

1. Introduction

Albumin is the most abundant plasma protein (usual concentration 36-50 g/l). It's derived from the liver, and the increase in its synthetic rate is highly responsive to any change in requirements consequent upon loss of circulating albumin. It has a relatively long half-life of 2-3 weeks.(Peter and Sally, 1998)

1.1 Albumin function

Albumin is the most abundant plasma protein (usual concentration 36-50 g/l). It's derived from the liver, and the increase in its synthetic rate is highly responsive to any change in requirements consequent upon loss of circulating albumin. It has a relatively long half-life of 2-3 weeks. Its main functions are to maintain colloid osmotic pressure within the intravascular compartment, and as transporter for ion, water insoluble substances such as lipids and non-esterified fatty acids, hormones and drugs. Catabolism of albumin takes place predominately in the liver and the kidney (Peter and Sally, 1998)

1.2 Mechanisms of urinary protein excretion

Under physiological conditions normal urine contains no more than one millionth of the 12 600 g of protein filtrated daily by the glomeruli. This reflects the efficiency of the glomerulus as a sieve, and the reabsorptive capacity of the tubular cells. Perhaps no more than 60 % of urinary protein excretion is normally derived from the glomerular ultra-filtrate of the plasma. The remainder produced by the kidney and lower urinary tract. Glomerular and to lesser extent tubular protein handling are the most important determinant of abnormal patterns of protein excretion (Peter and Sally, 1998).

The glomerular filtrate is an ultra-filtrate of the plasma; (similar composition to plasma except that it is almost free of proteins). This is because the endothelium provides a barrier to red and white blood cells, and the basement membrane although permeable to water, and low molecular weight substances .is largely impermeable to macromolecule. The impermeability is related to both molecular size and electrical charge. Proteins of molecular weights lower than that of albumin (68KDs) are filterable; negatively charged molecules are less easily filtrated than those bearing al positive charges (William and Stephem, 2007), so Low proteins molecular weight (less than 25000 kd) are extensively filtered by the glomeruli, taken up by the tubules, and subsequently handled by proximal tubular degradation (Robert, 2007).

Individual characteristics of plasma proteins (plasma concentration, size, charge, configuration and rigidity) and the integrity of the golomerular filtration barrier also determine the composition of the ultra-filtrate (Peter and Sally, 1998).

Both diffusion and filtration (bulk flow) contribute to transglomerular protein passage. The selectivity index is based on the fractional excretion of two molecules of different size and charge, such as albumin and IgG. (Vito, 1997)

Factors such as non-enzymatic glycation (*vide infra*) may alter the charges of proteins and of the filtration barrier, thereby modifying this process. Molecular heterogeneity of individual proteins may also alter glomerular handling in healthy subjects. For example there is evidence that excessive binding of non-estrified Fatty acids to albumin leads to distinctive changes in conformation, size, charge and ligand reactivity, with subsequent increased excretion and different chromatographic patterns of albuminuria (Peter and Sally, 1998).

It does appear that proteins may compete for uptake, with increased filtration of either low or high molecular weight proteins leading to increased urinary excretion of other proteins (Peter and Sally, 1998).

Following reabsorption, partial intracellular hydrolysis to amino acids and then secretions into the circulation takes place. Under certain condition, transtubular transporter of intact protein may occur. Fractional reabsorption of low molecular weight proteins in healthy individuals have been estimated at 99.97% whereas that of albumin varies between 92 % and 99% (Peter and Sally, 1998).

1.3 Microalbuminuria

The term microalbuminuria refers to the presence of a relatively small quantity of protein in the urine. The term was coined by Viberti in 1982 to describe subclinical increase in urinary albumin excretion (UAE) that is below the detection limit of dye-binding assays which are used in a dipstick analysis (Carl and Vijay, 2011).

According to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KNF-KDOQI) guidelines MAU is defined as urine albumin excretion (UAE) between 30-300 mg/day if measured in 24 h urine collection, or 30-300mg/g, if measured by the preferred method of spot albumin to creatinine (Jhon, 2011).

Microalbuminuria is a relatively common finding in the general population, with a prevalence rate of around 7 %. The level of urinary albumin excretion is a powerful predictor of both all-cause and CV mortality in the general population, even in the absence of diabetes. Community based- surveys have shown that the prevalence of MAU for people with diabetes ranges between 16-28 %. It appears that both the absolute level and the rate of progression of urinary albumin excretion are independent predictors of all- cause mortality and renal and CV events in individuals with diabetes (Richard, 2012).

