamination. Urea is excreted mostly by the kidneys. Elevations in blood urea nitrogen concentration are seen in inadequate renal perfusion, shock, and diminished blood volume such as in patients with Diabetic nephropathy (12). Determination of blood urea nitrogen is the most widely used screening test for renal function (5).

The creatinine concentration was higher in type 2 diabetes mellitus patients with DN than those without DN ($730 + 4.0$) and ($250 + 2.1$), P – Value 0.03 respectively. These results were consistent with those reported in the study done by Friendman et al., (2008) to find out if creatinine clearance was a reliable index for glomerular filtration rate (GFR). Creatinine is produced endogenously from creatine and creatine phosphate as a result of muscle metabolic processes (5). It is excreted by glomerular filtration during normal renal function. An abnormality in the functioning of the kidney such as in diabetic nephropathy will result in elevated amounts of blood creatinine concentration. When urea is used in conjunction with serum creatinine determinations, it can aid in the differential diagnosis of the three types of azotemia: prerenal, renal, and postrenal (17).

The mean urine albumin concentration of participants with DN (12.4 ± 3.3) in this study was not significantly higher than the concentration in the control group (12.2 ± 2.9), P – Value 0.168. The low urine albumin concentration found in the patients with DN is not consistent with Fersblom's report (2008) of elevated urine albumin concentration as one of the markers of degenerating kidney function among diabetic patients. The reason for the difference in albumin concentration observed in this study could be as a result of population difference in which this study and others were done.

DN also known as Kimmestiel – Wilson syndrome, is a condition characterized by intercapillary glomerulonephritis (infection of the glomeruli) and is a progressive disease caused by angiopathy of capillaries in the kidney glomeruli and this leads to the compromise in the glomerulus filtration rate (GFR) system leading to the kidney becoming leaky to blood proteins such as albumin. Levels of creatinine, urea and urine albumin are increased following renal damage such as in DN, resulting in hyper perfusion and hyper filtration leading to the leakage of macro molecules such as proteins from the glomerulus into the urine. According to this study, 72(59%) of the participants had diabetic nephropathy (had Creatinine $>120\mu\text{mol/l}$, Urea $>8.3\text{mmol/l}$ and Urine albumin $>30\text{mg/l}$). out of these results 67(55%) participant results were true positives, implying that both the renal profile test results and the MCV results were abnormal. 2(1.6%) of the results were false positive test results with the MCV but the patients did not have diabetic nephropathy because renal profile results were below (Creatinine $< 120\mu\text{mol/l}$, Urea $< 8.3\text{mmol/l}$ and Urine albumin $< 30\text{mg/l}$). 5(4.1%) of the participant results were false negative, implying that the RDW test results were negative but the participants had diabetic nephropathy in the actual fact. 48 (39%) negative MCV test results were true negatives because renal profile results were also negative, implying that patients had no diabetic nephropathy.

MCV had a sensitivity of 93% (95% CI [88.0 – 96.7]), PPV 97% (95% CI [91.9 – 98.7]), NPV 91% (95% CI [83.6 – 94.5]) which were all acceptable parameters to support the suitability of MCV as a marker for DN.

This study revealed that MCV test had few false negative results leading to high and better sensitivity of 93% (95% CI [88.0 – 96.7]) this implies that MCV test is capable of detecting 93% of DN cases among type 2 diabetes mellitus patients and only 7% will be missed out as this will be reported as negative. Therefore, MCV test has a high probability of detecting diabetic nephropathy in type 2 diabetes mellitus patients.

MCV test also had an acceptable specificity of 96% (95% CI [88.8 – 98.2]). This means that, the probability of type 2 diabetes mellitus patients not having DN was 96% which means 2 (4%) tests gave false positive results.

MCV test had a high PPV 97 % (95% CI [91.9 – 98.7]). This can be interpreted to mean that 67 (97%) positive MCV test results were truly DN cases.

91% NPV results obtained for MCV in this study means that 48 (91%) of negative MCV test results were true negatives (no DN) while 5 (9%) were false negatives (had DN). From the available literature searched so far, no diagnostic study has been done to specifically evaluate the suitability of MCV in the detection of DN in type 2 diabetes mellitus patients. Long T.W., (2009) reported that the acceptable sensitivity, PPV and NPV should be above 90%. From the results obtained in this study, MCV was found to be a suitable diagnostic test for DN in type 2 diabetes mellitus patients because all parameters used for detecting suitability were above 90%. Positive and negative predictive values vary according to the prevalence of the condition under study (3). Therefore, it would be wrong for predictive values determined for one population to be applied to another population with a different prevalence. In this case, MCV test results could be used among type 2 diabetes mellitus patients and not the general population because DN may be absent hence low predictive values even if the test is highly sensitive and specific.

