AGE DETERMINED CHANGES IN GLOMERULAR FILTRATION RATE AMONG BLACK ETHNIC NORMOTENSIVE AND HYPERTENSIVE NIGERIANS: A CROSS SECTIONAL STUDY

BY

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COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NIGERIA

DECEMBER, 2025

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A THESIS SUBMITTED TO THE COLLEGE OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY (PhD) DEGREE IN PHYSIOLOGY, UNIVERSITY OF BENIN, NIGERIA

DECEMBER, 2025

CERTIFICATION

This research work titled **'Age Determined Changes in Glomerular Filtration Rate among Black Ethnic Normotensive and Hypertensive Nigerians: A Cross-Sectional Study'** submitted to the College of Postgraduate Studies, University of Benin, Benin City, Edo State, Nigeria, for the award of Doctor of Philosophy is original research carried out by AZUBIKE Chukwuemeka Ogugua in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, under the supervision of Prof. F.O. Agoreyo and Prof O.K. Uche. The research work has not been submitted previously in whole or in part to qualify for any other academic award.

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DEDICATION

I humbly dedicate this Thesis to the memory of my Father, late Chief Edward Samuel Ogugua Azubike, my Mother late Mrs. Felicia Ndirika Azubike (nee Mmuogbogu), my Grandmothers; late Madam Grace Udugwu Mmuogbogu and Madam Eunice Mmaegbunem Azubike.

ACKNOWLEDGEMENT

I must always be humble and grateful in thanking GOD Almighty because His banner over me is the greatest love that is beyond human understanding. THANK YOU YAHWEH, THANK YOU JESUS CHRIST AND THANK YOU HOLY SPIRIT for guiding, providing and protecting me through these years.

I immensely thank my Supervisors; Prof. F.O Agoreyo and Prof. O.K Uche for their tireless efforts at steering this research. Their collaboration and strictness on following accepted standards was excellent. I cannot be grateful enough to this team and neither can words be enough to express the gratitude in my heart but I will continue to show good examples of their tutelage. May God Almighty bless them marvelously.

I thank all the participants who contributed their data to this research because they endured pain and inconveniences no matter how minimal. I sincerely thank Mrs. Sophia Osama from Department of Anatomy who was tireless in guiding the participants on submission of their data on anthropometric measurements and some logistics through the period.

I humbly thank all my Teachers in this Department who have been of immense assistant to me in various ways: Prof LFO Obika who was my Supervisor and Head of Department during my MSc degree period in 1998/2000, Prof AC Ugwu, Prof (Mrs) ADA Ighorodje, Prof KA Onyia (late), Prof AB Ebeigbe (late), who were my lecturers then. I specially thank Prof (Mrs.) MI Ebomoyi who was then Head of Department when I was employed in 2009.

I thank all my colleagues who were of immense encouragement and help from the day I joined this Department to date; Prof EB Ezenwanne, Prof FO Agoreyo, Prof OI Ajayi, Prof OK Uche, Prof A Omorogiuwa, Dr. (Mrs.) MI Omigie, Dr.(Mrs.) BO Eiya, Dr. FO Ebojele, Dr. (Mrs.) RO Aikpitanyi-Iduitua, Dr. OD Fajoriju, Dr. (Mrs.) JO Nzoputam, Dr. OEO. Aloamaka, Dr. EO Aighie, Dr.(Mrs.) PE Akpe, Dr (Mrs.) MO Obayuwana, Miss LA Okoli, Mrs GNJ Azekhumen, Mr. D Edeha, Mr.WJ Silas, Mrs. AZ Aliu and Mr. AA Abayomi.

I thank immensely Prof. VI Iyawe (late) who encouraged and employed me in the Department in 2009 and started supervision of this PhD research. May God Almighty accept the souls of Prof. VI Iyawe and Prof. AB Ebeigbe and Prof KA Onyia into His paradise. May their gentle souls Rest in the perfect peace of our merciful GOD Almighty in the name of Jesus Christ.

I thank my Siblings, my wife (Ifeoma Scholastica) and my children (Chidimma Glory, Nnamdi Emmanuel, Agbomma Bera, Obehi Angel and Chizobam Esther) who have been of steady prayers and anticipation for this victory.

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ABSTRACT

Glomerular filtration rate (GFR) is the volume of non-protein plasma filtered by the glomeruli per unit of time (average of 125 ml/min/1.73 m^2 of body surface area in adults). Age-related physiological changes influence GFR and results in age determined annual rate of Decline (ADARD) of about 1ml/minute/year. The GFR decline varies among populations due to genetic and environmental factors but the extent and pattern of this decline among ethnic black Nigerians had not been characterized. This study evaluated age-determined changes in GFR among adult black Nigerians and compared values in normotensive and hypertensive individuals by using measured creatinine clearance (mCrCl) and GFR estimating equations as Cockroft-Gault equation (CG), modification of diet in renal disease (MDRD) and National Kidney Foundation chronic kidney disease epidemiology collaboration (NKF CKD-EP!). Two hundred and seventy (270) apparently healthy volunteers (18-70 years), were recruited and arranged 30 per grouped (15 males and 15 females) for 9 age groups (18-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-60, 61-65, 66-70 years). Their age, gender, Pulse rates (PR), blood pressure and mean arterial pressure (MAP) were documented. Ten (10) milliliters (ml) of spot urine was obtained for assays of sodium ion and potassium ion by Ion selective electrode. Spectrophotometric methods were used for creatinine (Cr) and albumin (for calculation of mean urine albumin creatinine ratio, mUACR). Ante-cubital venipuncture was done for 10 ml of venous blood (2 ml anti-coagulated and 8ml coagulated for serum extraction). About 4 ml of serum was used for assays of serum Cr and CystatinC (ELISA). Subjects provided 12 hours timed urine for mCrCl in ml/min. The Mean ± Sem of the data were calculated on excel worksheet and further statistics as analysis of variance (ANOVA), Student's t-Test, regression analysis and graphs were done with SPSS-29. The mean GFR in ml/min/1.73 m^2 were; mCrCl (124.86 ± 5.09), CG (85.22 ± 1.69), NKF CKD-EP! Cr (82.95 ± 1.27), NKF CKD-EP! Cystatin C (72.90 ± 3.88), NKFCKD-EP! Cr-cystc (79.62 ± 2.64) and MDRD (93.44 ± 1.01). The ADARD in GFR (in ml/min/yr) were significant ($P<0.01$) for; mCrCl 3.64, CG equation (0.7501), NKF CKD-EP!cr 2021 (0.4398) and MDRD (0.503). Twenty-one percent (21.1%) of the subjects were hypertensive (MAP>100) and 90.5% had mean Urine Albumin Creatinine ratio (mUACR) of 81.12 ± 3.58 (>30 mg/g). The annual rate of increase in UACR was 1.4457 mg/g ($P<0.001$). The GFR was significantly lower in hypertensives ($P<0.05$) while UACR was significantly higher in hypertensives ($P<0.01$). The ADARD in GFR was significant and associated with significant increase in UACR indicating a strong relationship between these CKD. Hypertension and increased mUACR reduced GFR significantly and increased ADARD in GFR. Early on-set of CKD manifested in this population as increase in mUACR before decrease in GFR occurred. Assessment of GFR must include UACR.

CHAPTER ONE

1.1 INTRODUCTION

The kidneys are two reddish brown bean shaped organs in humans (also referred to as renals) which are necessary for formation and excretion of most excess water soluble electrolytes and metabolites in urine (Barrett *et al.*, 2010). Each is located bilaterally in the retroperitoneal spaces. They embryologically originate and develop from the intermediate mesoderm and are fully developed by 32 to 36 weeks of gestation. Each kidney has about one million three hundred thousand nephrons (1.3million) and in addition to the calyces, pelvis, ureters and bladder constitute the renal system (Barrett *et al.*, 2010). Each nephron comprises of the Malpighian corpuscle or glomerulus (the site of fluid filtration) and the tubules (the sites for reabsorption and secretion). Glomerular filtration rate (GFR) is the volume of plasma water (with electrolytes, nutrients and metabolic wastes) filtered by all glomeruli per unit time, usually expressed as ml/min/1.73m^2 (Hall and Hall, 2021).

Glomerular filtration rate (GFR) is a key indicator of adequate renal function (Bertram *et al.*, 2011). Using a suitable exogenous indicator (that is freely filtered, not reabsorbed nor secreted by renal tubules e.g. Inulin as Gold standard) GFR can be measured (mGFR) as the clearance of the substance in urine per unit time (Jamie *et al*., 2006). The average in adult human is about 125ml/min/1.73m^2. It is the most important measurement of kidney function (Jamie *et al*., 2006). The mGFR procedures are near accurate but cumbersome and expensive for easy clinical uses. #Glomerular filtration rate can also be estimated with formula (eGFR) by using single plasma sample of an endogenous metabolite such as creatinine which is more convenient for clinical practice (e.g. Cockcroft and Gault formula, 1976). Age, sex, race and body weight influence the results of GFR and has made it necessary to incorporate these factors most eGFR equations as; (a) Cockroft-Gault (CG), (b) Modification of Diet in Renal Disease (MDRD), (c) CKD-EPI, and (d)

Lund-Malmo-Rev which are serum creatinine based eGFR equations (Levey *et al.,* 2006). There are also currently accepted cystatin C based equations which are; (a) simple cystatin C equation (Sebastjan *et al.,* 2011) and combined creatinine cystatin C (Stevens *et al.,* 2008). Due to diseases, kidney functions can decrease acutely within a few hours or days less 90 days (acute kidney injury, AKI) or over period greater than three months (chronic kidney disease, CKD) both leading renal insufficiency (azotemia or clinical uremia). Age associated gradual decline in GFR among apparently healthy population was first reported by Davies and Shock in 1950. The inulin mGFR studies showed that it occurred mostly in age-group above 30 years (Morrissey and Yango, 2006).

The National Kidney foundation (NKF2002) guidelines (Levey *et al.,* 2006) and Kidney Diseases: Improving Global Outcomes (Stevens *et al.,* 2013) classified five stages of chronic kidney diseases as stages 1 to 5. Assessment of renal function is necessary for clinical purposes, determination of drug dosing, documentation for health insurance, research, grading of chronic kidney disease in the population and enrolment into dialysis and kidney transplantation (Stevens *et al.,* 2013). The National Kidney foundation guidelines for evaluation, classification and staging of CKD (NKF KDOQI., 2002) and Kidney Diseases Improving Global Outcomes (KDIGO, 2012) have classified five stages of chronic kidney disease for understanding of the clinical features, management methods and prognosis of therapy (stages 1 to 5) as noted in table 1.1.

Table 1.1: Stages of Chronic Kidney Disease with associated metabolic changes (NKF K/DOQI, 2002 guidelines, Levy *et al.,* 2003).

Stage	GFR (ml/min/1.73m^2)	Description	Metabolic Consequences
1	≥90	Kidney with normal or ↑ GFR	Normal or may have DM, HTN, microalbuminuria etc.
2	60 - 89	Kidney damage with mild ↓ in GFR	↑PTH , onset of renal bone disease
3	30 – 59	Moderate ↓ in GFR	↓ in Ca^{2+} absorption. ↓ lipoprotein lipase activity, Onset of malnutrition, LVH, Anaemia
4	15 – 29	Severe ↓ in GFR	↑Triglycerides, ↑PO4, ↑H+, ↑K+
5	<15	Kidney failure or End Stage Kidney Failure	Azotemia or Uremia

Chronic kidney disease is defined as kidney damage or GFR < 60 ml/min/1.73 m^2 observed for a period of 3months (NKF K/DOQI guidelines, 2002). There are kidney pathologic abnormalities or markers urine tests, abnormalities in blood tests or kidney imaging studies (NKF K/DOQI guidelines, 2002).

Urinary markers of kidney disease include: albuminuria measured as urinary albumin: creatinine ratio (ACR > 3 mg/mmol or mg/g), hematuria of presumed or confirmed renal origin (Levy *et al.,* 2003). There are also serum electrolyte abnormalities due to tubular disorders, renal histological abnormalities, structural abnormalities detected by imaging or a history of kidney transplantation (Levy *et al.,* 2003).

The KDIGO guidelines in 2012, modified the five stages by including urinary albumin creatinine ratio for risk stratification of CKD (Stevens *et al.,* 2013). Individuals are classified as G1- G5 based on their GFR and A1 – A3 based on their ACR (Stevens *et al.,* 2013) shown in table 2.

Table 1.2: Classification of CKD using GFR and ACR categories by KDIGO CKD work group (Stevens *et al.,* 2013)

Classification of chronic kidney disease using GFR and ACR categories

GFR and ACR categories and risk of adverse outcomes			ACR categories (mg/mmol), description and range		
			<3 Normal to mildly increased	3–30 Moderately increased	>30 Severely increased
			A1	A2	A3
GFR categories (ml/min/1.73m²), description and range	≥90 Normal and high	G1	No CKD in the absence of markers of kidney damage		
	60–89 Mild reduction related to normal range for a young adult	G2			
	45–59 Mild–moderate reduction	G3a[1]			
	30–44 Moderate–severe reduction	G3b			
	15–29 Severe reduction	G4			
	<15 Kidney failure	G5			

Increasing risk

Increasing risk

[1] Consider using eGFRcystatinC for people with CKD G3aA1 (see recommendations 1.1.14 and 1.1.15)

Abbreviations: ACR, albumin:creatinine ratio; CKD, chronic kidney disease; GFR, glomerular filtration rate

Adapted with permission from Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group (2013) KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney International (Suppl. 3): 1–150

It was reported that after the age of 30-40 years, the GFR declines gradually at the rate of 1ml/min/1.73m^2/year resulting in an inulin clearance of 65ml/min/1.73m^2 at the age of 90 years in apparently healthy Caucasians (Morrissey and Yango, 2006). The rate of age determined GFR decline has not been studied among apparently healthy ethnic black Nigerians in order to compare with reported Caucasian value. There are urine expressed peptidomic biomarkers of kidney origin (e.g. albuminuria, epidermal growth factor, kidney injury molecule -1, monocyte chemo-attractant protein-1 and alpha-1microglobulin in kidney injury) which are important

molecules that may pre-date or predict development of decline in kidney functions (Vittorio *et al*., 2020). Total Antioxidant Capacity and Oxidative Stress appear to have positive and negative effects respectively on kidney aging and progressive deterioration by generation of advanced glycation end products (Martin and Sheaff, 2007 and Lu *et al*, 2004). In our ethnic black population, we are yet to examine the impact of age, peptidomic biomarkers, antioxidant capacity and oxidative stress on mGFR and eGFR.

1.2 STATEMENT OF PROBLEM

There was no obvious pilot, large scale studies or Meta-analysis on age associated changes in GFR among apparently healthy adult Nigerians. Although a previous hospital based study by Azubike and Unuigbe (2012) involved diabetic hypertensive patients in a longitudinal study using only Cockroft-Gault eGFR equation. Renal clearance or GFR studies are usually peculiar or localized for the specific environment (Lesley *et al*., 2008). There is an obvious problem for Nigeria Renal Health Registry, Sports, Insurance, pharmaceutical research, drug studies and prescriptions peculiar to Nigerian environment which this study attempted to solve. This cross-sectional study utilized Crcl mGFR procedure and some eGFR equations as listed above. This study also examined the impact of age, peptidomic biomarkers, antioxidant capacity and oxidative stress on mGFR and eGFR among our ethnic black population which had not been done. This study is likely a pioneering effort.

1.3 JUSTIFICATION OF STUDY

This study is justified because it has generated data on Age Determined Rate of Decline in GFR (in apparently healthy Normotensive and Hypertensive subjects) among ethnic black population of Edo state, which was likely a pioneering study in Nigeria. The study utilized CrCl (as mGFR), eGFR formulas and Urine Albumin Creatinine Ratio (UACR). It also examined the relationship between oxidative stress markers and Na+/K+ ratio on GFR. The results of this study generated data for Nigeria National Renal data Registry and may inspire further studies in this line.

