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RESEARCH ARTICLE

PREVALENCE OF UROTHELIAL CELL CANCER AND ASSESSMENT OF DIABETIC PATIENTS ATTENDING CLINIC AT A TERTIARY HOSPITAL SOUTHEASTERN NIGERIA

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ABSTRACT

Background: Type 2 Diabetes Mellitus has been proved to increase the risk of bladder cancer among diabetics, yet studies in Nigeria are scarce. **Objectives to Aim:** To determine the prevalence of urothelial cell cancer and assessment of Diabetic patients who attend clinic at University of Nigeria Teaching Hospital, Ituku/Ozalla, Enugu southeastern Nigeria. **Materials and methods:** Ethical clearance and informed consents were appropriately obtained. Fasting Blood Sugar of the participants (165 diabetics and 140 non diabetics) were determined, then terminal urine samples were collected in a sterile universal container by the participants. Self administered questionnaires were issued. Urine samples were centrifuged at 4000rpm for 10 minutes. The sediments were used to make smear on well labeled charged slides and stained using May-Grunwald Geimsa staining technique for cytology. Urinalysis and urine microscopy were also carried out on the urine sediments. **Results:** Hyperglycaemia was noticed in 81.05% of the diabetics. Prevalence of abnormal urothelial cell among the diabetics was 3.03%. **Conclusion:** There was low prevalence of urothelial cell cancerand poor control of blood sugar level among diabetics in this locality. There was low post diagnosis survival rate.

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INTRODUCTION

Diabetes mellitus commonly referred to as diabetes is a group of metabolic diseases in which there is high blood sugar level due to defects in insulin secretion, insulin action, or both (American Diabetes Association, 2014). Diabetes mellitus could be due to either the pancreas not producing enough insulin or the cells of the body not responding properly to insulin produced (Gardner, 2011). On this basis, Diabetes mellitus has been classified into various types: type 1, type 2, and gestational diabetes. According to "update 2015'' of International Diabetes Federation, as of 2015, an estimated 415million people had diabetes worldwide, with type 2 diabetes mellitus making up about 90% of the cases (Shi, 1948). This represents 8.3% of the population with equal rates in both women and men. Diabetes mellitus doubles a person's risk of early death. From 2012 to 2015, about 1.5 to 5.0 million persons die yearly as a result of diabetes (World Health Organisation, 2018). The global economic cost of diabetes in 2014 was estimated to be US dollar 612 billion. In the United States, diabetes cost 245 billion US dollars in 2012 (American Diabetes Association, 2013).

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In Africa, the International Diabetes Federation estimated that over 5 million people suffer from the disease and the number is estimated to increase to 15 million by 2025 (International Diabetes Federation, 2013). The prevalence in Nigeria varies from 0.65 in rural Mangu village to 11.0% in Urban Lagos (Akinkgbe, 1997). WHO suggest that Nigeria has the greatest number of people living with diabetes in Africa, with an estimated burden of about 1.7 million which will increase to 4.8 million by 2030. Symptoms of hyperglyceamia include frequent urination (polyuria), increased thirst (polydipsia) and increased hunger (polyphagia), weight loss. If left untreated, diabetes can cause many complications. Acute complications include; diabetic ketoacidosis, nonketotic hyperosmolar hyperglcaemic syndrome or death. Serious long-term complications include heart disease, stroke, chronic kidney failure, foot ulcer, damage to the eyes, autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction. Diabetics have an increased risk of atherosclerotic, cardiovascular, peripheral arterial and cerebrovascular disease (American Diabetes Association, 2014). Urine cytology is the scientific study of cells in voided urine. It is also the branch of pathology, the medical specialty that deals with making diagnosis of diseases and conditions through the examination of cells in urine. More than five decades ago, Dr George Papanicolaou hypothesized that

microscopic evaluation of exfoliated cells in urine was a potentially useful method to detect urinary tract malignancies (Blick, 2012). Urinary cytology is most helpful in diagnosing high-grade tumors and Carcinoma In Situ (CIS). Low-grade non-invasive tumors may be missed by routine cytologic analysis. Approximately 70% of bladder urinary cytology is non-invasive, papillary tumors that are usually morphologically categorized as low grade urothelial carcinoma. They have good prognosis, but may be associated with recurrence and progression to high-grade 10-15% of cases. The remaining 30% are muscle-invasive which are histologically categorized as high-grade (Van Rhijn, 2012). Bladder cancer is a heterogenous tumor due to transitional cell carcinoma. Hematuria is the most prevalent symptom that occurs in 80%-90% of cases and is usually presented without other urinary symptoms (Park, 2014).