Conclusion

This study revealed that type 2 diabetes mellitus patients with DN had higher levels of MCV and abnormal renal profile test results (creatinine $>120\mu\text{mol/l}$, Urea $> 8.3\text{mmol/l}$ and Urine albumin $> 30\text{mg/l}$) than healthy non diabetic control participants. Using diagnostic sensitivity and specificity, PPV, NPV and efficiency, it was found that MCV was a reliable and suitable biomarker of detecting

diabetic nephropathy in type 2 diabetes mellitus patients. In addition, MCV is cheap, readily available as part of the full blood count test and easy to perform.

Acknowledgements

I wish to sincerely thank Kabwe Mine Hospital management and staff for contributing to the success of this study in various ways. Others include; Dr Joseph muyangana, Dr. Edgar Mufaya Mrs. Ireen mushili Nombwende, Mrs. Hilda Bwalya.

All laboratory staff at Kabwe Mine Hospital.

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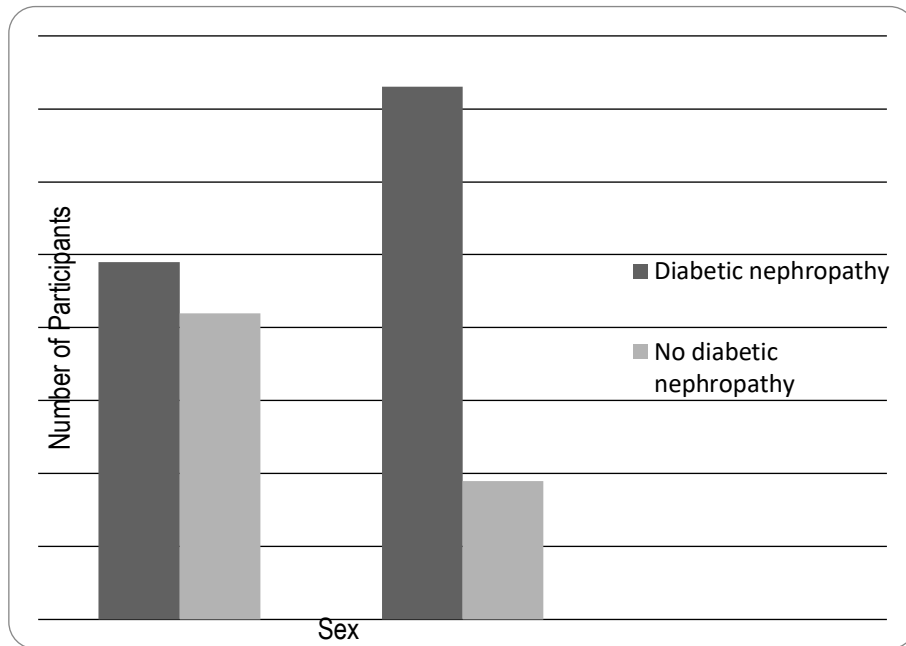