1.4 AIM OF THIS STUDY

The aim of this study is to examine Age Determined Rate of change in mGFR and eGFR among apparently healthy normotensive and hypertensive ethnic black adults of Nigerian origin in a cross-sectional study and compare with standard Caucasian value of 1ml/min/1.73m^2/year (Morrissey and Yango, 2006).

1.5 OBJECTIVES OF THE STUDY

The objectives of the study were to:

1. Determine mean mGFR and mean eGFR (in ml/min/1.73 m^2) of the subjects;
2. Calculate age determined annual rate of decline (ADARD) in mGFR and eGFR (ml/min/year) among the subjects;
3. Determine gender differences in ADARD in Glomerular Filtration Rate (GFR);
4. Suggest preferable mGFR or eGFR as clinical tool;
5. Determine proportion of hypertensive and normotensive subjects and compare GFR values among them;

6. Determine annual rate of increase of urine albumin creatinine : ratio (mUACR) among the subjects;

7. Determine the difference in mUACR among normotensive and hypertensive subjects;

8. Determine the effects of age on; (a) human Urine-Alpha-1 microglobulin (HU -α -1m) and (b) human urine monocyte chemo-attractant protein-1 (UMCP-1) and their effects on ADARD of GFR;

9. Determine the relationship between age, total antioxidant capacity (TAC) and malondialdehyde (MDA) as indicators of oxidative stress and their effects on GFR;

10. Determine relationship between age and urine sodium/potassium ratio (UNa^+/K^+) as a measure of renal tubular function , salt intake and effects on GFR;

1.6 RESEARCH QUESTIONS

1. What is the rate of age determined decline in mGFR and eGFR?
2. Were there significant differences in GFR decline between male and female subjects?
3. Were there significant differences in GFR among the age groups of subjects?
4. Could the various eGFR and mGFR results be compared to identify a preferred sensitive clinical tool?
5. Were there any effects of age on the expression of urine peptidomic biomarkers?
6. Were there effects of urine peptidomic biomarkers expression on eGFR and mGFR?
7. Were there any relationship between age on Total Antioxidant Capacity and Oxidative Stress?

8. Were there effects of Total Antioxidant Capacity and Oxidative Stress on GFR?
9. Were there significant differences in GFR between normotensive and hypertensive subjects?
10. Were there any relationships between Na+/K+ ratio and GFR
11. Were there differences in Urine Na+/K+ ratio between male and female subjects

CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy of Normal Kidneys

There are two multifunctional, bean-shaped, reddish-brown kidney organs found in vertebrates (Lote, 2012). Each is located bilaterally within the right and left retroperitoneal spaces with dimensions of about 10 to 12 centimeters in length and 5.5 to 6.5 centimeters in width among adult humans (Mescher, 2016). A report measured the median renal length as 11.2cm on the left and 10.9cm on the right while the median renal volumes were 146cm^3 and 134cm^3 for the left and right respectively (Emamian *et al*, 1993).

Located on each superior pole of the kidneys are the suprarenal glands (made up of adrenal cortex and adrenal medulla). The right kidney is slightly lower and smaller than the left and slightly more medial than the left (Glodny *et al.*, 2009). The left kidney length covers vertebral bone levels T12 to L3 but the right kidney is slightly lower (Glodny *et al.*, 2009).

Renal parenchyma which is the functional substance of the kidneys are divided into the outer renal cortex and an inner renal medulla (Walter, 2004). The vertical section of the kidney shows eight to eighteen cone shaped renal lobes in which renal cortex surround a portion of medulla called renal pyramid (Walter, 2004). The renal columns are projections of the cortex between the pyramids (Clapp, 2009). The nephrons which are the main urine forming and functional units of the kidneys extend through the cortex and medulla (Glodny *et al.*, 2009).

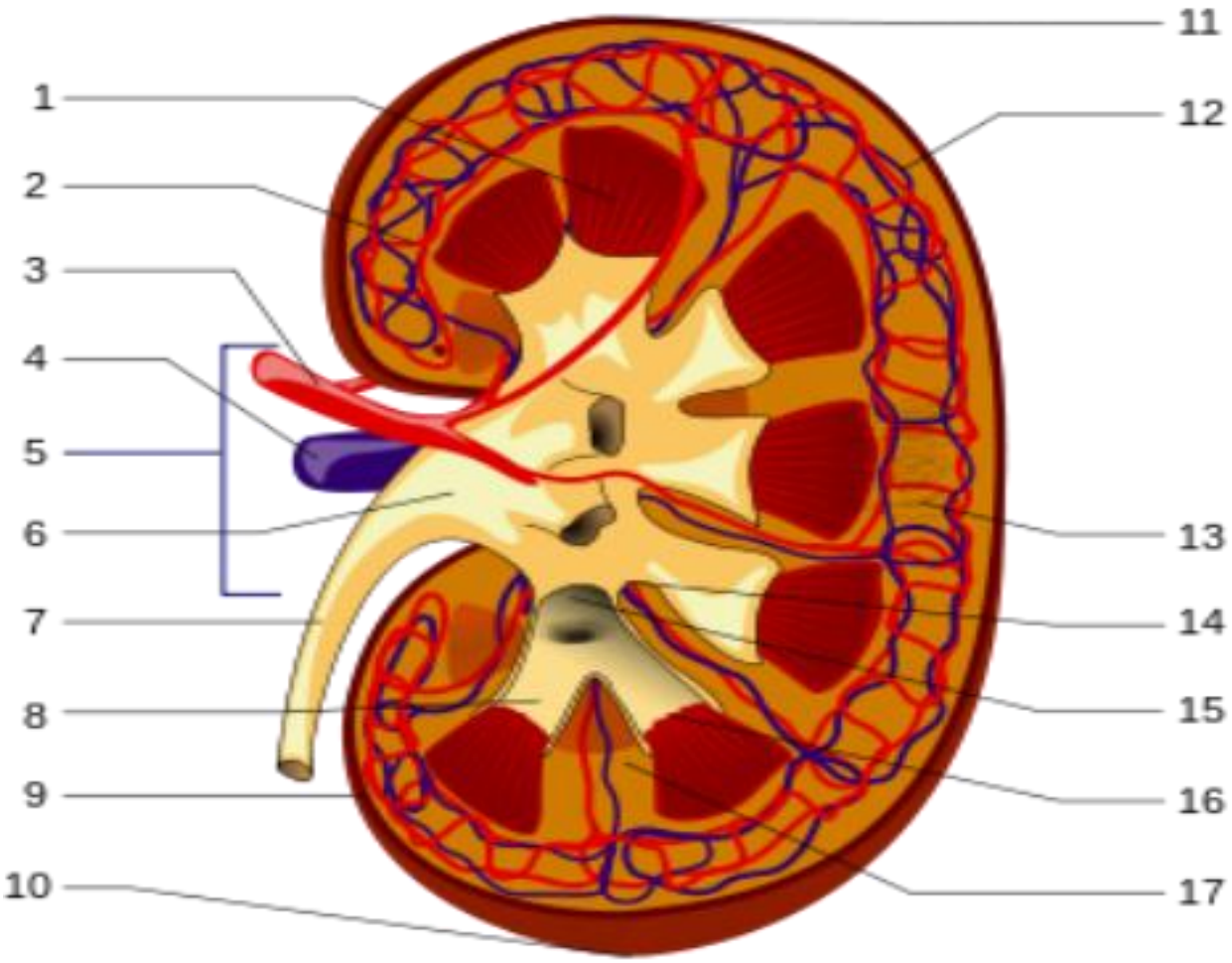

Fig. 2.1: Diagram of a kidney exposing structure of a longitudinal surface as listed below:

(1) Renal pyramid (2) Interlobular artery (3) Renal artery (4) Renal vein (5) Renal hilum

(6) Renal pelvis (7) Ureter (8) Minor calyx (9) Renal capsule (10) Inferior renal capsule

(11) Superior renal capsule (12) Interlobular vein (13) position of a Nephron (14) Renal sinus

(15) minor calyx (16) renal Papilla (17) renal column (Walter, 2004).

Nephron starts as the renal corpuscle (the filtration portion) located in the cortex and continues as the renal tubule which stretches into the renal medullary (Walter, 2004). Collection of renal tubules, called medullary ray, drains into a single collecting duct within the cortex (Clapp, 2009).. The renal papilla is the tip of each pyramid which drains urine into a minor calyx while the minor calyces empty urine into major calyces (Clapp, 2009). The major calyces extend into renal

pelvis which exits the kidney at the renal hilum with the renal vein while the renal artery enters the kidney (Walter, 2004). Each pelvicalyceal segment is continuous with a tube-like ureter that transports urine into the hollow bladder situated in the abdomino-pelvic region (Glodny *et al.*, 2009). The structures in the hilum are surrounded by fat and lymph nodes in the renal sinus which accommodates renal pelvis and calyces but separates these structures from renal medulla (Clapp, 2009).

Normally, the left and right renal arteries originate bilaterally from abdominal aorta to vascularize the left and right kidneys through the renal hilum respectively as the kidneys receive about 20% of cardiac output (Walter, 2004). The branches of each renal artery are about three segmental arteries which further divide into interlobar arteries that penetrate the renal capsule and ramify the renal columns between the renal pyramids as they divide into arcuate arteries which pass between the renal cortex and medulla (Clapp, 2009). Each arcuate artery divides into several interlobular arteries which further supply several afferent arterioles that form and supply the glomerular capillaries (Walter, 2004). A peculiar vascular formation in renal medulla associated with the juxtamedullary nephron is vasa recta which drains excess water to increase the concentration of medullary interstitium (Walter, 2004).

The renal veins drain venous blood from the kidneys into the inferior vena cava (Clapp, 2009). The efferent arterioles channel filtered blood from glomerular capillaries into network of peritubular capillaries and renal venules into interlobular veins which run beside interlobular arteries (Walter, 2004). The interlobular veins drain into the arcuate veins which empty into interlobar veins that form renal veins (Walter, 2004).

The autonomic nervous system innervates the kidneys through the renal plexus which are meshwork of microscopic nerves fibers embedded along renal arteries to ramify each kidney

(Bard *et al*, 2003). The renal plexus contains fibers of sympathetic and parasympathetic nerves (Shrier *et al.,* 1972. Sympathetic stimulation triggers vasoconstriction within the kidneys which reduces renal blood flow. The renal branches of vagus nerves supply parasympathetic fibers for which the function may be vasodilation (Shrier *et al.,* 1972*).* The sensory afferents of the kidneys reaches T10-11 of the spinal cord and renal pain is referred to corresponding dermatome as flank pain (Bard *et al*., 2003).

2.2 Development of the kidneys (Nephrogenesis)

Mammalian kidneys develop from intermediate mesoderm and goes through four successive phases; the archinephros, pronephros, mesonephros and metanephros or the primordial permanent kidney (Bruce, 2004). The archinephros is considered the primitive kidney while the pronephros is the most immature form of kidney (Bruce, 2004).

The pronephros develops from the cervical or cranial region of the intermediate mesoderm of embryo which appears about day 22 of human gestation (Bruce, 2004). The two pronephri are composed of epithelial cells that are arranged in tubules called nephrotomes which join laterally to pronephric duct which are non-excretory therefore nonfunctional in humans (Bruce, 2004).

The mesonephros develops as a consequence of tissue induction caused by cranio-caudal development of pronephric duct (Bruce, 2004). The pronephric duct induces nearby intermediate mesoderm in the thoracolumbar area to transform into epithelial tubules called mesonephric tubules which receives blood supply from a branch of the aorta and transforms into a capillary tuft similar to the glomerulus of definitive nephron (Bruce, 2004). Mesonephric tubule forms a capsule around the capillary tuft which allows for blood filtration and flow of filtrate through the mesonephric tubule that drains into continuation of pronephric duct which has transformed into

mesonephric duct or Wolffian duct (Bruce, 2004). The previous nephrotomes of pronephros degenerate while the mesonephric duct grows caudally to attach to the cloaca (Bruce, 2004).

The Metanephros develops during the fifth week of gestation (Bruce, 2004). The mesonephric duct develops as an outward pouch or the ureteric bud close to its attachment to the cloaca (Bruce, 2004). This bud is also called the metanephrogenic diverticulum which elongates and becomes metanephric duct to become the ureter (Bruce, 2004). The cranial portion extends into the intermediate mesoderm and develops series of branches to form collecting duct system of the kidney in addition to the major and minor calyces and renal pelvis (Bruce, 2004). This branching ureteric bud makes contact with undifferentiated intermediate mesoderm to form metanephrogenic blastema (Bruce, 2004). The signals released from ureteric bud induce the differentiation of metanephrogenic blastema into renal tubules which will grow to make contact with and join connecting tubules of collecting duct system (Bruce, 2004). During the same period, precursors of vascular endothelial cells invade the tips of the renal tubules and differentiate into cells of definitive glomerulus (Bruce, 2004).

During the 32 to 36 weeks of gestation all the branches of ureteric bud and the nephronic units have become formed but they are immature and their maturity continues after birth (Bertram *et al.,* 2011).. At maturity in humans, there are about one million nephrons per kidney ranging about 200,000 to 2.5 million per kidney (Bertram *et al.,* 2011).

After inducing the metanephric mesenchyme the lower portions of the nephric duct start caudal (or downward) migration to connect with the urinary bladder thereby form the ureters which channel urine from kidneys to the bladder for excretion from the fetus into the amniotic sac (Bertram *et al.,* 2011).. As the fetus develops, the body elongates and the kidneys rotate and

migrate upwards within the abdomen while the length of the kidney increases (Bertram *et al.,* 2011).

2.3 Nephron and the Functions

Nephron is the functional unit of the kidney (Guyton and Hall, 2006). This means that each nephron has the minute ability to perform the various functions attributed to the whole kidney (Pocock and Richards, 2006).

These microscopic structural and functional units of the kidney are about 1 to 1.5 million in each healthy adult kidney (Lote, 2012). The component parts are a renal corpuscle (which is a tuft of capillaries also called glomerulus surrounded by Bowmans's capsule) and a renal tubule (which extends from the Bowman's capsule (Lote, 2012). The word renal is an adjectival reference to the kidneys as derived from French or Latin (Cotran *et al.*, 2005).

The major component parts of the nephron are the; glomerulus, Bowman's capsule, proximal convoluted tubule, proximal straight tubule, loop of Henle (which has thin descending loop and thick ascending loop of Henle), distal convoluted tubule, connecting tubule and collecting ducts (Mescher 2003). The nephron segments have varied lengths with different urine concentrating abilities (Lote, 2012). (a) The long juxtamedullary nephrons have their renal corpuscle located in the inner two third of renal cortex close to the cortico-medullary margin with their long renal tubules extending deep into the renal medullary papillae (Lote, 2012). They are fewer than cortical nephrons (ratio of 1:6) but have greater ability for urine concentration (Lote, 2012). (b) The short cortical nephrons have their renal corpuscle located in the outer one third of the cortex with maximum length of loop of Henle located in the outer one third of renal medullar (Barrett *et all.,* 2010).