1.4 Microalbuminuria as a risk factor for renal dysfunction

The development of sensitive immunoassays for urine albumin and their application to diabetic patients revealed that patients with albuminuria below the detection limit of chemical urine protein methods, now described as MAU, were at increased risk of later developing overt proteinuria and renal failure (Peter, 2008).

Early identification of diabetic patients with MAU allowed aggressive treatment to improve glycemic control, lower blood pressure and serum lipids, and has been shown to improve the outcome. More recently, large scale studies have shown that screening for MAU in non-diabetic populations identifies patients at increased cardiovascular risk. Furthermore, MAU has been found to be a predictor of outcome in critically ill patients following insults such as major surgery, trauma or sepsis. The pathogenetic link between MAU and outcome in such apparently diverse groups of patients appears to be that MAU reflects the systemic microvascular endothelial dysfunction that, in various forms, is common to all these conditions. (Peter, 2008)

1.5 Measurement and expression of microalbuminuria

Urine micro albumin measurement is important in the management of patients with diabetic mellitus, who are at serious risk for developing nephropathy over their lifetime (Kara, 2013). Quantitative albumin-specific immunoassays, usually using nephelometry or immunoturbidimetry are widely used for 24 h urine collection. 30 to 300 mg of albumin is diagnostic of MAU, a 24 h urine collection is preferred but a random urine sample that uses ratio of albumin to creatinine can also be used. An albumin to creatinine ratio of $>30\text{mg/g}$ is diagnostic of MAU (Kara, 2013).

1.5.1. Radioimmunoassay

Most RIA are saturation assays performed in liquid phase with an excess of an antigen being added, although solid-phase assays have been described. Separation of bound and free albumin may be by precipitation with polyethylene glycol or a second antibody. Several commercially produced RIA kits are available. The sensitivity of an albumin RIA is generally $<1\text{mg/l}$ (Peter and Sally, 1998).

1.5.2 Radial immunodiffusion

In this technique antigen-antibody reaction takes place in the antibody-containing well. The antigen travels through the gel to reach equilibrium, at which point antigen-antibody complexes precipitate when antibody is present in excess. The complexes are then stained and the diameter of antigen-antibody ring measured manually. (Peter and Sally, 1998)

1.5.3 Immunoturbidimetry

This is a kinetic assay where by the rate of formation of antigen-antibody complex in solution and in the presence of antibody excess is measured by changes in absorbance of transmitted light at 340nm. Sensitivity is around 2- 4mg/l (Peter and Sally, 1998).

1.5.4 Laser immunonephlometry

Laser immunonephelometry quantifies large scattering caused by antigen-antibody complexes precipitated in a liquid phase. Several methods have been described. Sensitivity is generally less than RIA, but accuracy and precision are similar (Peter and Sally, 1998).

1.5.5. Enzyme linked- immunosorbant assay

An early publication of this technique described in 'three-site' assay, with the first anti-human albumin antibody being immobilized on a solid phase. After added has bound to this first antibody, the second antibody, and goat anti-human albumin is added, a third anti-goat antibody conjugated to an enzyme label is then added. Subsequent publications have detailed a simplified 'two-site' immunoassay, whereby bound complexes are detected and quantified by addition of a second antibody conjugated to an enzyme label. Other competitive variations of this technique, in which standard or sample are added at the same time as the second antibody, have been described. Most conjugates contain horse-radish peroxidase which, on addition of o-phenylenediamine and hydrogen peroxide, generates a quantifiable color reaction (Peter and Sally, 1998).

1.5.6 Non- immunological methods

Because of the complexities of the immunological methods, other techniques of detecting low concentrations of albumin in the urine have been sought. These however measure total protein rather than albumin. A colorimetric method using pyrogallol red-molybdate has been described, based on the shift in the absorption spectrum of the pyrogallolred-molybdate complex when protein is bound. The method has a range of 10-1000mg/l and is simple and easy to perform. a comparison of a modification of this method , with a nephelometric assay specific for albumin showed that all samples with a urinary protein concentration greater than 60mg/l by the pyrogallol red method had an albumin concentration >30 mg/l (Peter and Sally, 1998)

1.6 Risk factors of Microalbuminuria

1.6.1 Cardiovascular risk

MAU is a marker for generalized vascular endothelial dysfunction (Peter Gosling, 2008), numerous clinical studies on non-diabetic populations have found an association between microalbuminuria and cardiovascular risk factors, target organ damage and the presence of

cardiovascular disease in large population studies, urine albumin was significantly associated with C-reactive protein (CRP) concentrations (using highly sensitive assays) , which is consistent with the view that MAU reflect systemic endothelial dysfunction mediated by inflammatory process (Peter, 2008).