The risk of bladder cancer is approximately 40% higher in individuals with diabetes than individual without diabetes[11]. However, some short comings of these studies have to be emphasized including a mixture of case-control and cohort studies, a mixture of bladder cancer, incidence and mortality, lack of differentiation between type 1 and type 2 diabetes and small numbers of bladder cancer case in most included studies. Type 2 diabetes is also associated with a higher number of bladder tumors and a higher grade. The insulin-like growth factors 1 receptor and the insulin receptor play an important role in tumor growth, differentiation, motility and protection from apoptosis (Belfiore, 2011). Insulin-like growth factor 1 receptor is also over-expressed in malignant bladder cells and is indirectly stimulated by insulin, as insulin increases circulating levels of the insulin-like growth factor1receptor ligand, IGF-1 (Rochester, 2007). Through this mechanism, hyperinsulinemia in individuals with type 2 diabetes may more strongly promote bladder cancer than in non-diabetic individual (Giovannucci *et al*., 2010). A meta-analysis of 29 cohort studies conducted in 2012 indicated that compared with non-diabetic or general population, individual with diabetes may have more than 29% increase incidence of bladder cancer (Zhaowei *et al*., 2012). Also, findings of another meta-analysis of fifteen cohort studies showed that diabetes is associated with an 11%increase risk of bladder cancer. Emerging epidemiologic evidence from Canada and Denmark suggest the risk of cancer in individuals with type 2 diabetes changes over time following diagnosis (Johnson *et al*., 2011). Compared to individual who do not have diabetes, the risk of being diagnosed with bladder cancer in the first year after a new diagnosis of type 2 diabetes is increased by 30% (Colmers *et al*., 2013). Ever since then, there have been many high quality cohort studies, researches, findings, evaluations on this association but incompatible results still reigns.

Justification: However, due to the incompatible and inconsistency of the existing literature and the insufficient statistical power of primary studies, we performed a research studies to obtain a more precise estimation of the relationship between diabetes and risk of bladder cancer. Therefore, the cytological evaluation of diabetic urine is necessary to check if there is any tumor associated with early or later stage of diabetes mellitus. A study of this kind would help in providing alternative means to ascertain earlier symptomless lesion of urothelial cells in diabetes mellitus. Hence assessment of Diabetics on management and urine cytology was employed in the examination of diabetic urine to check for the predisposing effect of diabetes to precancerous or cancerous tumors.

Aim: The aim of this work was to cytologicallyevaluate diabetics urine and assess the response of diabetics to management in our locality.

Objectives

- To determine the prevalence of urothelial cell malignancy, among Diabetes Mellitus patients in our locality.
- To assess the control Diabetes Mellitus patients in our locality.

MATERIALS AND METHODS

Study Design: This study was a prospective, observational, group, analytical cohort study.

Study Location: The study location was University of Nigeria Teaching Hospital Ituku/Ozalla, Enugu, Southeastern Nigeria. Urine samples of both male and female of different ages were collected from patients attending clinics at University of Nigeria Teaching hospital Ituku Ozalla Enugu after explanation of the research and due written consent obtained from willing participants.

Sample population and sample size: Urine samples were randomly collected from 165 diabetic patients and 140 non diabetic patients who serve as control participants.

Minimum sample size (n)

The value of p was taken as 11.0% (International Diabetes Federation, 2013)

$Z = 1.96$; $q = 0.89$; $d = 0.05$; $n = 165$

Sampling and data collection: The clinical presentations and biodata of participants were obtained using self administered questionnaire. Laboratory parameters of diabetic out-patient and non diabetics were evaluated. The clinical presentations studied included types of diabetes, blood pressure status, urine frequency, years of post diagnosis and treatment type. Laboratory data assessed included urinalysis, urine microscopy, Fasting Blood Sugar and urine cytology.