Figure.1. Distribution of participants according to sex

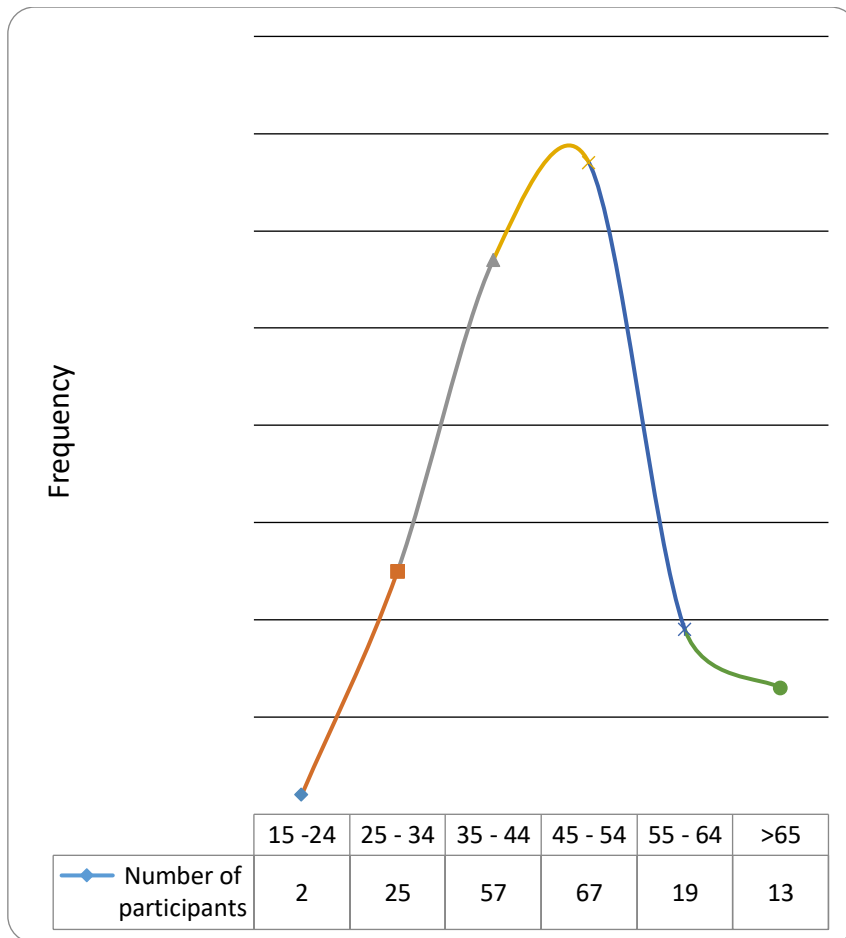


Figure.2. Age distribution (according to years) of respondents

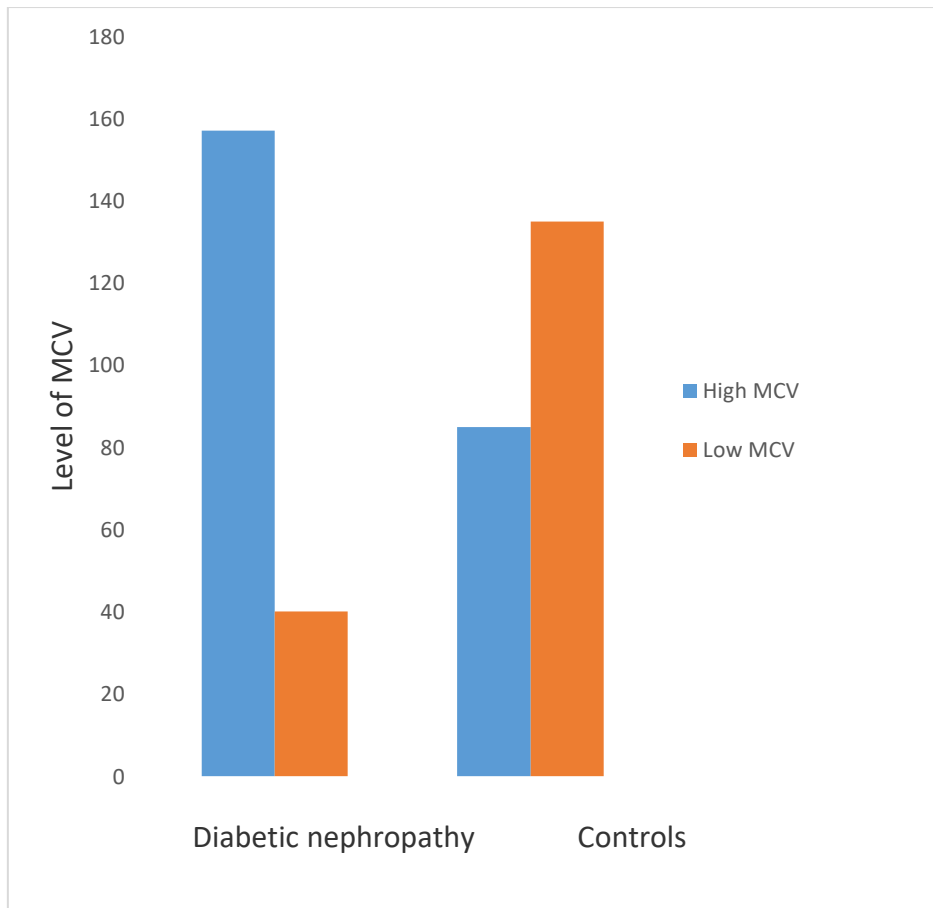


Figure 3. Association between mean corpuscular volume and Diabetic nephropathy

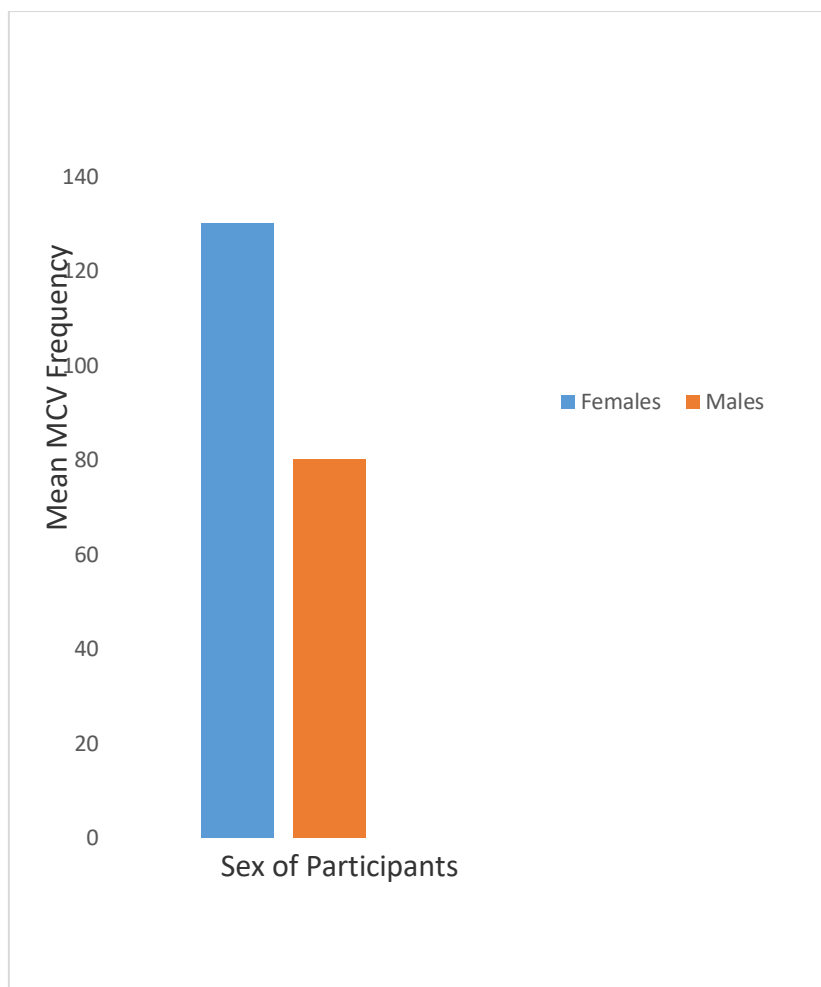


Figure 4. Comparison of average mean corpuscular volume between male and female patients with diabetic nephropathy

Table 1. Mean renal profiles of creatinine, urea, urine albumin and mean corpuscular volume (MCV) in patients with diabetic nephropathy and control subjects

	Status	Number of participants	Mean	P - Value
Creatinine($\mu\text{mol/L}$)	Control	61	250+2.1	0.003
	DN	122	730+4.0	
Urea (mmol/L)	Control	61	2.2+1.5	0.002
	DN	122	4.2+2.4	
Urine albumin (mg/L)	Control	61	12.2+2.9	0.168
	DN	122	12.4+3.3	
MCV	Control	61	14.7+3.8	0.001
	DN	122	32.2+4.2	

Table. 2. Comparison of renal profile results and MCV test results in patients with diabetic nephropathy

MCV test	Renal profiles		Total