Most studies have found a positive correlation between MAU and left ventricular mass, however it remains to be shown whether MAU is an independent risk factor for cardiovascular disease, or is linked by association with other factors such as obesity, ethnicity, insulin resistance, lipids and in particular blood pressure (Carl and Vijay, 2011)

Currently, MAU can be considered not only as risk factors for progressive renal damage, but as providing an integrated assessment of long term damage to the cardiovascular system, and as a result are being increasingly used in cardiovascular risk-assessment clinics. What remains to be shown is whether targeted treatment of MAU reduces cardiovascular morbidity and mortality. However, there is general consensus that evaluation of microalbuminuria is useful for the assessment of overall cardiovascular risk in hypertension, since albumin excretion measurement appears to be a cost-effective way to identify patients at higher risk for whom additional preventative and therapeutic measures are advisable (Peter, 2008).

1.6.2 Smoking

Several studies have suggested that cigarette smoking is a risk factor associated with MAU, both in type 1 and type-2 diabetes, there is some evidence that smoking could exacerbate endothelial changes and damage, and therefore induce MAU. (Carl E Mogensen, 2012)

There may be several mechanisms by which smoking may affect chronic kidney disease (CKD), chronic smoking could lead to micro vascular atherosclerotic disease which could potentially be an initiating etiology of nephrosclerosis, and this may accelerate progression of pre-existing CKD. Micro vascular disease could lead to hypertension, which can further hasten CKD progression .renal susceptibility genes as well as gen-environment interactions, may differentially affect the nephrotoxic effect of smoking. There are more than 4000 chemicals in the form of particles or gases found in cigarette smoke, and many of these could be responsible for smoking's nephrotoxic effects (Jhon, 2011).

There are also data indicating that cigarette smoking contribute to the development of diabetic nephropathy. cigarette smoking increase the urinary albumin excretion (UAE) in patients with type 1 diabetes and also in type 2 , in addition smoking cessation is associated with reduced UAE in patients with type 1 diabetes ,also some data indicates that cigarette smoking predicts faster progression of type 2 established diabetic nephropathy despite angiotensin-converting enzyme inhibition. However as to whether smoking has a significant

impact on renal functional deterioration in type 1 diabetic patients is somewhat controversial (Agens, 2007).

Chase et al looked at the effects of cigarette smoking on diabetic renal and retinal complication in 359 type 1 diabetic patients. The prevalence of increased albumin excretion rate was 2.8 times higher in smokers than non-smokers, even after correction for glycohaemoglobin level and duration of diabetes; smoking was a significant factor in a logistic regression model for albuminuria. Significant improvement was also seen when subjects ceased smoking (Chauhirun*et al.*,) also showed that type 2 diabetic patients who smoked had a faster decline of a renal function and increased urinary albumin excretion than non-smokers, despite blood pressure control and use of ACE-inhibitors ,they also found that cessation of smoking ameliorates the progressive renal injury caused by continued smoking. This is an addition to the well-established effects of smoking on cardiovascular and pulmonary disease. It's thus important that every patient be made aware of the importance of quitting this habit (Robert, 2007).

1.6.3 Lipids abnormalities

Lipids abnormalities in hypertensive patients are associated with MAU. In vitro and in vivo animal and human studies indicate that hyperlipidemia can directly and indirectly contribute to the pathophysiology of micro vascular and macro vascular renal injury. Glomerular injury caused by oxidized lipids and elevated fatty acids lead to MAU and possibly potentiates excessive protein losses. elevation in urinary albumin excretion are associated with elevations in serum triglycerides, elevated low-density lipoprotein cholesterol, and low high-density lipoprotein cholesterol levels although the specific pathogenesis of hyperlipidemia in individual with MAU and proteinuria is currently not well understood, increased lipids intake as well as increased compensatory hepatic lipoprotein and lipid synthesis in response to increased urinary protein losses may figure prominently (Sunita and Eddie, 2004). So the explanation for the association between MAU and hyperlipidemia is that hyperlipidemia causes renal damage and the increase in urinary excretion of albumin. Hyperlipidemia is a very well-known independent risk factor for atherosclerosis and cardiovascular disease. Many authors now believe that lipid abnormalities may contribute to renal damage by a mechanism analogous to atherogenesis. This hypothesis would require that increased levels of lipoproteins accelerate generalized atherosclerosis as well as intrarenal micro- and macro vascular disease, resulting in MAU. Recent studies have shown that hyperlipidemia may play a role in the progression of renal disease, both in experimental nephropathy in animals as well as in patients with diabetic or with non-diabetic nephropathy (Vito *et al.*, 1997).