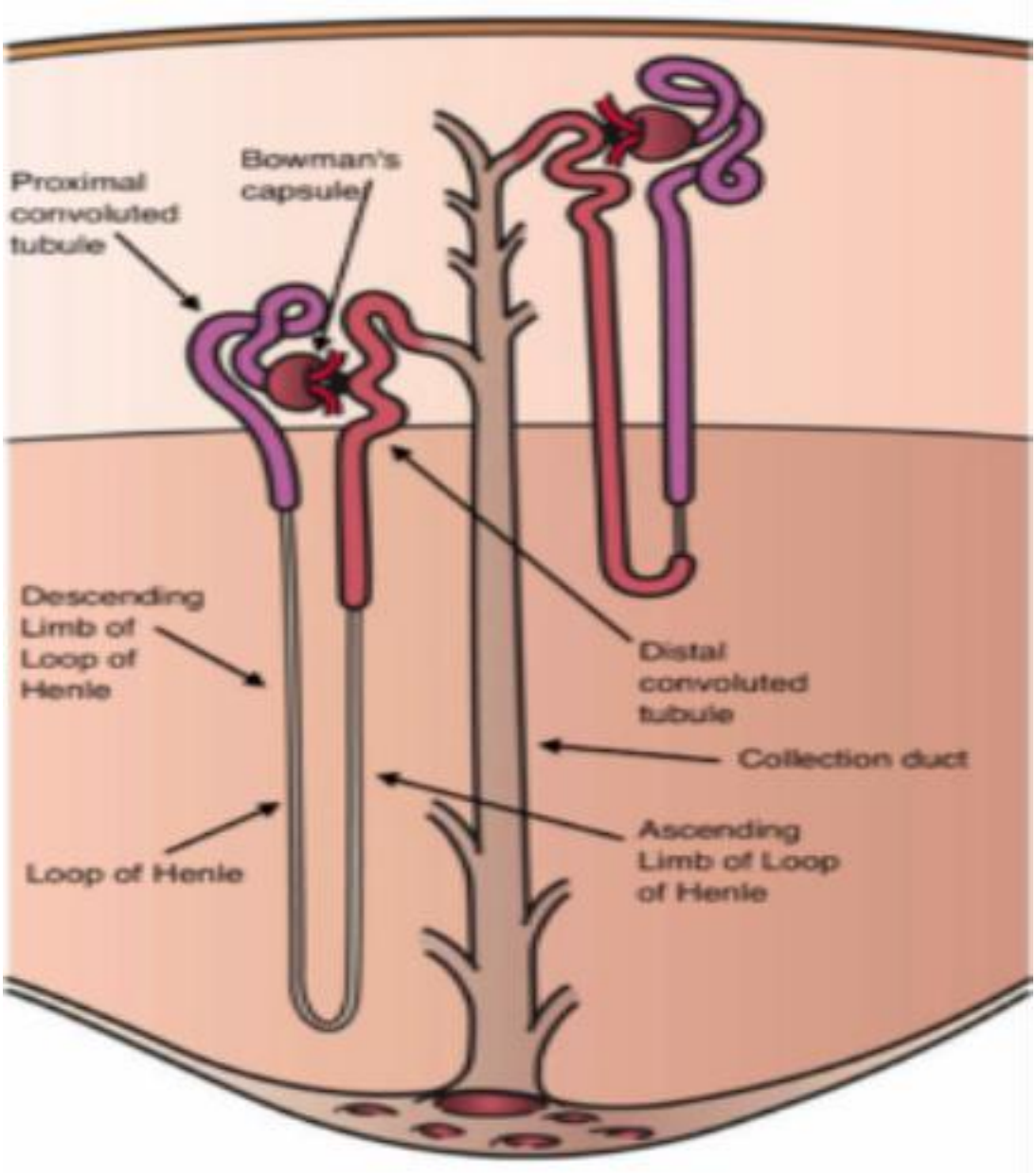

Fig. 2.2: Major component segments of a nephron
The nephron with labeled components parts except the gray connecting tubule located after the (dark red) distal convoluted tubule and before the large (gray) collecting duct.

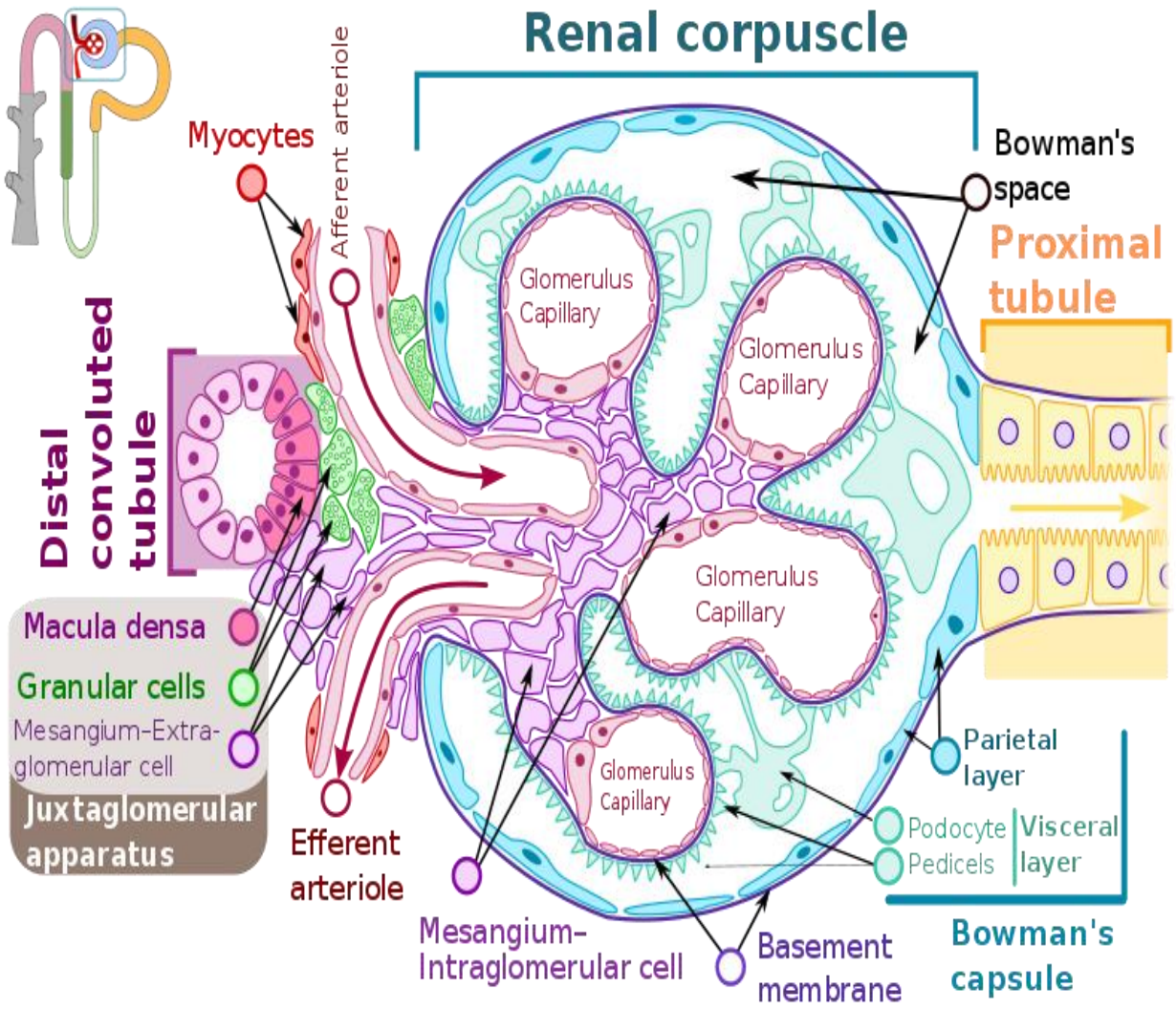

Fig. 2.3: Diagram of Component parts of Glomerulus

With regards to blood supply to the nephron, each efferent arteriole divides into capillary network called glomerulus and supplies arterial blood for filtration to the glomerular capillaries while efferent arteriole drains blood from it (Lote, 2012).. Blood is filtered of plasma through three layers called glomerular filtration barrier (which comprise of fenestrated capillary endothelial cells, its basement membrane and between foot processes or podocytes lining the Bowman's capsule membrane) as shown in fig 3 and 4 (Lote, 2012).. Surrounding the

glomerular capillaries are supporting mesangial cell. The glomerular filtrate collects within Bowman's capsular space before flowing into the proximal renal convoluted tubule (Lote, 2012). The nephron produces urine through four processes; glomerular filtration, tubular reabsorption, tubular secretion and excretion through collecting ducts into the minor calyces (Barrett *et al.,*2010), as shown in fig 4. Filtration is mostly passive but also dependent on intracapillary blood hydrostatic pressure and plasma oncotic pressure (Starling forces or intracapillary flow dynamics, Barrett *et al.,* 2010). About 20% of cardiac output constitute renal blood flow while about one-fifth (20%) of the plasma is filtered as blood passes through the glomerular capillaries (Barrett *et al.,* 2010). The remaining 80% flows into the efferent arteriole and peritubular capillaries (Lote, 2012). Normally, plasma water, electrolytes, aminoacids and dissolved metabolic wastes are filtered into Bowman's capsule while the cellular components (red blood cells, white blood cells, and platelets) and blood proteins (except few low molecular weight proteins) are not filtered (Barrett *et al.,*2010). More than 150 liters of fluid are filtered into the Bowman's spaces of an adult per day but 99% of the water in the filtrate is reabsorbed in various parts of the tubules (Barrett *et al.,* 2010). For all substances filtered, the process of tubular reabsorption can be by passive diffusion or active transport depending on the substance and tubular segment (Barrett *et al.,* 2010). Renal tubular secretion occurs by active process. Substances reabsorbed are water, sodium chloride, glucose, amino acids, lactate, magnesium, calcium phosphate, uric acid, bicarbonate and low molecular weight proteins(Barrett *et al.,*2010). Secreted substances are urea, creatinine, potassium ion, hydrogen and uric acid. There are hormones which alter reabsorption or secretion rates in the tubules in response to maintenance of homeostasis (Barrett *et al.,* 2010). Antidiuretic hormone can increase water reabsorption in response to increase in plasma osmolality or decrease in blood volume (Barrett *et al.,*2010).

Aldosterone stimulates sodium chloride reabsorption in addition to hydrogen and potassium ion secretion in distal convoluted tubule (Hall and Hall, 2021). Parathyroid hormone facilitates calcium reabsorption but inhibit phosphate reabsorption (Hall and Hall, 2021). Both atrial and brain natriuretic peptides increase sodium secretion in the tubules in response to increased blood and atrial volume as a consequence of increase in plasma sodium concentration (Barrett *et al.,* 2010).

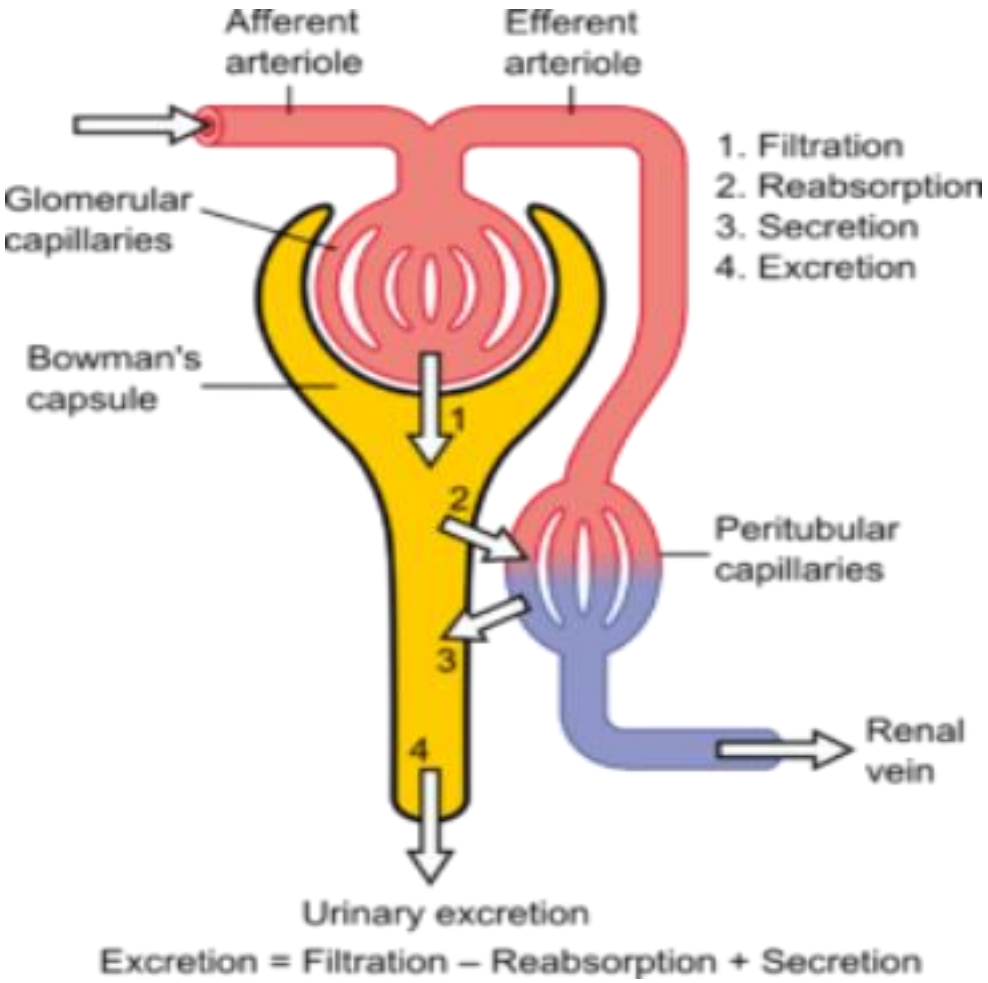

Fig 2.4: Diagram of the Nephron showing three important processes in urine formation and excretion

The renal medullary countercurrent system (which is generated by the peritubular capillaries, vasa recta and long tubules of juxtamedullary nephron) produce a hypertonic medullary interstitium that enables reabsorption of solute free water from the nephron into venous system (Guyton and Hall, 2006).

2.4 Glomerular Filtration Barrier

The ultrastructure of the glomerular filtration barrier (GBF) became evident with invention of electron microscope and other high-definition microscopic equipments which revealed the GBF as a highly specialized blood filtration interface that has high conductance to small and medium sized solutes in plasma but retains relative impermeability to macromolecules (Madhav *et.al.,* 2012). The integrity is maintained by physicochemical and signaling interplay among the three core constituents which are;

(1) Glomerular endothelial cell layer of the vascular capillaries

(2) The basement membrane

(3) Visceral epithelial cell (podocyte) layer of Bowman's capsule membrane

The understanding of pathomechanisms of inherited and acquired human diseases as well as experimental injury models of this barrier have helped to expose the interdependence of these three layers (Madhav *et.al.,* 2012) . Notedly, the consequences of disruption of the integrity of the glomerular filtration barrier is the appearance of significant amount of proteins in urine (initialy albuminuria) which correlates with glomerular disease progression and cardiovascular mortality (Azubike and Unuigbe, 2012).