Sample collection: Urine samples were collected using a sterile urine container by the patient in a secluded area. The patients were advised on how to collect the last part of urine (terminal urine). The urine specimen collected were appropriately labeled and sent immediately to the laboratory for investigation within one hour of collection. Urinalysis was carried out using Cambi-10 urine strip. Urine samples were centrifuged and wet preparation used for microscopy. The remaining sediments were used to make smear on two well labeled slides and preserved in fixative for subsequent cytology staining using May-Grunwald Giemsa cytology staining technique. The stained slides were sent for microscopy and photomicrography. Fasting blood sugar test was carried out using glucometer (accu-check advantage)

Sample analysis

Urine test strip or dipstick test

- The urine was collected in a clean sterile urine container

- It was mixed well for evenly distribution of its constituent.
- The test strip was dip inside the container containing urine and supported the edge of the strip over the mouth of the container to remove excess urine.
- The strip was then left to stand for the time necessary (2 minutes) for the reaction to occur. The colour changes of each pad on the strip was compared against the chromatic scale provided by the manufacturer.
- The matched colour was observed and recorded.

Urine microscopy

- The urine was mixed well and with a pasture, 10ml of urine was placed into the plastic conical tube labelled with patient number and the tube was covered with tight fitting cover.
- The tube was placed in centrifuge and the centrifuge machine was switched on.
- The urine specimen was centrifuged at 4000 RPM (evolution per minute) for 10 minutes.
- After the centrifuge has stopped, the tubes were removed and the supernatant decanted leaving only sediment in the bottom of the tube
- With a plastic pipette the remaining liquid and sediment were mixed and a few drops of the mixture were removed.
- One drop of the sediment solution was placed on a glass slide and covered with cover slip
- Using the microscope, the sediments were examined using phase contrast under low (10x) and high (40x) power, scanning several fields to obtain average number of formed elements.

Slide preparation for cytology

On a clean grease free slide, one drop of egg albumin was smeared on the slide and allowed to air dry. With a pasture pipette, from the remaining urine sediment above, one drop of the sediment was used to make a thin smear on the slide containing egg albumin and allowed to air dried.

Staining

May-Grunwald Giemsa staining technique

- The air-dried smear slide was fixed in methanol for 15 minutes
- It was stained with may-Grunwald working solution for 5 minutes
- It was stain with Giemsa working solution for 12 minutes
- It was assessed with clean buffered water for 2,5, and 2 minutes
- The slides were dried in an upright position at room temperature.
- The slide was mounted with a coverslip using DPX mountant
- The mounted slide was then examined microscopically using 10x and 40x objectives.

Point of care test

Fasting blood sugar using glucometor

- The patient was advised not to eat for at least 8 hours (the test was done in the morning before taking breakfast)
- The thumb (finger) was chosen because it has more blood supply.
- The fingers was disinfected by cleaning with alcohol swab
- The glucometer was reset by fitting its code which matched with the code on the strip.
- The strip was inserted on the glucometer and the finger was pricked with the aid of lancet.
- It was allowed sometimes for blood to rush out from the pricked area.
- A drop of blood was allowed on the test strip (at the test spot) already inserted in the glucometer.
- The glucometer then tested the sample immediately and the result shown on the screen was recorded.

RESULTS

The results of this research comprised of the age-sex distribution of diabetic participants; years of post diagnosis; Fasting Blood Sugar level of diabetic participants; urine frequency before commencing ofmanagement and during management (day and night); present symptoms, signs and complications; urine strip analysis of diabetic participants; urine sediment microscopy and urine cytology of diabetic participants. The age-sex distribution of diabetic participants was as shown on chart one. Fasting Blood Sugar level of diabetics was as shown on chart two. Cytology report of diabetics was as shown on chart three. Urine strip analysis was as shown on chart four. Figure one is photomicrograph showing normal and abnormal urothelial cells. Figure two is the photomicrograph of abnormal urothelial cells. Analysis of urine frequency before diagnosis and during control revealed, no previous frequencyamong 5(3.25%) diabetic participants (2 males and 3 females); previous frequency among 149(96.75%) diabetic participants (61 males and 88 females). Present frequency among 47(29.19%) diabetic participants (18 males and 29 females) where as presently no frequency among 114(70.81%) diabetic participants (45 males and 69 females). Analysis of the present signs, symptoms and complications revealed, polydipsia among 40(22.5%) diabetic participants (14 males and 26 females); polyuria among 41(26.8%) diabetic participants (15 males and 26 females); polyphagia among 34(22.2%) diabetic participants (16 males and 18 females); easily fatigued among 45(29.4%) diabetic participants (16 males and 29 females); numbness among 28(18.3%) diabetic participants (10 males and 18 females); leg ulcer among 2(1.3%) diabetic participants (2 males and 0 female); weight loss in 1(0.85%) diabetic participant (1 male and 0 female); family history of diabetes 29(19.0%) diabetic participants (10 males and 19 females); history of hypertension among 2(1.3%) diabetic participants (2 males and 0 female).