A substantial body of evidence supports the hypothesis that lipids may be involved in glomerulosclerosis and the progression of renal disease. Cholesterol-enriched diets may cause albuminuria and glomerulosclerosis in different animal species, particularly when combined with hypertension. In addition pharmacologic agents that lower serum lipids ameliorate renal injury in several experimental models of renal disease (Vito *et al.*, 1997).

1.6.4 Hypertension

Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated (Robert, 2007). Hypertension is classified as either primary (essential) hypertension or secondary hypertension; about 90–95% of cases are categorized as "primary hypertension" which means high blood pressure with no obvious underlying medical cause (Peter, 2008). Hypertension plays an important role in renal damage, whether manifested as proteinuria, reduced glomerular filtration rate (GFR) or progression to end-stage kidney disease (ESKD). However the manner by which hypertension injures the kidney and the frequency of renal damage arising from hypertension are not settled (Norman, 2003).

Reports estimate that the prevalence of MAU in patients with essential hypertension ranges from 6% to 40 %. In addition MAU is strongly associated with presence of early end organ damage in non-diabetic hypertensive patients, including left ventricular hypertrophy and increased carotid artery wall thickness. Major Cardio vascular events associated with MAU in hypertensive patients include the development of left ventricular hypertrophy, coronary artery disease (74%), peripheral vascular disease (44%) and fatal and nonfatal coronary events (Vito, 1997).

The increased cardiovascular risk in hypertensive patients with MAU is multifactorial .higher BP levels occur in hypertensive patients with MAU. Moreover, the nocturnal dip in BP is either blunted or absent in hypertensive with MAU and may increase the likelihood of earlier end stage damage (Rober, 2007).

1.6.5 Diabetes mellitus

Diabetes is a group of metabolic diseases characterizes by high blood sugar due to problems in insulin secretion or action (Kara, 2013). About 50 different types of diabetes mellitus have been identified the majority of cases being type1, type2, or gestational diabetes mellitus (Grieset *al.*, 2003).

1.6.5.1 Type1 diabetes mellitus

Type 1 DM is defined as insulinopenic (low-insulin) diabetes resulting from chronic autoimmune beta cell destruction (type 1 diabetes) or idiopathic processes (type 1B diabetes). Marks of beta-cell autoimmunity include islet cell cytoplasmic auto antibodies, insulin autoantibodies, glutamic acid decarboxylase auto antibodies and tyrosine phosphatase IA-2 and IA-2 beta auto antibodies (William and Maria, 2002).

1.6.5.2 Type 2 diabetes mellitus

80% of patients with diabetes mellitus suffers from type 2 DM. the clinical form of this disorder are heterogeneous. The primary underlying lesion is insulin resistance, caused by 'post receptor defect' that has not been fully characterized. in addition to peripheral insulin resistance , patients with type 2 DM also have a diminished or absent first phase of insulin secretion , which can often be detected years before diabetes becomes clinically overt (Battegay and Spinas, 2007).

1.6.5.3 Gestational diabetes

Gestational diabetes mellitus is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It's usually developed because of a faulty physical interaction between the mother and the baby. During the second trimester, somewhere between 24-28 weeks, the placenta begins producing many hormones. One of these hormones may block the action of insulin in mother, thus creating insulin resistance. if the mother cannot produce enough extra insulin to overcome this resistance , her blood sugar will rise , the mother's high blood sugar then stimulate the baby to make more insulin and move more sugar into his or her cells. Causing him or her to gain extra weight, if left unregulated, these changes can have serious harmful effects on both the mother and the child(Bob and Robert, 2002).

1.6.5.4 Pathophysiology

Diabetes mellitus can affect the kidneys in a number of ways leading for example to glomerular disease, obstruction, tubular disease and a predisposition to infection. But it's the diabetic glomerular disease that is the most important. In the early stages of diabetic renal disease, there is a supernormal GFR, reflecting hyper filtration, which may be associated with MAU with adequate treatment, the GFR falls towards normal and MAU disappears. However MAU returns during periods of hyperglycemia. As the disease progresses MAU becomes persistent and occasionally clinical albuminuria (i.e.>250 mg/l) appears during periods of poor glycamic control and after exercise. Diabetic nephropathy can be averted by careful glycamic control using (Peter Gosling, 2008).