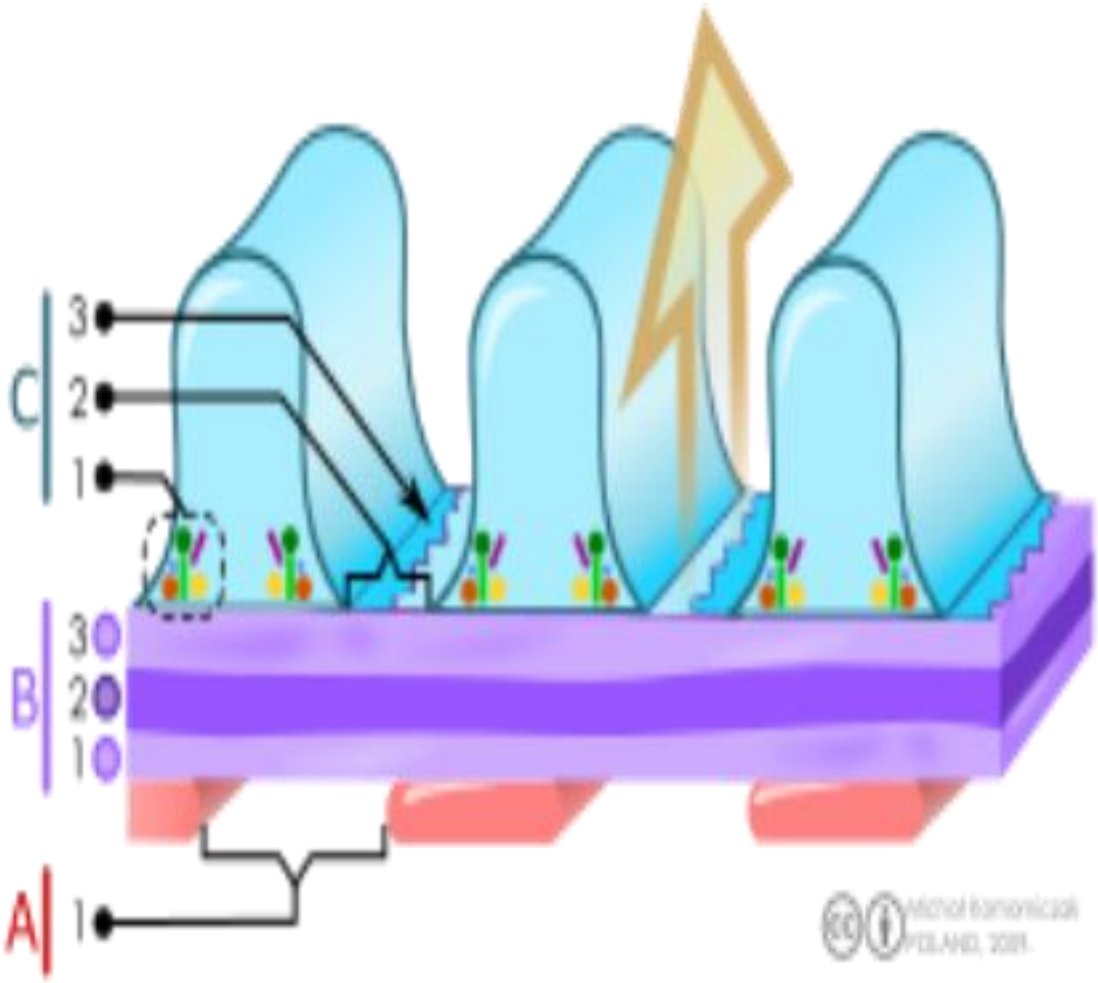

Fig. 2.5: Diagram of the glomerular filtration barrier (GFB)

It shows; A endothelial cell layer of the glomerulus with 1 as the endothelial pore (fenestra).

B. Glomerular basement membrane: (1) lamina rara interna, (2) lamina densa and (3) lamina rara externa

C. Podocytes: (1) enzymatic, (2) structural proteins and (3) filtration slit (Jarad and Miner, 2009).

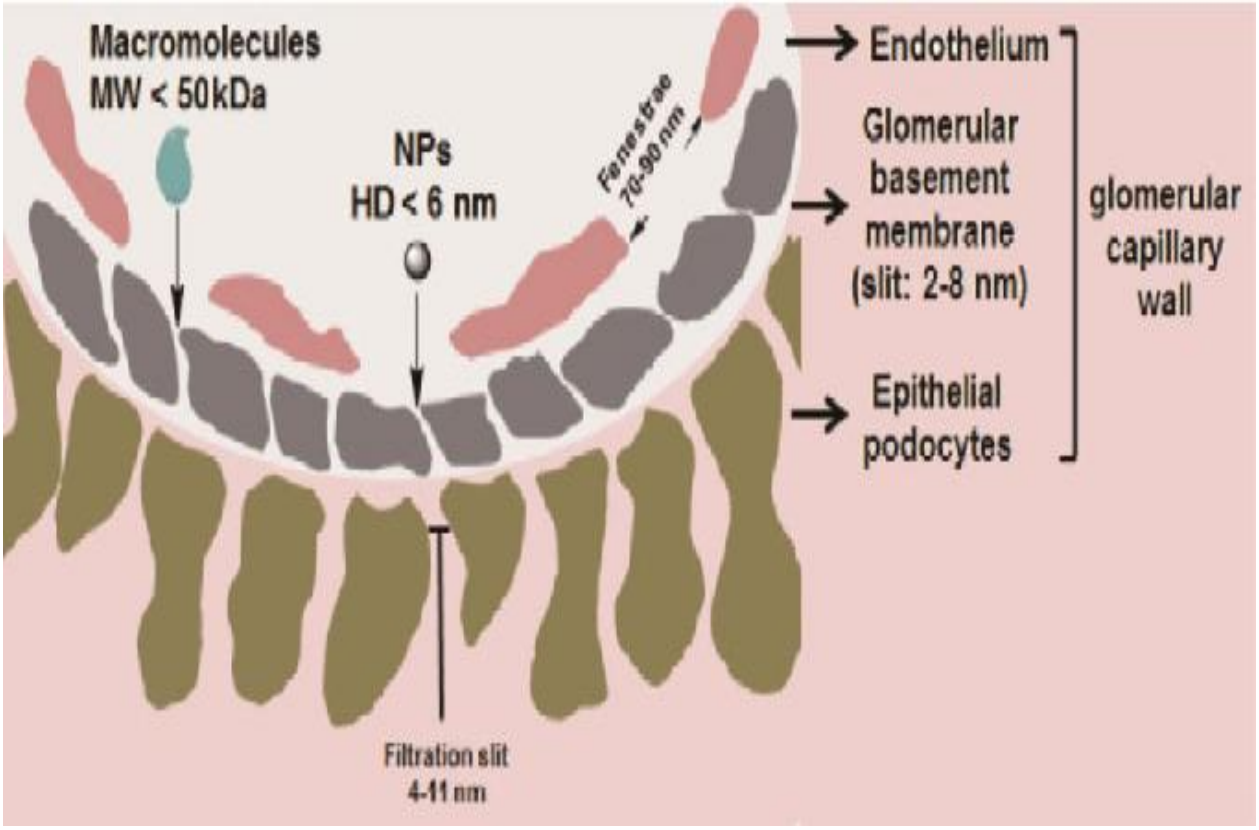

Fig. 2.6: An illustration of the glomerular filtration barrier showing as follows; (a) Fenestrated endothelium, (b) Glomerular basement membrane, (c) Epithelial podocytes

The illustrations reveal the molecular nature of GFB in Fig 5 and dimensions of filtrations spaces in fig 6. The capillary endothelial fenestra is 70-90 nm, the glomerular basement membrane slit is 2-8nm while epithelial podocyte filtration slit is 4-11nm (Fiden *et al.,* 2011).. Only macromolecules of less than 50kDa or less than 6nm in diameter may filter into the Bowman's space. The fenestrations in glomerular endothelial cell layer are 50-100nm and accounts for about 20% of the surface area. This layer was initially not regarded as permeability selective but current studies noted that its coating of glycocalyx layer is composed principally of proteoglycans (Fiden *et al.,* 2011). Intra glomerular lipid injection studies show this glycocalyx

layer to extend 200nm into the capillary lumen (Fiden *et al.,* 2011).. Disruption of this glycocalyx layer by hyaluronidase with Adriamycin was shown to induce proteinuria (Hjalmarsson *et. al.*, 2004). Arterial hypertonic saline infusion in rat kidneys which displaces the non-covalently bound particles of this layer resulted in increased (12-fold) filtration of albumin (Fiden *et al*., 2011). Primary endothelial cell activation is a component of many immune-mediated glomerular diseases (Fiden *et al.,* 2011).. In human disease phenotypes, the glomerular endothelium has appeared to be particularly susceptible to complement-mediated injury (Fiden *et al.,* 2011).

2.5 Histological Features and Functions of Renal Tubules

2.5.1 Proximal Tubule

The proximal tubule is divided into the initial coiled or convoluted segment (pars convoluta) and straight segment (pars recta) because of some differences in cell outlines and functions (Boron and Boulpaep, 2005).. Considering the ultrastructure, the proximal tubule has three segments (S1, S2, S3). The S1 and S2 have higher cell complexity and found in the proximal convoluted tubule while the S3 has lower cell complexity and dominate straight segment (Boron and Boulpaep, 2005).

2.5.2 Proximal convoluted tubule (pars convoluta)

The proximal convoluted tubule (PCT) located in the renal cortex extends from the renal pole of Bowman's capsule to join the straight proximal straight tubule (PST) is coiled and has a lumen surrounded by simple cuboidal epithelia which have brush borders (Wang, 2006). Numerous microvilli characterize the luminal brush border (visible by light microscope) which increase the luminal area of reabsorption and putative flow sensing (Wang, 2006). The proximal tubular cell

cytoplasm is densely populated by mitochondria which gives the cell acidophilic appearance and may correlate with the high energy activity required for active transport of sodium ions out of the cells into plasma in peritubular capillaries (Wang, 2006). This creates high concentration gradient that allows sodium ions movement into the cell from the luminal fluid (glomerular filtrate). Water passively moves with the sodium out of the cell along its concentration gradient (Wang, 2006). These Cuboidal epithelial cells have extensive lateral interdigitations between cells which presents a histological appearance of no discrete cell margins under light microscope (Wang, 2006). Injury induced resorption of the proximal tubular organelles occur after ischemia of the capillaries surrounding the renal tubules (Wang, 2006). This causes disturbance of cellular morphology of the proximal tubule cells and ejection of cell nuclei into the tubule lumen (Wang, 2006).

2.5.3 The Proximal Straight tubule (pars recta)

This segment extends into the outer zone of renal medulla as the termination point essentially demarcates the boundary between outer and inner medulla (Wang, 2006).

2.5.4 Functions of Proximal tubule

2.5.5 Absorptive functions

The proximal tubule regulates the pH of the filtrate by exchanging hydrogen ions in the interstitium for bicarbonate ions in the filtrate in addition also secret organic acids, such as creatinine and other bases, into the filtrate while the fluid in the filtrate entering the proximal convoluted tubule is reabsorbed into the peritubular capillaries (Aronson, 2002). This is driven by active sodium transport from the lumen into the blood by the Na+/K+ ATPase in the basolateral membrane of the cells (Aronson, 2002). Sodium reabsorption is primarily driven by

this P-type ATPase and 60-70% of the filtered sodium load is reabsorbed in the proximal tubule through active transport, solvent drag, and paracellular electrodiffusion (Aronson, 2002). Active transport is mainly through the sodium/hydrogen antiporter-3 (NHE3), Aronson (2002). Paracellular transport increases transport efficiency, as determined by oxygen consumed per unit of Na+ reabsorbed, thus playing a part in maintaining renal oxygen homeostasis (Pei *et al.,* 2016).

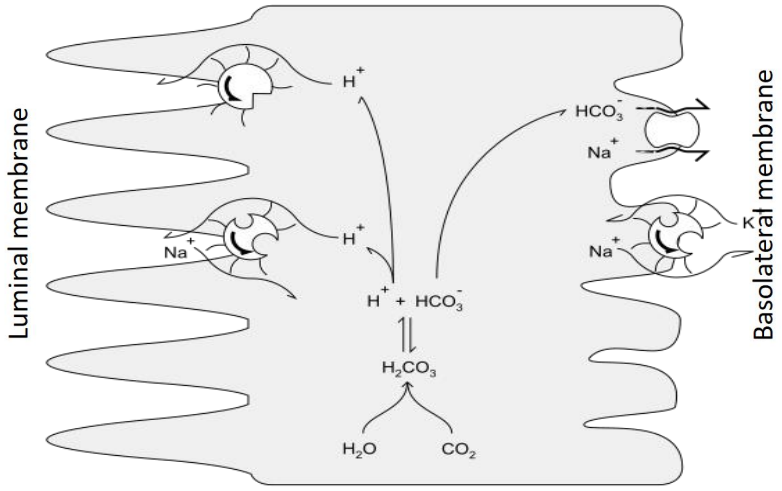

Fig. 2.7: Proximal tubule cell showing pumps involved in acid base balance, left is the lumen of tubule

Table 2.1: Showing absorptive capacity for molecules in proximal tubule

Substance	% of filtrate reabsorbed	Comments
Salt and water	Approx. 2/3	Much of the mass movement of water and solutes occurs through the cells, passively across the basolateral membrane via transcellular transport, followed by active resorption across the apical/luminal membrane via the Na+/K+/ATPase pump (Boron and Boulpaep, 2005). The solutes are absorbed isotonically because the osmotic potential of the fluid leaving the proximal tubule is the same as that of the initial glomerular filtrate (Boron and Boulpaep, 2005).
Organic solutes (primarily glucose and amino acids)	100%	Glucose, amino acids, inorganic phosphate, and some other solutes are resorbed via secondary active transport through co-transporters driven by the sodium gradient out of the nephron(Boron and Boulpaep, 2005).
Potassium	Approx.65%	Most of the filtered potassium is resorbed by two paracellular mechanisms - solvent drag and simple diffusion (Boron and Boulpaep, 2005)
Urea	Approx. 50%	Paracellular fluid reabsorption sweeps some urea with it via solvent drag (Boron and Boulpaep, 2005). As water leaves the lumen, the concentration of urea increases which facilitates diffusion in the late proximal tubule (Boron and Boulpaep, (2005)
Phosphate	Approx. 80%	Parathyroid hormone reduces reabsorption of phosphate in the proximal tubules, but, because it also enhances the uptake of phosphate from the intestine and bones into the blood, the responses to PTH cancel each other out, and the serum concentration of phosphate remains approximately the same.
Citrate	70%–90%	Acidosis increases absorption. Alkalosis decreases absorption.

2.5.6 Secretory functions

Most of the ammonium that is excreted in the urine is formed in the proximal tubule via the breakdown of glutamine to alpha-ketoglutarate which occurs in two steps, each of which generates an ammonium anion; the conversion of glutamine to glutamate and the conversion of glutamate to alpha-ketoglutarate (Rose and Rennke, 1994). The alpha-ketoglutarate generated in

this process is then further broken down to form two bicarbonate anions, which are pumped out of the basolateral portion of the tubule cell by co-transport with sodium ions (Rose and Rennke ,1994):. Various medications are secreted in the proximal tubule.