Analysis of years of post diagnosis revealed <1 year among 7(45.8%) diabetic participants (2 males and 5 females); 1-5 years among 61(39.9%) diabetic participants (23 males and 38 females); 6-10 years among 50(32.7%) diabetic participants (20 males and 30 females); 11-15 years among 22(14.4%) diabetic participants (12 males and 10 females); 16-20 years among 10(6.5%) diabetic participants (5 males and 5 females); 21-25 years among 3(2%) diabetic participants (1 male and 2 females). Analysis of urine sediment microscopy (RBC/HPF) revealed, 1-2 RBCs/HPF among 10(6.5%) diabetic participants; 3-4 RBCs/HPF among 15(9.8%) diabetic participants; 5-6 RBCs/HPF in 1(0.65%) diabetic participant;

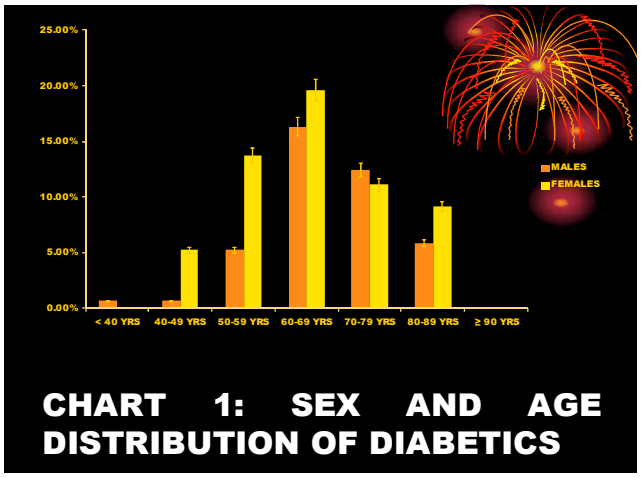

CHART 1: SEX AND AGE DISTRIBUTION OF DIABETICS

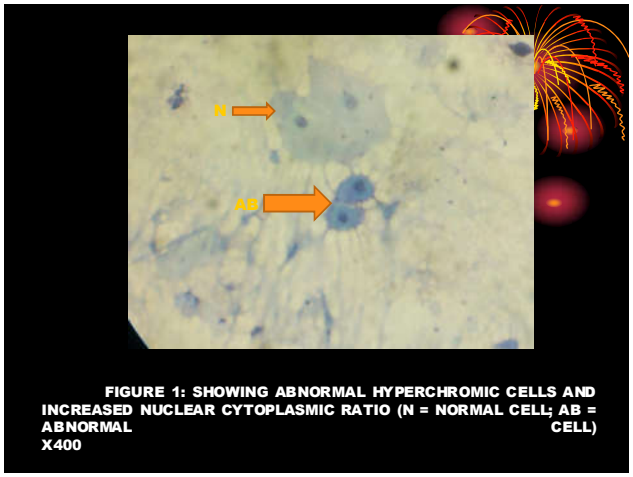

FIGURE 1: SHOWING ABNORMAL HYPERCHROMIC CELLS AND INCREASED NUCLEAR CYTOPLASMIC RATIO (N = NORMAL CELL; AB = ABNORMAL CELL) X400