	Diabetic nephropathy	No diabetic nephropathy	
	Creatinine >120µmol/l, Urea >8.3mmol/l, Urine albumin >30mg/l	Creatinine <120µmol/l, Urea < 8.3mmol/l, Urine albumin < 30mg/l	
High tests	TP 67	FP 2	TP + FP 69
Low tests	FN 5	TN 48	FN + TN 53
Total	TP + FN 72	FP + TN 50	TP + FP + FN + TN 122

Key: **TP:** True positive = test positive in actually positive cases, **TN:** True negative = test negative in actually negative cases, **FP:** false positive = test positive in actually negative cases, **FN:** false negative = test negative in actually positive cases.

Calculations

- Sensitivity = $TP/TP + FN \times 100 = 67/72 \times 100 = 93\%$
- Specificity = $TN/FP + TN \times 100 = 48/50 \times 100 = 96\%$
- PPV = $TP/TP + FP \times 100 = 67/69 \times 100 = 97\%$
- NPV = $TN/TN + FN \times 100 = 48/53 \times 100 = 91\%$
- Efficiency = $TN + TP/TP + FN + FP + TN \times 100 = 115/122 \times 100 = 94\%$

Table.3. Reliability of Mean Corpuscular Volume as a biomarker of diabetic nephropathy.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency (%)
MCV	93	96	97	91	94