Table 2.2: Drugs secreted in the proximal tubule of kidney

Medication	**Location**	**Acid** or **Base**	**Anion** or **Cation**	**Percent Excreted**
para-aminohippurate	proximal tubule (Pei *et al.,* 2016)	Acid	anion	
Furosemide	proximal tubule (Pei *et al.,* 2016)	Acid	Anion	75-100
Glucuronic acid-conjugates	proximal tubule (Pei *et al.,* 2016)	Acid		
Glycine conjugates	proximal tubule (Pei *et al.,* 2016)	Acid		
Indomethacin	proximal tubule (Pei *et al*., 2016)	Acid	Anion	
methotrexate	proximal tubule (Pei *et al.,*2016)	Acid		75-100
penicillin	proximal tubule (Pei *et al.,*2016)	Acid	Anion	50-75 (benzylpenicillin)
Probenecid	proximal tubule (Pei *et al.,* 2016)	Acid	Anion	
Sulphate conjugates	proximal tubule (Pei *et al.,* 2016)	Acid		
Thiazide diuretics	proximal tubule (Pei *et al.,* 2016)	Acid		
Uric acid	proximal tubule (Pei *et al.,* 2016)	Acid		
Amiloride	proximal tubule (Pei *et al.,* 2016)	Base	Cation	
Dopamine	proximal tubule (Pei *et al.,* 2016)	Base		
Histamine	proximal tubule (Pei *et al.,* 2016)	Base		
Mepacrine	proximal tubule (Pei *et al.,* 2016)	Base		
Morphine	proximal tubule (Pei *et al.,* 2016)	Base	Cation	
Pethidine	proximal tubule (Pei *et al.,* 2016)	Base		
Quaternary ammonium compounds	proximal tubule (Pei *et al.,* 2016)	Base		

Table 2.3: Drugs secreted in the proximal tubule of kidney (cont)

Medication	**Location**	**Acid** or **Base**	**Anion** or **Cation**	**Percent Excreted**
Quinine	proximal tubule (Pei *et al.,* 2016)	Base	Cation	
5-hydroxytryptamine (serotonin)	proximal tubule (Pei *et al.,* 2016)	Base		
triamterene	proximal tubule (Pei *et al.,* 2016)	Base		
Gentamicin				75-100
Atenolol				75-100
Digoxin				75-100
cimetidine			Cation	50-75
Tetracycline				50-75 (oxytetracycline)
Neostigmine				50-75
Atropine	proximal tubule (Boron and Boulpaep, 2005)		Cation	
1-Nicotinamide	proximal tubule (Boron and Boulpaep, 2005)		Cation	
Paraquat	proximal tubule (Boron and Boulpaep, 2005)		Cation	
Procainamide	proximal tubule (Boron and Boulpaep, 2005)		Cation	
tethraethylammonium	proximal tubule (Boron and Boulpaep, 2005)		Cation	
Chlopromazine	proximal tubule(Boron and Boulpaep 2005)		Cation	

2.5.7 Clinical significance

Most renal cell carcinomas (the most common form of kidney cancer) arise from the proximal convoluted tubules (Tomita, 2006). Acute tubular necrosis occurs when Proximal Tubular Epithelial Cells (PTECs) are directly damaged by toxins such as antibiotics (e.g., gentamicin), pigments (e.g., myoglobin) and sepsis (e.g., mediated by lipopolysaccharide from gram-negative bacteria), Tomita (2006). Renal tubular acidosis (proximal type), called Fanconi syndrome, occurs when the PTECs are unable to properly reabsorb glomerular filtrate so that there is increased loss of bicarbonate, glucose, amino acids, and phosphate (Tomita, 2006). Proximal tubular epithelial cells are damaged in progressive tubulointerstitial injury due to glomerulonephritis, ischemia, interstitial nephritis, vascular injury, and diabetic nephropathy (Pei *et al.,* 2016). In these situations, PTECs may be directly affected by protein (e.g., proteinuria in glomerulonephritis), glucose (in diabetes mellitus), or cytokines like interferon-γ and tumor necrosis factors (Tomita, 2006). The PTECs may respond by: producing cytokines, chemokines, collagen, undergoing epithelial mesenchymal trans-differentiation and necrosis or apoptosis (Tomita, 2006).

2.5.8 Functions of Loop of Henle

The loop of Henle or Henle's loop or nephron loop or ansa nephron (in Latin), as described by Friedrich Gustav Jacob Henle, is the portion of the nephron that continues from the end of proximal convoluted tubule to distal convoluted tubule (Dunn *et.al.,* 2011). The major function is to create concentration gradient in the renal medulla by generation of high renal medullary urea concentration, deep in the papillary duct, by the use of countercurrent multiplier system and electrolyte pumps (Dunn *et.al.,* 2011). The actual functional segment in this regard extends from the end of straight portion of proximal tubule epithelial cells (Dunn *et.al.,* 2011).. The actual loop

of Henle is divided into four portions: (1) Thin descending limb which is the first part of the loop of Henle and located in the renal medullar (Barrett *et al.,* 2010). The epithelium is simple squamous which can be differentiated from vasa recta by absence of blood cells and from thick ascending limb by differential thickness of the cells of this part (Barrett *et al.,* 2010). It has low permeability for sodium and chloride ions, moderate permeability to urea and highly permeable to water by osmosis (Barrett *et al.,* 2010). The loop has a sharp bend (like hair pin end) in the renal medulla leading from descending to ascending thin limb (Barrett *et al.,* 2010). Due to these features and osmotic drag of water, the concentration of the luminal fluid increases intensely in the descending limb (Barrett *et al.,* 2010). Osmolality, it can increase from 300mOsmol/kg up to 1400mOsmol/kg from the proximal tubule to the end of descending limb (Barrett *et al.,* 2010).

(2) Thin ascending limb; the thin ascending limb epithelium is simple squamous cells which is a direct continuation from descending limb and located in the renal medulla (Barrett *et al.,* 2010). It is impermeable to water but permeable to ions allowing for sodium reabsorption from the luminal fluid because Na+/K+-ATPase is present at very low levels in these cells thus ionic reabsorption may be through passive diffusion (Sands and Layton, 2013). Salt moves out of the tubular fluid into the medullary interstitium due to osmotic pressure created by the countercurrent mechanism (Sands and Layton, 2013)..

(3) Thick ascending limb epithelium consist of squamous cells but thicker (in size) than those of thin descending and thin ascending limbs of loop of Henle but has more mitochondria and microvilli with parts of thick ascending limb located in renal medullar and cortex although functionally similar (Sands and Layton, 2013). Sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions are reabsorbed from the tubular fluid or urine by secondary active transport through the Na-K-Cl cotransporter (NKCC2), (Sands and Layton, 2013). The electrical and concentration

gradients drive more reabsorption of Na+, as well as other cations such as magnesium (Mg2+) and calcium (Ca2+), (Sands and Layton, 2013).

The medullary thick ascending limb is largely impermeable to water but Na+, K+ and Cl− ions are reabsorbed by active transport (Mount, 2014). The predominant mechanism of active transport in this segment is through the Na+/K+/Cl− co-transporter (NKCC2) as well as the sodium/hydrogen exchanger NHE3 (Mount, 2014). This segment reabsorbs approximately 25-30% of Na+ along the nephron (Mount, 2014). The clinical importance is that commonly used "loop diuretics" act by inhibiting the NKCC2 (Wile, 2012). This active transport enables the kidney to establish an osmotic gradient that is essential to the kidneys ability to concentrate the urine and make it hypertonic (Wile, 2012). Potassium ion is passively transported along its concentration gradient through a K+ leak channel in the apical aspect of the cells, back into the luminal fluid of the ascending limb (Wile, 2012). This K+ "leak" generates a positive electrochemical potential difference in the lumen which drives more paracellular reabsorption of Na+, as well as other cations such as magnesium (Mg2+) and importantly calcium Ca2+ due to charge repulsion into medullary interstitium (Wile, 2012). This part of the tubule also generates Tamm-Horsfall protein during renal injury although the function of this protein is not well understood, but is responsible for creating urinary casts (Wile, 2012).

(4) Cortical thick ascending limb segment is mostly found in the cortical than the juxtamedullary nephron and the functions are similar to that of medullary thick ascending segment, also drains urine into the distal convoluted tubule (Dunn *et al.,* 2011).

2.5.9 Functions of Distal Convoluted Tubule (DCT)

This is the coiled portion between loop of Henle and the collecting tubule of the nephron (Barrett *et al.,* 2010). In both cortical and juxtamedullary nephron segment of the kidney it lies between afferent and efferent arteriole (Barrett *et al.,* 2010). The DCT is partially responsible for the regulation of plasma K+, Na+, Ca^{2+} and pH. The luminal surface of apical membranes in these cells have thiazide-sensitive Na-Cl cotransporter and are permeable to Ca+ through transient receptor potential cation channel subfamily V member 5 (TRPV5) channel (Dunn *et al.,* 2011). There are also at the basolateral surface (peritubular capillary side) an ATP-dependent Na/K antiporter pump, a secondary active Na+/Ca+ transporter and an ATP dependent Ca transporter (Dunn *et al.,* 2011). The basolateral ATP dependent Na+/K+ pump produces the gradient for Na to be absorbed from the apical membrane through the Na/Cl symporter, and for Ca to be reabsorbed into the blood by the Na/Ca basolateral antiporter (Dunn *et al.,* 2011). It regulates pH by absorbing bicarbonate and secreting protons (H+) into the filtrate, or by absorbing protons and secreting bicarbonate into the filtrate (Dunn *et al.,* 2011).

Sodium and potassium levels are controlled by secreting K+ and absorbing Na+ but Sodium absorption by the distal tubule is mediated by the hormone aldosterone which increases sodium reabsorption. Sodium and chloride reabsorption are also mediated by a group of kinases called WNK kinases (Aylin and Andreas, 2017). There are 4 different WNK kinases (these are lysine deficient protein kinases) which are, WNK1, WNK2, WNK3, and WNK4 that are involved and these kinases also participate in calcium regulation by reabsorbing Ca2+ in response to parathyroid hormone (Aylin and Andreas, 2017) . Parathyroid hormone (PTH) effect is mediated by phosphorylation of regulatory proteins and enhancing the synthesis of all transporters within the distal convoluted tubule (Shekarabi *et al.,* 2017).

Clinically, thiazide diuretics inhibit Na+/Cl− reabsorption in DCT by blocking the thiazide-sensitive Na-Cl cotransporter (van den Ouweland *et al.*, 1992). On inhibition of the transporter, thiazide diuretics increase the gradient potential for Na by increase in sodium concentration in the luminal fluid or urine (van den Ouweland *et al.*, 1992). This increases the activity of the basolateral Na/Ca antiport and causes the increase in calcium reclamation associated with thiazide diuretics (van den Ouweland *et al.*, 1992). Arginine vasopressin receptor2 (AVPR2) which activate aquaporin channels are present in the DCT which responds to arginine vasopressin by stimulating mechanisms that absorb water and concentrate urine thereby maintain water homeostasis in the animal (van den Ouweland *et al.*, 1992). The Loss of AVPR2 function results in nephrogenic diabetes insipidus (van den Ouweland *et al.*, 1992).

2.5.10 Functions of Collecting Duct System (CDS)

The CDS comprises of multiple tubules and ducts which connect various nephrons to a minor calyx or directly to the renal pelvis (Imai, 1979).. The CDS is the last portion of the nephron and functionally participates in electrolyte and fluid balance in addition to conduction of urine delivery into the renal pelvis (Imai, 1979). Reabsorption and secretion occur in the processes of electrolyte and fluid balance which are regulated by aldosterone and vasopressin (Imai, 1979). The three components of CDS are (a) connecting tubules (CNT) (b) cortical collecting ducts and (c) medullary collecting ducts (Barrett *et al.,* 2010).

The connecting tubule also called junction tubule or arcuate renal tubule is continuous with DCT. Several connecting tubules from adjacent nephron merge to form cortical collecting ducts (CCD) while connecting tubules of some juxtamedullary nephron will arch upward to form an arcade or arcuate feature (Imai, 1979). The CNT originated from the metanephric blastema while the

cortical and medullary collecting ducts were derived from the ureteric bud thus the CNT can be considered as part of the nephron rather than part of the collecting duct system (Mitchel, 2009).

The CNTs are associated with regulation of water and electrolytes including sodium and chloride (Eaton and Pooler, 2004). It is sensitive to both isoprotenerol (more in cortical collecting ducts) than arginine vasopressin (less in cortical collecting ducts), (Eaton and Pooler, 2004). The cortical collecting mainly determines the role in water reabsorption (Eaton and Pooler, 2004).

The cortical collecting ducts (CCD) are formed by merging of several adjacent CNTs. The CCDs receive filtrate or urine from multiple initial CNTs and descend into the renal medulla to form medullary collecting ducts (Eaton and Pooler, 2004).

The medullary collecting ducts are divided into outer and inner portions but the extent of water reabsorption in the two portions depends on body fluid balance and hormonal sensitivity in addition to the reabsorption and secretion of sodium, potassium, hydrogen and bicarbonate ion (Barrett *et al*., 2010). The urea transport is passive out of duct which creates concentration gradient of 500mOsm between medullary interstitium and tubular fluid (Boron, 2005).

The outer segment of the medullary collecting duct follows the cortical collecting duct. It reaches the level of the renal medulla where the thin descending limb of loop of Henle borders with the thick ascending limb of loop of Henle and the inner segment is the part of the collecting duct system between the outer segment and the papillary ducts (Boron, 2005).

The papillary (collecting) ducts are structures of the kidneys that were previously known as the ducts of Bellini and are the most distal portion of the collecting duct which receive renal filtrate (precursor to urine) from several medullary collecting ducts and empty into a minor calyx

(Mescher, 2013). Papillary ducts continue the work of water reabsorption and electrolyte balance initiated in the collecting tubules (Mescher, 2013).

The medullary collecting ducts converge to form a central (papillary) duct near the apex of each renal pyramid to exit the renal pyramid at the renal papillae and drain the renal filtrate it carries drains into a minor calyx as urine (Mescher, 2013). The cells are similar to the collecting system which are simple columnar epithelium resting on a thin basement membrane (Mescher, 2013). The epithelium is mostly principal cell and alpha-intercalated cells (Gartner, 2014). There is transition of the simple columnar epithelium of the collecting duct system into urothelium at the junction of papillary duct and a minor calyx (Mescher, 2013). These cells work in close collaboration to reabsorb water, sodium into the renal interstitium and secrete acid and potassium into tubular fluid (Mescher, 2013).

These processes are mediated by hormones (aldosterone, and vasopressin) and the osmolarity of the surrounding medulla (Mescher, 2013). In the medullary collecting duct, vasopressin primarily up-regulates urea transporter A1 which increases the concentration of urea in the surrounding interstitium and increases the osmolarity (Costanzo, 2011). Osmolarity drives the force that pulls (reabsorbs) water from the papillary duct into the medullary interstitium which is especially important in the papillary ducts(Costanzo, 2011). Osmolarity increases from the base of the renal pyramid to the apex. It is highest at the renal apex (up to 1200 mOsm). Thus the force driving the reabsorption of water from the collecting system is the greatest in the papillary duct (Costanzo, 2011).

2.5.11 Functions of cells of the Collecting Duct system (CDS)

The various cells of the collecting duct system are; intercalated cells (which is present in all segments), principal cells (which are present in the collecting ducts) and segment specific cells such as connecting tubule cell and inner medullary collecting duct cell (May *et.al*, 1997).

The Principal cells mediate the collecting duct's influence on sodium and potassium balance via sodium channels and potassium channels located on the cell's apical membrane(May *et.al*, 1997). Aldosterone determines expression of sodium channels (especially the ENaC on the collecting tubule). Increase in aldosterone multiplies expression of luminal sodium channels (May *et.al*, 1997). Aldosterone also increases the number of Na^+/K^+-ATPase pumps which allows active sodium reabsorption and potassium secretion (Hall and Hall. 2006). Vasopressin determines the expression of aquaporin channels that provide a physical pathway for water to pass through the principal cells (Schlatter and Schafer, 1987). Aldosterone and vasopressin permit the principal cells to control the quantity of water reabsorption.

2.5.12 Intercalated cells are the alpha, beta and non-alpha non-beta types.

They participate in renal mediated acid-base homeostasis (Kim *et.al*, 1999). The intercalated cells play tremendously roles in acid-base homeostasis especially during renal response to acidosis and alkalosis (Kim *et.al*, 1999). Damage to the α-intercalated cell's ability to secrete acid can result in distal renal tubular acidosis (RTA type I or classical RTA). The intercalated cell population is also extensively modified in response to chronic lithium treatment, including the addition of a largely uncharacterized cell type which expressed markers for both intercalated and principal cells (Christensen *et.al*, 2004 and Himmel *et.al*, 2018).