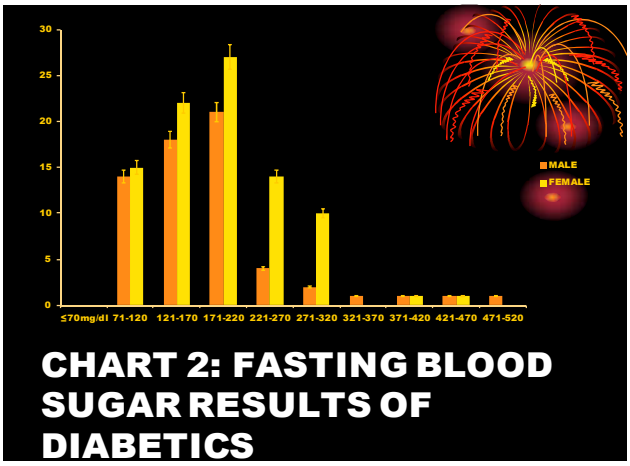

CHART 2: FASTING BLOOD SUGAR RESULTS OF DIABETICS

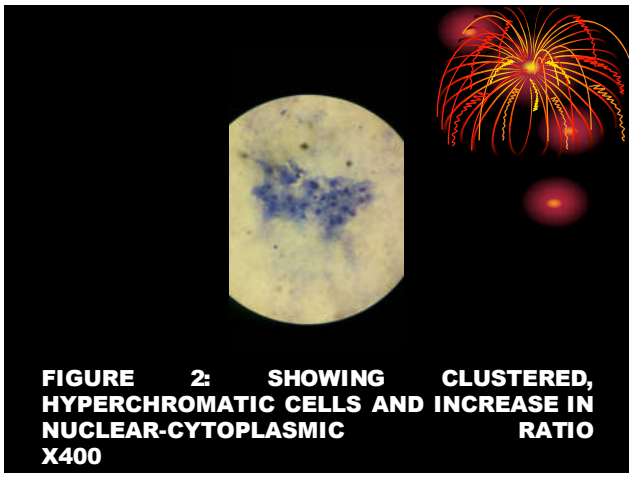

FIGURE 2: SHOWING CLUSTERED, HYPERCHROMATIC CELLS AND INCREASE IN NUCLEAR-CYTOPLASMIC RATIO X400

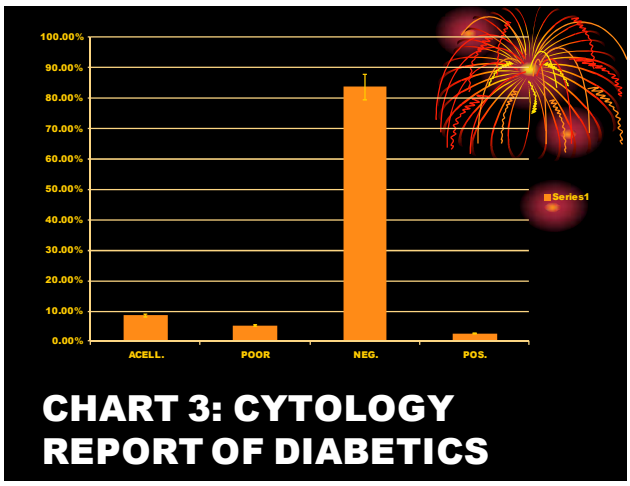

CHART 3: CYTOLOGY REPORT OF DIABETICS

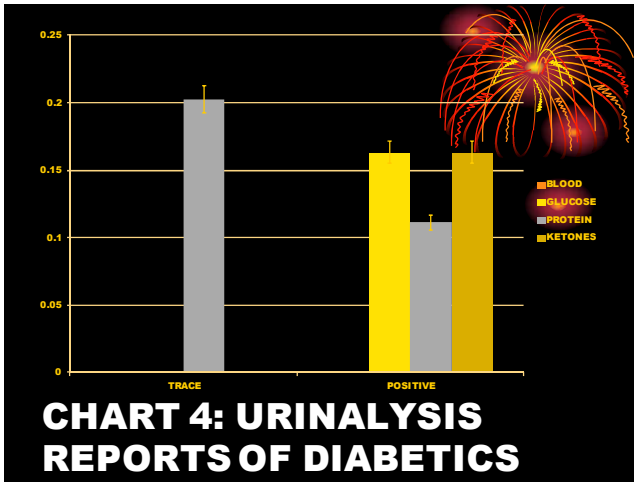

CHART 4: URINALYSIS REPORTS OF DIABETICS

7-8 RBCs/HPF in 1(0.65%) diabetic participant; 9-10 RBCs/HPF in 0 diabetic participant; ≥10 RBCs/HPF in 1(0.65%) diabetic participant. The abnormal urothelial cells showed few clusters and individual cells with high nucleus: cytoplasmic ratio and irregular nucleus . Others showed large irregular hyperchromatic nuclei. The cytology reports of the controls had no abnormal urothelial cells.