The significance of MAU was first described in 1982 by Viberti et al. who showed a 24 fold increased risk of development of clinical proteinuria in type 1 insulin dependent diabetic patients with MAU. This finding was later confirmed by other medium to long term longitudinal studies, not only in patients with type 1 diabetes but in type 2 noninsulin dependent diabetes. The prevalence of MAU is approximately 5-20% and 20-40% in the type 1 and type 2 diabetes (Jeannie and Trevisan, 2000).

So MAU is associated with insulin resistance in those with and without diabetes (Stephan and Gian, 2010).

In type 1 diabetes ,diagnosis is usually made early in life and MAU rarely occurs within 5 years of diagnosis , so annual monitoring of urine albumin is generally recommended after 5 years disease duration, patients with type 2 diabetes are usually order at presentation and defining the date of onset is difficult . For this reason, annual monitoring of urine albumin is recommended from the time of diagnosis (Peter, 2008).

Although type 1 diabetic patients risk complications including nephropathy, type 2 diabetes is the most common cause of renal failure throughout the world. Increased urinary albumin excretion occurs in 30-40%of patients with type 2 diabetes and the presence of kidney disease increases the mortality from cardiovascular disease. MAU is independent cardiovascular risk factors for these patients, probably because the increased levels of urinary albumin excretion represent more generalized vascular damage than renal micro vascular injury alone. Once MAU or albuminuria is present, control of intraglomerular pressure using ACE inhibitors can delay the onset of end-stage renal failure. Aggressive management of cardiovascular risk factors also slows the progression of nephropathy and prevents cardiovascular events in both type 1 and type 2 diabetes (Peter, 2008).

So urine micro albumin measurement is important in the management of patients with diabetes mellitus, who are at a serious risk for developing nephropathy, over their lifetime. In the early stages of nephropathy, there is a renal hypertrophy, hyper function, and increased thickness of the glomerular and tubular basement membrane. In this early stage, there are no overt signs of renal dysfunction. in the next 7 to 10 years , there is progression to glomeulusclerosis , with increased glomerular capillary permeability , this permeability allow small (micro) amounts of albumin to pass into the urine , if detected in this early phase , rigid glucose control , along with treatment to prevent hypertension , can be instituted and progression to kidney failure prevented (Kara, 2013).

1.7 The effect of smoking in renal cells

The association between smoking and renal damage in type 2 diabetes patients has been confirmed in different studies in different countries and nations. In 2006 in United Kingdom they studied Risk Factors for Renal Dysfunction in Type 2 Diabetes Of 5,102 U.K. Prospective Diabetes Study (UKPDS) participants, prospective analyses were undertaken in those without albuminuria or with normal plasma creatinine at diagnosis. Stepwise proportional hazards multivariate regression was used to assess association of putative baseline risk factors with subsequent development of albuminuria or renal impairment. Over a median of 15 years of follow-up 1,544 of 4,031 patients developed albuminuria and 1,449 of 5,032 developed renal impairment. Of 4,006 patients with the requisite data for both outcomes, 1,534 developed albuminuria and 1,132 developed renal impairment (Ravietal., 2006). So smoking is a part of the risk factor pattern related to microalbuminuria in type 2 diabetes (Owing, 2005).

In June 15, 2005 in Australia they studied Effects of smoking on renal function in patients with type 1 and type 2 diabetes mellitus on 185 patients with type 1 or 2 diabetes mellitus and with or without signs of overt renal disease for at least 3 years, median 5.1 years. Cases were patients who were smoking at the time the survey was started. Controls were patients who had never smoked. (GFR) was estimated using the MDRD formula. They found out that smokers were younger, $P < 0.01$), and had a lower GFR than non-smokers $P < 0.05$). Mean GFR remained constant during follow-up in non-smokers), but decreased significantly in smokers $P < 0.0001$). (Stephanetal., 2005), So they came into a conclusion that Cigarette smoking is associated with a decrease in GFR in diabetic patients with normal or near-normal renal function (Venkat, 2006) in 2006, in Italy they studied the relationship between cigarette smoking and GFR in a large cross-sectional study carried out in male subjects with type 2 diabetes. 158 current smokers and 158 never smokers with type 2 diabetes were consecutively recruited. They found out that the proportion of patients affected by low GFR was significantly higher in current smokers ($P = 0.03$). The adjusted risk (odds ratio [OR]) of low GFR in current smokers was, ($P = 0.02$) and markedly higher in patients from the first tertile of disease duration ($P = 0.02$). So they came out into a conclusion that In a large population of male patients with type 2 diabetes, the risk of low GFR is markedly enhanced by smoking. (Salvatore *etal.*, 2006) In 2007 in Japan they studied the Risk Imparted by Various Parameters of Smoking in Japanese Men with Type 2 Diabetes on Their Development of Microalbuminuria. Data from 357 normoalbuminuric male patients with type 2 diabetes who had been followed for at least 3 years. 179 of their 357 patients were