Table 2.4: Intercalated cells subtypes and their functions

Type of cell	**Secretory function**	**Reabsorption function**
α-intercalated cells	Acids as hydrogen ions via apical H^+-ATPase and H^+/K^+ exchanger	Bicarbonate via band 3 protein, a basolateral Cl^-/HCO_3^- exchange r
β-intercalated cells	Bicarbonate via pendrin a specialised apical Cl^-/HCO_3^-	Acid via basal H^+-ATPase
Non-α non-β intercalated cells	Acid (via an apical H^+-ATPase and H^+/K^+ exchanger) and bicarbonate (via pendrin), (Kim *et.al,* 2002) and (Wall *et.al*, 2003)	

This wide variation in water reabsorption levels for the collecting duct system reflects its dependence on hormonal activation (Takito *et.al*, 1996). The outer medullary and cortical collecting ducts are significantly impermeable to water except if activated by antidiuretic hormone (Takito *et.al*, 1996). In the absence of ADH, water in the renal filtrate increases the volume of urine (promoting diuresis) but presence of ADH activates aquaporins which permits water reabsorption thereby inhibiting diuresis (Takito *et.al*, 1996). The collecting duct system also participates in the regulation of electrolytes, including sodium, chloride, potassium, hydrogen ions, and bicarbonate (Takito *et.al*, 1996). An extracellular protein called hensin protein mediates the regulation of secretion of acid by alpha intercalated cells in acidosis, and secretion of bicarbonate by beta intercalated cells in alkalosis (Takito *et.al*, 1996). Collecting duct carcinoma is a rare subtype of renal cell carcinoma (RCC) occurring in about1% of all RCCs. Many occurred in younger age groups of third, fourth, or fifth decade of life, found in the medulla but many are infiltrative but can extend into the cortex (Takito *et.al*, 1996). At the time

of diagnosis most are high grade, advanced and symptomatic with poor response to conventional therapy. Immuno-histochemical and molecular analyses suggest that collecting duct RCC may resemble transitional cell carcinoma, and some patients with advanced collecting duct RCC have responded to cisplatin- or gemcitabine-based chemotherapy (Takito *et.al*, 1996).

2.5.13 Systemic Functions of the Kidneys

The kidneys summarily filter blood to produce urine. The study of functions of the kidneys is the field of Renal Physiology and which includes as follows;

(a) Regulation of body fluid volume, Osmolality, various electrolyte concentrations (plasma sodium, potassium, calcium and phosphate) and acid base balance.
(b) Removal of toxic metabolites such as urea, creatinine, uric acid and middle molecules.
(c) Removal exogenously ingested toxins and drug metabolites
(d) Conversion of vitamin D to calcitriol (the active form)
(e) Synthesis of erythropoietin and prostaglandins
(f) Synthesis, storage and secretion of renin (an enzyme required for activation of angiotensin1 and secretion of aldosterone) necessary for activation of thirst and regulation of blood pressure.

2.5.14 Effects of Aging on the Kidney Functions

There are known impacts of age and race on renal functions for which these factors are considered in various eGFR formulas. Renal aging is associated with alterations in renal morphology and a decline in functions (Zhou *et al*, 2008). There are glomerular, tubular, interstitial and endocrine function defects, jointly or separately. It has been reported that after the

age of 30-40 years, the GFR begins to decline at an average rate of 1ml/min/1.73m^2/year resulting in an inulin clearance of 65ml/min/1.73m^2 at the age of 90 years in apparently healthy individuals (Morrissey and Yango, 2006). In the aging kidneys, there are reductions in urinary sodium and potassium excretion as the ability of the kidneys to maximally concentrate and dilute urine diminishes with age which explains the higher rate of nocturia in the aging population (Zhou *et al*, 2008). The mean lithium clearance (an indicator of proximal tubular function) was significantly higher in healthy young subjects when compared among three groups of elderly subjects, which were; healthy elderly, hypertensive elderly, and elderly with compensated mild to moderate heart failure (Fliser *et al*, 1997). Aging related decline in renal tubular functions manifest in multiple ways, example, expected reduction in urinary sodium excretion in response to deprivation of dietary sodium chloride is not significant in the elderly compared to young individuals (Zhou *et al.* 2008). Renal handling of Potassium or potassium adaptation is negatively affected. The mean lithium clearance (an indicator of proximal tubular function) was significantly lower than for healthy young subjects when compared among three groups of elderly subjects, which were; healthy elderly, hypertensive elderly, and elderly with compensated mild to moderate heart failure (Fliser D *et al.,* 1997). Another study among apparently healthy adult Caucasians predicted that annual decline in GFR occurs by 1ml/min/1.73m^2/year in the adult above 40years of age. Patients or people who lose renal function faster than this value tend to progress to end-stage renal disease more rapidly (Jun *et al.,* 2017). There are reduction in the ability for maximal concentration and dilution of urine which explains the higher rate of nocturia and dehydration in the aging population (Zhou *et al*, 2008). There is increase in prevalence of anaemia with decreasing renal function that is related to reduction in erythropoietin production by the kidney (Jun *et al.,* 2017). Kidney removes about 50% of insulin from systemic circulation

as the major site of insulin clearance which is impaired and predisposes the elderly to insulin retention and hypoglycemia. There is gross appearance of surface granularity and pitting in the aging kidney which is due to underlying arterial disease (Zhou *et al*, 2008. The major histological features of renal aging are; global glomerulosclerosis, tubular atrophy, interstitial fibrosis and renal arterial intimal fibrosis affecting interlobular arteries (Zhou *et al*, 2008) as shown in plate 1 below.

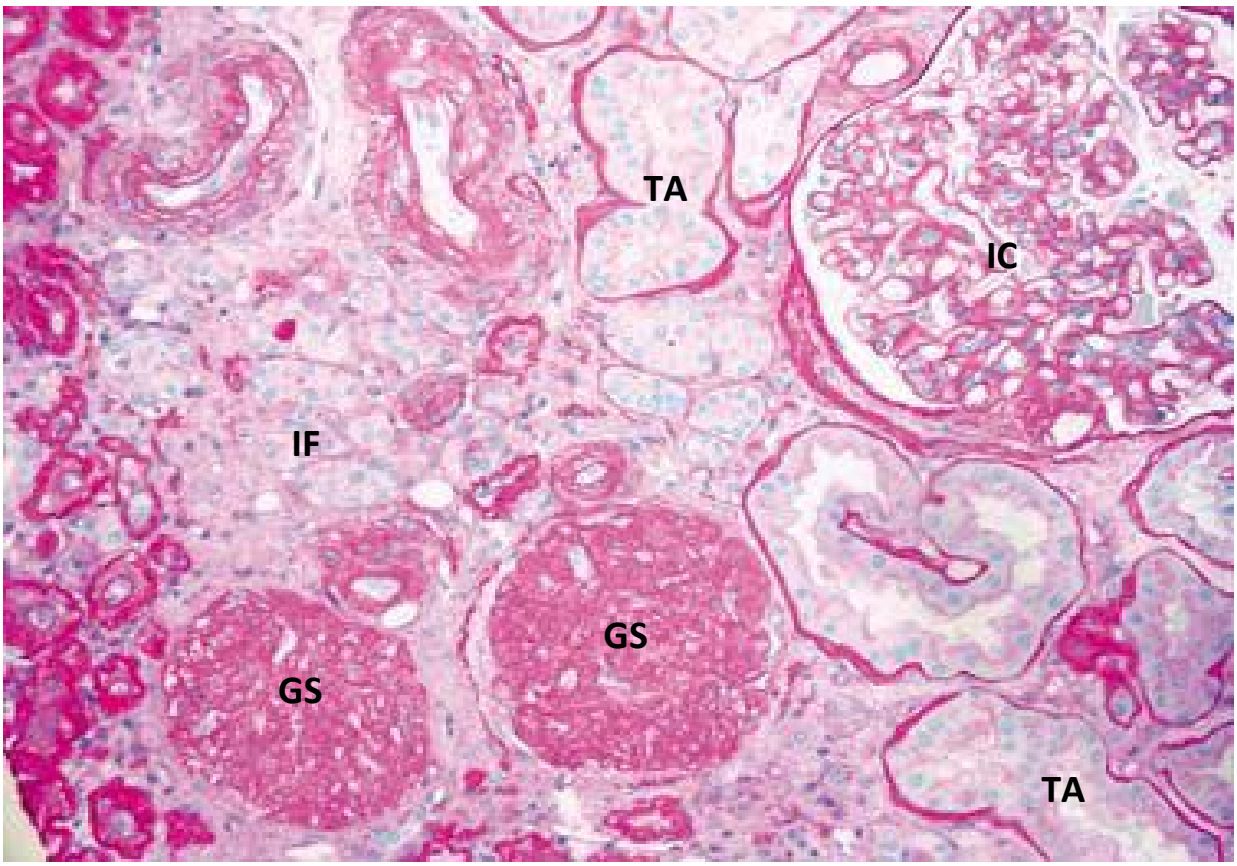

Plate 2.1: Showing an aging kidney histological section. Two glomeruli show solidified global glomerulosclerosis **(GS)**. The non-sclerotic glomerulus displays ischemic changes (**IC)**. There are also moderate renal tubular atrophy **(TA)** and interstitial fibrosis **(IF)**. The arterioles reveal significant hyalinosis (Periodic acid-Schiff stain; 200).

The pathogenesis of aging associated global glomerulosclerosis is multifactorial and the theories of renal senescence include; (a) genomic instability (b) oxidative damage (c) genetic programing and (d) cell death. The molecular basis of renal aging is a focus of active investigations (Zhou *et al*, 2008).

Aging kidneys can be affected by diseases like HTN, DM and sporadic insults as infections and drug injuries. Renal aging occurs in the absence of systemic or local diseases which can accelerate the natural process of aging. Cumulative oxidative stress is implicated in cellular aging process due to generation of advanced glycosylation end products (AGEs), and with their receptors which play their roles in aging process (Martin and Sheaff, 2007 and Lu *et al.*, 2004). In mice the anti-aging gene Klotho can suppress aging process with resultant increase in life span (Kuro *et al.*, 1997 and Kurosu et al, 2005). This suggests the possibility of Klotho gene abnormality or deficiency in aging.

2.5.15 Effects of Oxidative Stress and Total Antioxidant Capacity

MDA attack cell DNA to induce mutagenicity (Marnett, 1999), neurodegeneration (Patel and Chu, 2011), cardiovascular and diabetic (Andrea, 2024), and chronic kidney disease (Mariana *et al*, 2020). MDA is the major Oxidative Stress Biomarker assayed in plasma and serum (Nair, 2008). Age-related buildup of ROS and MDA overtime leads to cellular dysfunction, tissue degeneration and the progressive decline in physiological function found in aging (Andrea, 2024) also include CKD (Mariana et al, 2020). Age associated increase in MDA occurs in both health, ill health and identified in serum and urine (Dimitrios *et al*, 2023). Various endogenous antioxidant enzymes protect the cells from oxidative damage. These are superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), ceruloplasmin, and proteins such as metalothionins (Kusano and Ferrari, 2001).

Instead of separate antioxidant enzyme assays, Miller et al. (1993) developed a test to measure the Total Antioxidant Capacity (Trolox equivalent antioxidant capacity" (TEAC) method) which was later modified to minimize the effect of serum uric acid (Erel, 2004).

There was association between the oxidative balance score (OBS) and eGFR according to National Health and Nutrition Survey (2007–2018 NHANES. Mingda et al, 2024). A higher OBS suggests greater exposures to antioxidants. It had been noted that habitual increase of dietary TAC is associated with a lower risk of CKD (Parivash *et al*, 2020).

2.5.16 Urine Sodium / Potassium Ratio

There is reduced ability to maximally concentrate urine in both aging people and rats which is significantly related to the reduction in key transport proteins as noted in renal medulla aged rats namely; Aquaporin 2 (AQP2), serine-256-phosphorylated AQP2, Aquaporin3 (AQP3), Na^+-K^+-$2Cl^-$ cotransporter (NKCC2/BSC1), Urea Transporter gene A1 (UT-A1), Urea Transporter gene B (UT-B), and the Vasopressin2 (V2) receptor. These observations were linked to reduced response to supra-physiologic dose of desmopressin (dDAVP) [a specific V2 receptor agonist] or reduced urine concentration during water restriction in order to conserve body water (Sands, 2009).

In a population study that examined estimated 24-h urinary sodium and sodium-to-potassium ratio as predictors of decline in kidney function, Deriaz *et al* (2019) noted that there was a significant linear association between urinary sodium excretion and age-related kidney function decline but without significant association between urinary potassium excretion and age-related kidney function decline when taking into account baseline renal function. The Na/K ratio was a strong predictor of age related kidney function decline but strongly driven by the sodium

component of the ratio as it was less predictive than sodium alone (Deriaz *et al*., 2019). This suggested that reducing sodium intake and having a low-dietary Na+/K+ ratio may slow down the progression to CKD as there is also strong relationship between urinary Na+/K+ ratio and changes in blood pressure indices probably because it is an indicator of sodium/potassium consumption (Deriaz *et al*., 2019). A generalized linear model indicated that urinary Na+/K+ ratio changes were positively and significantly associated with SBP changes. In the non-antihypertensive urinary Na+/K+ ratio changes were significantly associated with SBP and DBP changes (Deriaz *et al*., 2019). In the antihypertensive medication user group, urinary Na/K ratio changes were also significantly associated with SBP changes. This obviously confirmed the association between changes in the Na+/K+ ratio and changes in BP (Abe *et al*., 2022).

Excessive intake of salt directly leads to worsening of hypertension (Elliott *et al*., 2007). It is noted that high salt intake increases the excretion of protein in the urine, resulting in a decrease in renal function (du Cailar *et al*., 2002). In an 11-year observational study of 2196 women with normal renal function, a higher intake of sodium based on questionnaire results showed a faster decline in GFR (Lin *et al*., 2010). In a longitudinal study involving individuals who participated in annual health check-up programs, urinary sodium-to-potassium (Na+/K+) ratio was found to be independently associated with incidence of chronic kidney disease, defined as an estimated glomerular filtration rate (eGFR) of < 60 mL/min/1.73 m^2 and an annual eGFR decline (Tabara, 2024).

In a Korean study, Koo *et al* (2018) reported that the higher the urinary Na/K ratio, the greater the risk of CKD progression. A low urinary Na^+/K^+ ratio may relate with lower CKD development risk in adults with preserved kidney function (Young *et al*., 2022).

2.5.17 Effects of Hypertension on Kidney Functions

The kidneys receive about 20-25 % of cardiac output from which it receives nutrients, fluid and removes metabolic wastes (Walter, 2004).

Kidney damage can be caused by or lead to hypertension and assessed routinely by renal function parameters (serum creatinine and eGFR) together with check for albuminuria (using dipstick or urinary albumin creatinine ratio [UACR]) with spot urine (Unger et al., 2020).

For adequate renal function, normal BP is accepted as 120/80 or less than 130/85mmHg (MAP ≤ 100mmHg). A BP value of ≥140/90 in adults < 60years or ≥ 150/90 for adults above 60 years is HTN according to JNC 8 classification (Armstrong, 2014) and 2020 ISH Global Hypertension Practice Guidelines (Unger et al,2020).

In a 2017 Nigerian nationwide survey, the overall age-standardized prevalence of hypertension was 38.1% (Odili et al., 2017). Incidence of CKD increases with proportion of HTN in a population (Klag et al.1996). Nigeria prevalence of HTN among adults 30-79 years was 28.9% (WHO 2023).

Prevalence of CKD in Nigeria was variously reported as 24-26% (Chukwuonye et al.,2018) and 15.432% (Apiyanteide et al., 2023).

After the age of 30-40 years, the GFR declines at an average rate of 1ml/min/1.73m2/year resulting in an inulin clearance of 65ml/min/1.73m2 at the age of 90 years in apparently healthy individuals (Morrissey and Yango, 2006). In a Nigerian study (Azubike and Unuigbe 2012), there was an annual eGFR reduction of 5.13ml/min/1.73m2 among treated diabetic hypertensive patients in a 12 years longitudinal study.

This study utilized creatinine clearance CrCl as mGFR procedure, eGFR equations and urine ACR (as primary urine renal biomarker).

2.5.18 Urine Peptidomic Biomarkers of CKD

Urinary Peptidomic Biomarkers in kidney Diseases have become important factors that pre-date development of decline in kidney functions associated with AKI and CKD (Vittorio S. et al 2020). As noted, significant rise in serum creatinine or decline in eGFR occurs after about 50% of kidney injury has occurred. The urine peptidomic biomarkers were already notably present long before this stage as exemplified by microalbuminuria and for which urine ACR was incorporated in the KDIGO CKD guidelines (2012).