Relative Risk (RR) =

Risk in exposed=0.0303$_{=}\infty$
Risk of non exposed0.00
RR > 1

Attributable Risk= 0.0303 – 0.00= 1
0.0303

AR in percent = 100

Comparing the cytology reports of the Diabetics and the controls

The calculated X^2= 3.98 at df = 1

The X^2 analysis of the control and test results showed that P < 0.05 hence there was significant difference.

DISCUSSION

A total of 165 diagnosed diabetes mellitus patients and 140 non diabetic control participants were used in this research. Out of the 165 Diabetes Mellitus participants, 12(7.27%) were disqualified due to inappropriate filling of questionnaire. Among the remaining 153, 63(41.18%) were male while 90(58.82%) were female.

This suggest that females are more health conscious compare to male since the relative percentage of females in most age groups were greater than that of males. The female preponderance (58.82%) observed is striking and does not reflect the pattern observed in studies involving diabetic patients outside the tertiary health centres in Nigeria where the male:female ratio is close to 1:1 (Okoro, 2002). This figure may be due to the pattern of health care financing in the country. Health care is largely financed by the individual patients (out-of-pocket). Women are more likely to be supported by relations and loved ones with financial assistance towards hospital visit than male patients (Atkins, 2005). The prevalence of type 2 diabetes mellitus is higher among women than men (Rosenbloom, 1999). From the sex-age distribution, it showed that most diabetics (35.95%) were between the ages of 60-69 years and most (99.35%) were type 2 diabetes mellitus hence juvenile onset diabetes mellitus is very rare (0.65%) in our area. Juvenile onset diabetes was being rarely reported in Nigeria as underscored by a study that was done in 1990 where only 6% of 756 registered diabetes at diagnosis[21]. Fasting blood sugar results showed that none of the diabetic patients was hypoglycaemic (≤ 70mg/dl) hence risk of hypoglycaemic coma is highly minimal in our area. 18.95% (9.15% male and 9.80% female) had their fasting blood between normal reference ranges of (71-120mg/dl), the remaining percentage 81.05% had their fasting blood sugar level above 120mg/dl. This suggested that the blood sugar level of diabetes mellitus patients in our area is poorly controlled. Majority (31.37%) had their fasting blood sugar as 171-220mg/dl. A previous study from Nigeria has also documented poor glycaemic control among diabetic patients (Rotimi *et al*., 2004).

Financial constraints is one of the key factors as most patients have to pay out-of-pocket for their drugs and for blood glucose tests and at a price which has been found to be much more higher than the cost of these drugs in other parts of the world (The diabetes declaration and strategy for Africa). Years of post diagnosis showed that majority 39.87% (15.03% male, 24.87% female) were post diagnosed between 1-5 years. After 6-10 years (32.68%), there was a drastic decline showed that survival rate of diabetes in our region is poor since only 11-15 years (14.38%), 16-20 years (6.54%), 21-25 years (1.96%) which was lowest. These low survival rate could be attributed to the poor control of blood sugar level as was seen in this research and in previous research. Urine cytology results for diabetes showed that 13(8.50%) slides were acellular, 8(5.20%) were poorly stained. Excluding them, we had 132(86.27%) slides. Out of the 132 slides, 128(96.97%) were negative that is, normal urothelial cells whereas 4(3.03%) were positive i.e abnormal urothelial cells related to malignancy. So there is low prevalence of urothelial malignancy (bladder cancer) in our area as compared to publications from other parts of the world. In 2006, Larrsson, *et al;* concluded that there is 40% higher risk of bladder cancer in individual with diabetes than individual without diabetes (Larsson, 2016). In 2012, Zhaowei *et al*; also concluded that there is 29% increase incidence of bladder cancer in diabetes mellitus (Zhaowei, 2012).

Conclusion

The prevalence of urothelial cell malignancy in our region is 3.03%. There is poor control of diabetes mellitus in our region and also low survival rate among diabetes patients which is optimal at 1-5 years and not more than 25 years.

Recommendation

Advanced diagnostic techniques such as immunocytochemistry and cytogenetics should be carried out on cytological examination of urothelial cells in Diabetes Mellitus patients. Careful research should be carried out to find out the actual cause of poor control of sugar in diabetics in our region and how to improve on it. We suggest that more awareness be created through public lectures, information dissemination through diabetic groups among patients to help improve on the control of diabetes in our region as this will go a long way to increase post diagnosis survival rate.

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