classified as current smokers and 74 as ex-smokers. During the mean follow-up period of 106 patients (never smokers/ex-smokers/current smokers: developed microalbuminuria, suggesting a crude incidence of 52.5/1,000 patient-years. Kaplan-Meier analysis revealed a difference in the incidence of microalbuminuria among never smokers, ex-smokers, and current smokers, with that between current smokers and never smokers being statistically significant by log-rank testing. Differences between ex-smokers and never smokers or between current smokers and never smokers were still statistically significant. All quantitative parameters determined, the number of cigarettes smoked per day (1.02 cigarettes/day), duration of smoking (1.02 per year), and pack-years smoked (1.01 per pack-year) were also significant. So they came out into a conclusion that smoking, both past and current, is a dose-dependent risk factor for the development of microalbuminuria in type 2 diabetic patients. (Kazumi *etal.*, 2007) In February 2007 in Japan they studied the association of smoking with urinary albumin excretion: an evaluation of premenopausal patients with type 2 diabetes mellitus. The study consisted of 20 premenopausal Japanese patients with type 2 diabetes mellitus in the current-smokers group (age, 45 ± 4 years). The control group consisted of 35 age-matched never-smoker patients (age, 45 ± 5 years). Urinary albumin excretion also was higher in the current-smokers group than in the never-smokers group ($P < .0001$). Multivariate logistic analysis revealed that urinary albumin excretion is independently associated with current smoking in Japanese premenopausal with type 2 diabetes mellitus ($P < .01$). The results of this study show that current smoking is associated with an increased level of urinary albumin excretion, suggesting that smoking was a risk factor in the development of increased urinary albumin excretion in these patients (Futoshi *et al.*, 2007).

1.8 Rationale

Smoking has become a major health problem in the world. It leads to many diseases such as heart disease, cancers and renal diseases. It has been recognized as a risk factor for diabetes, and Accelerate the progression of end stage renal disease (ESRD). This becomes a major Health problem in Sudan. This study will be done todetermine the association between smoking and progression of MAU among Sudanese adults; smokers with/without type 2 DM and non-smokers with type 2 DM. To our knowledge up to now research has been occurred to know the association between smoking and the progression of MAU, but not yet in our country.

1.9 Objectives

1.9.1 General objectives

To evaluate the microalbuminuria among Sudanese adult's smokerswith/without type 2 DM and non-smokers with type 2 DM

1.9.2 Specific objectives

1. To estimate MAU in smokers with type2 DM, non-smokers with type 2 DM and apparently healthy control subjects.
2. To compare mean concentration of MAU and MCR in study groups.

2.1 Materials

Study approach

2.1.1. Study area

The study carried out in Military hospital of Omdurman and Omdurman teaching hospital in Khartoum state during February 2015 to May 2015.

2.1.2 Study duration

The period from JAN 2015 to JUN 2015

2.1.3. Study design

Analytical case control hospital based study.

2.1.4. Study variables

Gender (males)

Age (measure by year)

2.1.5. Inclusion and exclusion criteria

2.1.5.1. Inclusion criteria

Adult males been diagnosed with type 2 diabetes mellitus, with normal renal, cardiac, lipids profile, HBA1c, and blood pressure, who do not smoke (appendix I).

Adult males been diagnosed with type 2 diabetes mellitus , with normal renal, cardiac, lipids profile, HBA1c, and blood pressure . Those are chronic smokers from 2 years or more, (See appendix I), apparently healthy adult males that are chronic smokers from 2 years or more (appendix I).

2.1.5.2 Exclusion criteria

Renal disease, cardiac disease, high blood pressure, hyperlipidemia, uncontrolled diabetes and any other systemic illness have been excluded from this study (See appendix I).

2.1.6 Ethical consideration

The research was granted ethical approval by Sudan University research ethics committee and informed consent was given by each participants.

2.1.7 Data collection

The data was collected by using a direct interviewing questionnaire (see appendix I), weighting scale, blood sample and urine sample.