Already studied urinary peptidomic substances in CKD were;

(a) Albuminuria

(b) Urine Epidermal Growth Factor (urine EGF) concentration

(c) Urine Kidney injury molecule -1 (urine KIM-1)

(d) Urine Monocyte chemo-attractant protein-1(urine MCP-1)

(e) Urine Alpha-1microglobulin (urine α -1 microglobulin)

Albuminuria; Albumin as large molecular weight plasma protein is not filtered by glomerulus into the urine in significant amount by healthy kidneys. Albuminuria is a sign of glomerular disease and means measurable amount of albumin in urine. It can be short-term in heart failure, fever, vigorous exercise, urinary tract infection, postural changes, sleep apnea and AKI or long-term in CKD (Robert, 2004). Microalbuminuria is abnormal increase in urine albumin above normal in the range of 30 – 299 mg/g creatinine, which is common in diabetic nephropathy

(Levy *et al,* 2003). It pre-dates progressive chronic decline in kidney functions. KDIGO has incorporated urinary albumin creatinine ratio (ACR) of A1 < 3, A2 < 3-30, A >30 (mg/mmol), in the current classification of eGFR for progressive decline in kidney functions (Table 2).

Microalbuminuria: Urine albumin: creatinine ratio (UACR) > 3 mg/mmol to 30mg/mmol or >30mg/g to 299mg/g, (NKF K/DOQI 2002 guidelines, Levy *et al,* 2003). UACR was usually assessed in diabetic CKD patients in order to stage the severity of CKD and also assessed in studies of CKD (Murton *et al.,* 2021). Minimal albumin can normally be filtered in urine but increase quantities are filtered if progressive glomerular epithelial membrane podocyte effacement occurs (Murton *et al.,* 2021).

Human Urinary Epidermal Growth Factor (EGF); urinary EGF is a globular protein that is generated in the kidney, particularly the loop of Henle and distal convoluted tubule (Norvik *et al.,* 2021). Although it is present in urine of healthy kidney but it is hardly detectable in plasma (Norvik *et al.,* 2021). It is a simple polypeptide of 53 amino acid residue which is involved in the regulation and cell proliferation and binds to target cell membrane EGF receptor (Norvik *et al.,* 2021). The urine excretion is about 1.5 micrograms/h or 25ng/mg creatinine. The EGF in urine is essentially derived from kidney synthesis and secretion (Norvik *et al,* 2021).

Human Urinary Kidney Injury Molecule – 1 (Urine KIM-1): It is a type 1 membrane protein comprising of an extracellular and a cytoplasmic portion found in the kidney, liver and spleen (Joseph, 2008). It is a specific and sensitive urine biomarker of kidney injury (Joseph, 2008). Studies in man show that kidney tissue expression and excretion of KIM-1 are specific and sensitive biomarkers of injury and predictors of outcome (Joseph, 2008). KIM-1 assay in urine is 0 – 2146 pg / ml.

Human Urinary Monocyte Chemoattractant Protein -1 (HUrine MCP-1): It is expressed by renal tissues and detectable in urine of patients with a variety of renal diseases (Tam and Kim, 2011). MCP-1 can be expressed by macrophages, neutrophils, fibroblasts, endothelial and epithelial cells (Tam and Kim, 2011).

Human Urinary Alpha-1-microglobulin (HUa-1m): Increase in concentration of U α -1m in urine is a signal for renal proximal tubule dysfunction. In ambulatory settings, high concentrations are associated with acute kidney injury (AKI), progressive CKD, cardiovascular events and mortality. It binds and degrades hem as a radical scavenger and reductase (Jonathan *et al*, 2011). Human Ua-1m is described as a circulating 'waste bin' which continuously removes free radicals and oxidizing agents, especially heme, from the tissues (Jonathan *et al*, 2011).

2.5.19 Glomerular Filtration Rate and the Measurements

The kidneys produce urine by filtering fluid and metabolic wastes through the glomeruli (which are subjected to the processes of reabsorption and secretion) as the filtrate flows through renal tubules until deposited into the renal calyces as urine. Through the efficiency of these processes the kidneys achieve the following as earlier listed:

(a) Maintenance of body acid-base balance

(b) Regulation of body fluid balance

(c) Regulation plasma sodium, potassium and other electrolytes

(d) Clearance and excretion of toxins

(e) Absorption of glucose, amino acids, vitamins and other small molecules

(f) Regulation of blood pressure

Other kidney functions include; Production of hormones such as erythropoietin, activation of vitamin D3, activation of renin-angiotensin-aldosterone and some prostaglandins

Glomerular filtration rate (GFR): This is essentially the quantity of cellular and protein free fluid filtered by the glomerular capillaries into the Bowman's capsular space per unit time (Guyton and Hall). The sum of fluid filtered by each nephron can be obtained by direct glass micro-puncture experiment of Bowman's capsule in lower animals. This procedure is mainly experimental but not feasible for routine whole animal and clinical measurements. The sums of GFRs in about two million nephrons in the two kidneys constitute all that is measured clinically.

Glomerular filtration rate (GFR) is an important measurement of adequate or inadequate kidney function. It is critical for detection evaluation and management of chronic kidney disease (CKD) (Lesley et al 2008). GFR is generally inversely proportional to plasma creatinine or serum cystatin C.

Very important in the maintenance of GFR is the difference in pressures between the afferent and efferent arterioles in addition to pressures exerted by plasma protein (oncotic pressure) and water content of blood and filtrate (hydrostatic pressure). The rate of filtration is dependent on the higher afferent arteriolar and hydrostatic pressure difference over the lower sum of efferent arteriolar pressure (due to lesser vasoconstriction) and oncotic pressure.

It is routine to measure endogenously generated metabolites or renal biomarker (e.g. creatinine or Cystatin C) that is easily filtered but not significantly reabsorbed or secreted by the nephron. Urea is not suitable because it is significantly reabsorbed and secrete by the tubular cells of the nephron as it determines renal medullar interstitial concentration which drives the countercurrent mechanism for urine concentration.

Glomerular filtration rate is equal to the renal clearance rate when a substance is freely filtered and is neither reabsorbed nor secreted by the kidneys. Such exogenously injected substances must not undergo reaction or exert toxicity in the body. They are usually injected in laboratory controlled experiments. Examples include; inulin, Radioactive tracers as (a) Chromium-51(51Cr-EDTA) (b) Technetium-99m (99mTc-DTPA) (c) Iodothalamide.

The rate measured is the quantity of the substance in the urine that originated from a volume of blood (plasma concentration cleared of the substance) which can be calculated. This principle can be related to the clearance equation for the substance used. Thus, the product of urine concentration of the substance and urine flow rate equals the mass of substance excreted during the timed urine collection. This mass is equal to the mass filtered at the glomerulus as none was secreted or reabsorbed in the nephron. Division of the mass by the plasma concentration of marker reveals the volume of blood plasma from which it was removed. This is the volume of plasma fluid that entered Bowman's capsule within the time specified. The GFR is typically recorded in units of volume per time, e.g., milliliters per minute (mL/min).

$$GFR = \frac{Urine\ Concentration\ of\ index\ substance\ x\ Urine\ Flow\ rate}{Plasma\ Concentration\ of\ index\ substance}$$

This can be interchanged with the renal clearance formula if the agent (*X*) used was not reabsorbed, secreted, did not undergo changes, was not bound to plasma proteins or toxic to the body. This is;

$C_x = (U_x)\underline{V/ P_x}$

Where,

- C_x is the clearance of X (normally in units of mL/min).
- U_x is the urine concentration of X.
- P_x is the plasma concentration of X.

- V is the urine flow rate.

The various agents used to measure renal clearance or glomerular filtration rate (mGFR) include;

(1) Inulin (an exogenous substance which is the gold standard)

(2) Creatinine (endogenous biomarker) by using 24 hours urine collection and renal clearance formula

(3) Radioactive tracers such as; (a) Chromium-51(as 51Cr-EDTA) (b) Technetium-99m (as 99mTc-DTPA) (c)

(4) Radiocontrast agent; Iodothalamide

(5) Cystatin C (endogenous biomarker)

Inulin Clearance: The renal clearance of inulin is the gold standard for experimental measurement of GFR. Inulin is an inert substance obtained from dahlia tuber. Inulin or its analog sinistrin can be injected into the bloodstream while its concentration in urine is measured over time. Inulin and sinistrin are not reabsorbed nor secreted, bound, undergo neither reactions nor toxic to the renal tubules after glomerular filtration. Their rate of excretion is directly proportional to the rate of filtration of water and solutes across the glomerular filter. Incomplete urine collection is an important source of error in inulin or sinistrin clearance measurement (Rose, 1969).

The Use of inulin to measure GFR (mGFR) is the "gold standard" for comparison with other means of estimating glomerular filtration rate (Hsu and Bansal, 2011).

The classic procedure for measuring inulin clearance is rigorous and includes a continuous intravenous infusion, multiple repeated blood and urine collections, and careful timing for blood sampling. Such direct measurement of GFR is cumbersome and repeatedly invasive; it is not practical for day to day clinical assessment.

Determination of systemic inulin clearance by the standard technique of constant intravenous infusion has long been accepted as a reliable method for measuring glomerular filtration rate (GFR) without urine collection, except in edematous patients. However, recent studies using standard clearance techniques have claimed that systemic inulin clearance is significantly greater

than renal clearance and therefore overestimates mGFR. Hence there is need to re-evaluate the relationship between systemic and renal inulin clearance using a different technical approach.

Systemic and renal inulin clearances were simultaneously evaluated, in healthy subjects and patients with edema and ascites, by analysis of the total area under the plasma concentration-time curve (AUC) following bolus intravenous injection. Renal clearance was calculated as the ratio of the total amount recovered in the urine to the AUC, and systemic clearance as dose/AUC (Orlando *et al,* 1998).

The interpretation of the Inulin Clearance Blood Test value depends on the age and gender of the individual. The ranges of values are as follows;

•Children less than 11 years: 82-122 mL/min

•Children aged 11-20 years: 84-125 mL/min

•Men aged 21-39 years: 90-168 mL/min

•Women aged 21-39 years: 84-150 mL/min

•Men aged 40-49 years: 78-162 mL/min

•Women aged 40-49 years: 82-146 mL/min (Maulik, 2019)

Creatinine Clearance: creatinine is naturally produced by the body as breakdown product of the skeletal muscle creatine phosphate. It is fairly distributed in body fluids and remains stable in plasma and serum over 24 hour period. General assay in serum (by Jaffe reaction method) gives a value of 0.4 -1.2 mg/dl.

In routine clinical practice creatinine clearance or estimates of creatinine clearance based on the serum creatinine level are used to measure GFR. It commonly requires a timed 24 hour urine collection (from an empty bladder) and venous blood sample within the period. Creatinine is freely filtered by the glomerulus but minimally secreted by the peritubular capillaries into renal tubule such that the clearance value overestimates actual GFR by 10% to 20%. There is an acceptable minimal margin of error that is due to the ease of measurement compared to inulin clearance procedure which requires constant infusion of inulin and blood sampling. Creatinine clearance (CrCl) is calculated from the creatinine concentration in the collected urine sample

(UCr), urine flow rate (Vdt) and the plasma concentration (PCr). Since the product of urine concentration and urine flow rate yields creatinine excretion rate, which is the rate of removal from the blood, creatinine clearance is calculated as removal rate per min (UCr×Vdt) divided by the plasma creatinine concentration. This is commonly represented mathematically as clearance formula above.

To allow comparison of results between people of different sizes, the CrCl is often corrected for the body surface area (BSA) and compared to the average sized man as ml/min/1.73 m^2. While most adults have a BSA that approaches 1.7 m^2 (1.6 m2 to 1.9 m2), very obese or slim individuals should have their CrCl corrected for their actual BSA.

The values of CrCl approximate well with GFR by inulin clearance but there are two drawbacks which include accurate urine collection and muscle mass. In healthy individuals, values alter between muscular young males, children, females, obese and elderly individuals. This is because the 24-hour creatinine excretion depends on recent meat ingestion and muscle mass which are variable among these groups. For individuals with high muscle mass, serum creatinine is higher for clearance rate which accounts for the relatively higher serum values in adult males than females.

2.5.20 Radioactive or Radioisotope tracers

Measured renal clearance (expressed as mGFR) studies using radionuclides as (a) Chromium-51(as 51Cr-EDTA) or (b) Technetium-99m (as 99mTc-DTPA) correlate very closely with Inulin clearance. Radionuclides are usually given as a bolus intravenous bolus doses and the GFR calculated by their rate of disappearance from plasma avoiding the need for urine tests and steady infusion.

2.5.21 The Radiocontrast agents

These were initially available in the 1960s but difficulties in chemical analysis and unacceptable amounts of free iodine in the preparations limited their application in favor of Radioisotope agents (previously mentioned). The problems have largely been abated with introduction of Iodothalamide. The common criticisms of the above measured GFR methods are; (a) allergic side effects (b) time consuming (c) not easily applicable for routine clinical use (d) inaccurate

urine collection and (e) need for sophisticated equipments which make them only suitable for research and kidney donor screening.

2.5.22 Cystatin C (endogenous biomarker)

Cystatin C is an inhibitor of cysteine protease is a common protein secreted by all nucleated cells in the body. It is a small molecule that is freely filtered by the glomerulus but it is mostly reabsorbed and catabolized by renal tubular epithelial cells therefore only small quantities are excreted in urine except there this significant renal tubular damage. Cystatin C levels are preferably measured in venous blood and common to calculate eGFR with serum Cystatin C levels. Among eGFR formula using single serum Cystatin C, **the 100/serum simple Cystatin C formula** is a reliable marker of GFR in the elderly and compares very well with creatinine formulas including the CKD-EPI formula (Sebastjan B. et al 2011). The eGFR equations have been developed to include consideration for sex, age, race adjusted Cystatin C and creatinine. The most accurate of the equations is the (sex, age and race) with adjusted Cystatin C, followed by (sex, age and race) with adjusted creatinine and the Cystatin C alone (Stevens et al. 2008).

2.5.23 Calculated or Estimated Glomerular Filtration Rate

The various methods recommended for Calculated or Estimated glomerular filtration rate (eGFR) are;

2.5.23.1 Serum Creatinine Based Equations; Serum creatinine is inversely related to GFR but highly deficient as a surrogate marker GFR. It is a small nitrogen molecule, equally distributed in serum, freely filtered by glomeruli but minimally secreted by renal tubules. It was observed that renal function would have deteriorated by about 50% before significant increase in serum occurred (Cockcroft and Gault, 1976). Creatinine has been used both as an endogenous marker for renal clearance and in the earlier development of eGFR because it compares favorably with Inulin clearance.

(1) Cockroft-Gault formula (using serum creatinine)
(2) Modification of Diet in Renal Disease (MDRD) formula
(3) NKFCKD-EP! (Chronic Kidney Disease Epidemiology Collaboration) formula
(4) Mayo Quadratic formula
(5) Schwartz formula

(6) Cystatin C formula

(1)Cockroft-Gault equation (using serum creatinine), Cockcroft DW and Gault MH.(1976)

It was developed in 1973 but published in 1976. It was said to be only useful for research purposes because results were not standardized for initial creatinine values and body surface area. It is gradually gaining acceptance due the ease of calculation both in clinical use and dosing for kidney excretable toxic drugs.

The eGFR = (140 – age in years) x Weight (kg) x constant/serum creatinine (umol/L). The constant is represented by 1.23 for males of any age and 1.05 for females of any age.