2.1.8 Sample size

Total of two hundreds subjects were enrolled in this study randomly. (100blood samples: 50 from diabetic smokers and 50 from diabetic non- smokers). (200 urine samples: 50 from healthy non -smokers, 50 from smokers without diabetes, 50 from diabetic non-smokers and 50 from diabetic smoker

2.1.9 Sample collection

Random blood samples were taken from participants. Under a septic condition 2.5 ml venous blood was collected in EDTA container from smoker's diabetic patients and non-smokers diabetic patients.

Spot urine sample were also collected from participants, in a clean dry wide mouth container from smoker with/without diabetic patients, non-smoker diabetic patients and controls (apparently healthy subjects).

2.1.10 Rejection criteria

Hemolysed and icteric samples were rejected.

2.2. Laboratory methods

2.2.1. Method of HBA1c:

HBA1c was done by Nycocard diagnostic medical equipment for quantitative determination (see appendix II).

2.2.2. Method of microalbumin:

Microalbuminuria was done with immunofluorescent method (I- chroma equipment) (see appendix III).

2.2.3. Quality control

For internal quality control a quality control material with nycocard HBA1c specific target values were used to confirm the efficiency of the reagents and the correct performance of the test. (See appendix II).

Wako albumin control was used for microalbumin test. (See appendix III).

2.2.4 Data analysis

SPSS version 16 was used for analysis of data

1. Descriptive
2. Anova
3. Independent T test

3. Results

Two hundreds classified as (50 nonsmokers, 50 smokers, 50 DM nonsmokers and 50 DM smokers), urine samples were collected and analyzed for microalbumin by fluorescence immunoassay and creatinine by Jaffe method and one hundred blood sample for HbA1c by Nycocard device

3.1 Microalbuminuria

- There is a significant increase in microalbuminuria mean between healthy (17 ± 1.8 mg/l) and smokers (19 ± 2.7 mg/l) ($P = 0.000$)
- There is a significant increase difference in the mean of microalbuminuria between diabetic smokers (42 ± 9.93 mg/l) and diabetic non-smokers (36 ± 8.08 mg/l). ($p = 0.000$).

3.2 Microalbumin/creatinine ratio (MCR)

- The significance increase in microalbumin/creatinine ratio mean between group one (16 ± 2.8 mg/g) and group two (18 ± 4.4 mg/g). ($p = 0.018$)
- The significance increase in microalbumin/creatinine ratio between group three (34 ± 5.3 mg/g) and group four (38 ± 5.3 mg/g). ($p = 0.002$).

Table 3.3 mean concentration of MAU and ACR in smokers group versus nonsmokers, results expressed as (Mean±SD) and significant difference considered as p-value ≤0.05.

Parameters	Non-Smokers Mean±SD	Smokers Mean±SD	P-value
Microalbumuria mg/l	17.2±1.8	19.3±2.7	0.000
Albumin/Creatinine Ratio mg/g	16.2±2.8	18.0±4.4	0.018

3.3 Independent T-test for the MAU and MCR for healthy and smokers

Table 3.4 mean concentration of MAU and ACR in diabetic non-smokers group versus diabetic smokers, results expressed as (Mean±SD) and significant difference considered as p-value ≤0.05.

Parameters	DM Non-Smokers Mean±SD	DM Smokers Mean±SD	P-value
Microalbumuria mg/l	35.6±8.08	42.2±9.93	0.000
Albumin/Creatinine Ratio mg/g	34.3±5.30	37.8±5.3	0.002

3.4 Table:-Independent T-test for the MAU and MCR for diabetic non-smokers and diabetic smokers

Descriptive statistics for the mean and SD of the MAU and MCR in all the groups (health, smokers, diabetic non-smokers and diabetic smokers)

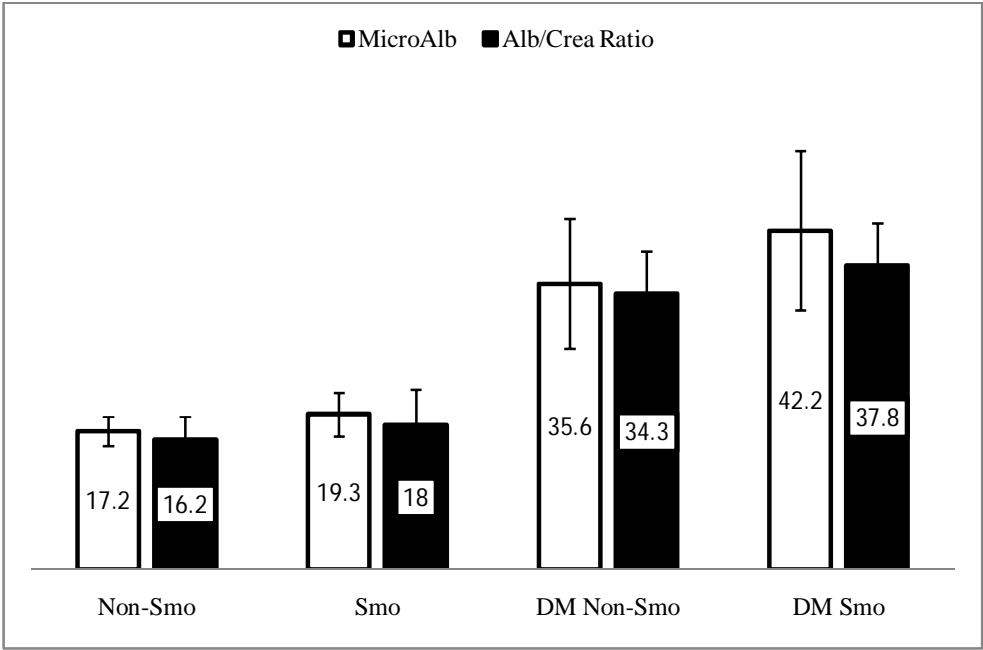


Fig 3.5:- descriptive statistics for the MAU and MCR for all study groups

4.1 Discussion

Smoking has become a major health problem in the world. It leads to many diseases such as heart disease, cancers and renal diseases. It has been recognized as a risk factor for diabetes, and accelerates the progression of end stage renal disease (ESRD). This becomes a major health problem in Sudan. Accordingly the present study conducted to evaluate microalbuminuria among adults healthy and type 2 DM smoker's Sudanese subjects.

The current study shows the relationship between smoking and the development of renal impairment and that is cleared by the significant increase in the MAU and MCR between the health and smokers groups (table 3.3)

This study revealed that higher levels of microalbuminuria associated with smoking in diabetic males. This is after adjustment of HBA1c. There is a significant difference in microalbuminuria when compared the diabetic smokers group with the control group.

The study was made on Sudanese males and found out there is a significant difference in MAU when compared the diabetic smokers with diabetic non-smokers ($P=0.000$) this is in agreement with Futoshietalstudy but it differs in that they made their study on premenopausal patients with type 2 DM and found out Urinary albumin excretion also was higher in the current-smokers group than in the control group ($P < .0001$). Also the effect of smoking on renal function was determined by comparing the MCR of the diabetic group with the control group. There was a significant increase in the MCR between the two groups ($P= 0.002$). I noticed that MY findings in agreement with Stephan etalstudy and Salvatore etal. But on their study they determined the risk on renal function by measuring the GFR and found out that the GFR decreased significantly in smokers than non-smokers.

Also this study can be used as a proof to ensure that diabetes alone can affect the kidney function, this is done easily by comparing the levels of MAU and MCR on diabetic non-smokers and health group. The mean of MAU in the healthy group and diabetic non-smokers was (17.2 ± 1.8 mg/l), (35.6 ± 8.08 mg/l) respectively. The mean of MCR in the healthy group and diabetic non-smokers was (16.2 ± 2.8 mg/g) and (34.3 ± 5.3 mg/g) respectively, so the great difference in the mean of MAU and MCR is a strong proof for the effect of diabetes on the kidneys.

Also the combined effect of smoking and diabetes is greater than that of diabetes alone, and this is cleared by comparing the means of both diabetic non-smokers and diabetic smokers with the healthy group as shown in fig 3.3.

The study proved that smoking and diabetes together is more dangerous on the kidney

The study shows that there is a significant difference in microalbuminuria results among diabetic smokers can be related to the smoking habits among diabetic patients which has an effect on the kidneys and accelerate the progression of kidney diseases, this is in agreement with all previous study

4.2 conclusions

This study concludes that there is a significant increase between elevation of the urinary albumin excretion in type 2 diabetic patients and chronic smoking (more than 10 years) when compared to control group in Khartoum state.

4.3 Recommendations

- 1- Chronic smokers with type 2 diabetes mellitus can be screened for microalbuminuria annually in the future.
- 2- A cohort study is highly recommended to document the relationship between chronic smoking and development of endstage renal disease.

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Appendix I

Questionnaire

Appendix II :

HbA1c method

Appendix III:

Microalbuminuria method