Or $eGFR_{CG}$ = (140 − age in years) × (weight in kilograms) × (0.85 if female) / (72 × serum creatinine)

(2)The Modification of Diet in Renal Disease equation (MDRD); Levey et.al (2006)

The MDRD study equation was developed in 1999 with the use of data from patients with chronic kidney disease. It estimates GFR adjusted for body-surface area, considered race as either black or not which reflected a higher average serum creatinine level in blacks (due to higher muscle mass). It was noted to be more accurate than either the use of the Cockcroft–Gault equation or the measurement of creatinine clearance (Levey, et al, 2006).

This equation requires plasma or serum sample for creatinine assay to calculate eGFR as follows;

$eGFR_{MDRD}$ = 175 × (standardized serum creatinine)$^{-1.154}$ × (age in years)$^{-0.203}$ × 0.742 [if female] × 1.212 [if Black].

Or $eGFR_{MDRD}$ = 186 x (serum creatinine) $^{-0.154}$ x (age in years) $^{-0.203}$ x (0.742 if female) x (1.21 if black)

Or MDRD study formula for Korean population;

$eGFR_{MDRD}$ = 107.904 x (serum creatinine in mg/dl) $^{-1.009}$ x age $^{-0.02}$ x 0.667; (Lee et al., 2010).

These current formulas differ from the previous 6 variable MDRD formula which comprised of age, sex, ethnicity, serum creatinine, urea and albumin.

(3)The NKF CKD-EP! (National Kidney Foundation Chronic Kidney Disease Epidemiology Collaboration) equations;

(a) The 2009 NKF CKD-EP! Cr equation applies serum creatinine, age, race, and gender to estimate GFR in adults ages 18years and older developed by NIDDK rely on creatinine assay by isotope dilution mass spectrometry (IDMS). An eGFR above 90ml/min/1.73m2 is considered normal kidney function.

$eGFR_{CKD\text{-}EP!} = 141 \times \min(Scr/k,1)^{\alpha} \times \max(Scr/k,1)^{-1.209} \times 0.993^{Age} \times 1.018$(if female)_1.159 (if black)

where Scr is serum creatinine, k is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/k or1

An online electronic calculator can be used to derive eGFR.

(b) NKF CKD-EP! Cr (2021) Expressed as a single equation excluded race factor and IDMS creatinine assay is not compulsory.

$eGFR = 142 \times \min(\text{standardized } S_{cr}/K, 1)^{\alpha} \times \max(\text{standardized } S_{cr}/K, 1)^{-1.200} \times 0.9938^{Age} \times 1.012$ [if female]

Where eGFR is estimated glomerular filtration rate in mL/min/ 1.73 m^2

S_{cr} (serum creatinine) = mg/dL, K = 0.7 (females) or 0.9 (males), α = - 0.241 (females) or - 0.302 (males),

min = indicates the minimum of S_{cr}/K or 1, max = indicates the maximum of S_{cr}/K or 1

An online electronic calculator was used to derive eGFR.

(4)MAYO Quadratic eGFR formula (MCQ)

The Mayo Clinic Quadratic equation is another formula which attempts to estimate GFR from variables including serum creatinine, age and sex (Vincent R. et al (2007).

$MCQ\ eq = \exp[1.911 + 5.249/SCr - 2.114/SCr^2 - 0.00686 \times \text{age (years)} - 0.205$ if female]

Where; exp is exponent and SCr is serum creatinine. All serum creatinine measurements were performed in the same laboratory and determined by the Jaffé alkaline picrate method (normal range 0.6–1.5 mg/dl), calibrated using the SET point Calibrator T13-1291 (Bayer, Barcelona, Spain).

2.5.23.2 Combined Creatinine and Cystatin C Equations

(a)The NKF CKD-EP! Equation (2021) combined serum Creatinine and Cystatin C

The combination of serum Creatinine and Cystatin C in the equation was developed by Stevens et.al (2008) and CKD-EP! (2008).This equation may provide more accurate estimates in patients with differences in diet, extremes of muscle mass (such as body builders or patients with muscle wasting), or those outside the boundaries of where the MDRD Study equation has been validated. It may also prove useful in estimating change in eGFR over time in people with changing muscle mass or diet. It May have a role in identifying persons with CKD who have the highest risk for complications.

CKD-EPI Creatinine-Cystatin Equation (2021)

$eGFR_{cr\text{-}cys} = 135 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-0.544} \times \min(S_{cys}/0.8, 1)^{-0.323} \times \max(S_{cys}/0.8, 1)^{-0.778} \times 0.9961^{Age} \times 0.963$ [if female]

where:

S_{cr} = standardized serum creatinine in mg/dL, $\kappa = 0.7$ (females) or 0.9 (males)

$\alpha = -0.219$ (female) or -0.144 (male), $\min(S_{cr}/\kappa, 1)$ is the minimum of S_{cr}/κ or 1.0,

$\max(S_{cr}/\kappa, 1)$ is the maximum of S_{cr}/κ or 1.0, S_{cys} = standardized serum cystatin C in mg/L, Age (years)

An online electronic calculator can be used to derive eGFR.

(b) Schwartz equation: The original Schwartz eGFR formula was devised in the mid-1970s to estimate GFR in children. This model was felt to overestimates GFR, likely a result of a change in methods used to measure creatinine. In 2009, Schwartz et al developed a revised estimated GFR formula using data from cohort of children in the Chronic Kidney Disease in Children (CKiD) study. This model is appropriate for children between the ages of 1 to 16 yr. The linear regression analyses generated a model that included the following: height (meters), serum creatinine (mg/dL), cystatin C (mg/L), Blood urea nitrogen (mg/dL) and Gender.

$GFR(ml/minper1.73m^2) = 39.1[height/creatinine]^{0.516}$ x$[1.8/cystatinC]^{0.294}$ $[30/BUN]^{0.169}$ $[1.099]^{male}$ $[height/1.4]^{0.188}$

Given the complexity of this formula, a "bedside" version requiring only height and creatinine was developed:

eGFR=0.413x(height/serumcreatinine),

where height is in cm, and creatinine is in mg/dL

2.5.24 Cystatin C Only Equations

(a) Simple Cystatin C formula (National kidney foundation 2002**)**

Simple Cystatin C formula: eGFR = 100/serum Cystatin C (mg/L) = eGFR in ml/min/1.73m^2

(b) NKF Cystatin C alone equation (Bevc *et al*, 2012**)**

NKF Cystatin C alone equation (2012): eGFR = 70.69 x (cys C) $^{-0.931}$ ml/min/1.73m^2

Where; cys C (or cystatin C) is in mg/L

2.6.1. Studies on Age Determined Decline in Glomerular Filtration Rate

In their earlier experimental studies Davies and Shock (1950) showed that there was progressive reduction in mean inulin clearance, diodrast clearance and diodrast Tm in males in between the ages of 60 and 80 years than in males 20-40 years of age (although cross-sectional studies). In the studies of Davies and Shock which were among American Caucasian males, the average decline in GFR was 0.96 ml/min/year or about 10 ml/min/decade. A similar report of 1ml/min/1.73m^2/year also among Caucasian population was published by Morrissey and Yango in 2006. Rowe *et al*. (1976) reported that creatinine clearance (Ccr) fell from 140 ml/min/1.73m2 at age 30 years to about 97 ml/min/1.73m^2 at age 80 years among 548 normal subjects in a cross-sectional study. In longitudinal studies involving three or more Ccr measurements done over 12–18 months in 293 "normal" subjects, similar pattern of decline of Ccr with age was observed, with acceleration in the rate of decline with advancing age (Linderman *et.al,* 1985). In a Rotterdam study, (van der Burgh *et al.,* 2021) which involved 12,062 participants with 85,922 eGFR assessments (mean age 67.0 years, 58.7% were women) with ACR measurements. The annual eGFR decline was 0.82 ml/min/1.73m^2 and the ACR increase was 0.05mg/g. All determinants as smoking and hypertension were detrimental for eGFR and ACR, except for prediabetes and higher body mass index which were detrimental for ACR. The first systematic review (between 1958 and 2021) that examined longitudinal decline in kidney function with age in apparently healthy individuals involved 12 reports from 7 countries (Sweden, Japan, Israel, Canada, USA, China and United kingdom) and Vidt 2011 data from 26 countries showed that normal decline in GFR was between −0.37 and −1.07 mL / min /1.73 m^2 / year in healthy adults without hypertension (Guppy *et al*., 2024). This research will contribute data from Nigeria.

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.2 MATERIALS

Important materials used in this study were;

Cotton wool

Tourniquet

Methylated spirit (70%)

5ml and 10ml Sterile Syringes with 21G needle

Four liter plastic containers for urine collection

10ml plain sterile plastic urine containers

Multistix for simple urinalysis (Newspring Multiscreen 11)

EDTA specimen bottles

Plain specimen bottles

10ml glass centrifuge tubes

Six bucket Centrifuge

Bedside weighing scale

Height meter rule

Accuson mercury column sphygmomanometer

Omron digital Automatic Blood Pressure monitor(Omron, China)

Glucometer (Newspring KF-B12 Cofos Medical Tech Ltd-China)

Ion Selective Electrode (ISE Analyzer 4000 SFRI) for Na^+ and K^+

Sinothinker SK8800 – Fully Auto Hematology Analyzer

Auto Elisa Plate Reader (AVI Labtech) for serum Cystatin C and Urine peptidomic biomarkers [Urine Monocyte chemo-attractant protein-1(urine MCP-1) and Urine Alpha-1microglobulin (Human urine α -1 microglobulin)] assays.

Spectrophotometer (VIS--7220G BIOTECH ENGINEERING MANAGEMENT CO. LTD. UK) for creatinine and urine albumin assays

Cystatin C ELISA kit Ref AD00492hu

Human AMBP ELISA KIT Ref AD00081hu for Urine Alpha-1microglobulin (Human urine α -1 microglobulin) assay.

Human MCP-1 ELISA KIT Ref AD01699hu for Urine Monocyte chemo-attractant protein-1(Human urine MCP-1) assays.

3.3 METHODOLOGY

3.3.1 Subjects and Sample Size

A sample size of two hundred and seventy (270) apparently healthy adult male and female volunteer subjects (18-70 years), calculated with Lwanga and Lemeshow (1991) formula were invited by open advert for medical check-up in various social media from within and outside the University of Benin Community.

- This population of subjects was determined with Lwanga and Lemeshow (1991) formula for sample size determination as stated below:
- N = Z^2 (1-α)/2 x P (1-P)/d^2
- Where, N = sample size,
- Z^2 (1-α)/2 at 95% confidence limit = 1.96
- P = Prevalence or Proportion of population in question (assume a value of 0.5 where the prevalence is not known). The prevalence of CKD in Nigeria was estimated at about 24 to 26% (Chukwuonye et al, 2018), therefore an average of 25% was applied for P.
- d = Absolute precision on either side of the proportion i.e. 5% or 0.05.

3.4 Ethical Approval

Ethical approval, **REC Approval No: CMS/REC/2025/900** dated 28th January, 2025 was obtained from College of Medical Sciences Research Ethics Committee.

3.5 Informed Consent

The individuals who submitted their Biodata and signed the consent form were accepted and enrolled as subjects if they met the inclusion criteria.

3.6 Inclusion and Exclusion Criteria

1 Apparently healthy Nigerians of age (18 to 70+ years) were included.

2 Female subjects in their menstrual period were deferred.

3. Pregnant females were excluded.

4. Sickle cell disease subjects were excluded.

5. Recently hospitalized, confirmed dialysis and post-kidney transplant patients were excluded.

3.7 Study Design

The Subjects were arranged in groups of 15 males and 15 females for 9 age groups in years (18-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-60, 61-65, 66-70 +). During the period the age of subjects in groups A and G were extended because of fewer respondents. The five years age grouping was used because of the usually assumed five–seven years interval in human physiological transitions (Gary, 2016). Glomerular filtration rate remains about the same range during adolescent to early adult life (Maulik, 2019, Jessica and Sharon, 2010).

Table 3.1: Distribution of the 270 subjects into groups

Group	Age group in years	Number	Males	Females
A	17--24	30	15Am	15Af
B	25—29	30	15Bm	15Bf
C	30—34	30	15Cm	15Cf
D	35—39	30	15Dm	15Df
E	40—44	30	15Em	15Ef
F	45—49	30	15Fm	15Ff
G	50—60	30	15Gm	15Gf
H	61--- 65	30	15Hm	15Hf
I	66 -- 70	30	15Im	15If

The subjects were asked to seat comfortably in the Physiology laboratory and enrolled into a questioner which contained information on their Biodata and social habits. Their age, gender, height, weight, body mass index (BMI), Pulse rate (PR), Systolic and Diastolic blood pressure (SBP and DBP respectively) and Mean Arterial Blood Pressure (MAP) were recorded in a form. Heights and Weights were measured in meters and kg respectively and BMI calculation was done as; BMI = Weight (kg) / Height (m^2)

3.7.1 Blood Pressure (BP) measurements expressed in mmHg Values were obtained with validated Omron electronic (oscillometric) method with upper-arm cuff device which is currently preferred (Unger, 2020).

Values were also cross-checked with auscultatory mercury column sphygmomanometer (Accuson). The 1st Korotkoff sound heard was the systolic blood pressure (SBP) while the muffling or the 5th Korotkoff sound was taken as the diastolic blood pressure (DBP).

Normal BP was accepted as 120/80. BP value ≥ 130/85 accepted as prehypertension (MAP ≥ 100mmHg). BP values of ≥140/90 or ≥ 150/90 were accepted as hypertension for adults below and above 60 years respectively according to JNC 8 (Armstrong, 2014).

Mean Arterial blood pressure was calculated as; MAP = (SBP-DBP)/3 + DBP. MAP ≥ 100 was accepted as hypertension (Heba, 2023).

3.7.2 Urine collection procedures

Fresh midstream 10ml spot urine sample was collected into a plain steril sample container for urinalysis and assays of albumin, creatinine, urine peptidomic biomarkers, Na^+ and K^+.

Subjects were instructed on 12-hours urine collection (6pm to 6am). Total urine volume was measured for calculation of urine flow rate per minute. At least 5ml was transferred into a plain specimen container for assay of creatinine.

3.7.3 Venipuncture procedure

Ten (10) ml of venous blood sample was carefully collected from an ante cubital vein and divided as follows; (a) 2ml into EDTA bottle which will be used for full blood count and ESR,

(b) 8ml into a 10ml plain centrifuge container which was allowed to clot until clot retraction was seen and centrifuged at 5000 rpm for 5mins. The serum was aspirated and transferred into a plain specimen container. The serum was used for assays of Cystatin C, Creatinine, electrolytes (Na+, K+), Total Antioxidant capacity and Malondialdehyde (MDA as oxidative stress marker).

3.7.4 Assays

The following investigations were done with the samples.

(1) Full blood count was done with Sinothinker SK8800 – Fully Auto Hematology Analyzer

(2) Serum and urine electrolytes (Na^+ and K^+ in mmol/l) were done by using Ion selective Electrode method (ISE Analyzer 4000 SFRI)

(3) Serum and Urine creatinine concentrations by spectrophotometric Jaffe alkaline picric acid reaction method

(4) Serum Cystatin C concentration was assayed with Human Cystatin C ELISA kit-AD00492hu and read with the Auto Elisa Plate Reader (AVI Labtech).

(5) Urine albumin concentration was done by using the Spectrophotometric Sulphosalicylic Acid method

(7) Urine alpha-1-microglobulin (Hu -α -1m) concentration was done by using Human Alpha-1-microglobulin ELISA kit (AD00081hu) , Zhang *et al.* (2018)

(8) Urine monocyte chemo-attractant protein-1 (uMCP-1) concentration was done by using the human monocyte chemo-attractant protein-1 ELISA kit (AD01699hu), Gudeta, *et al.,* (2015)

(9) Serum Total Antioxidant capacity assay was done by using the spectrophotometric Phosphomolybdenum method (Prieto et al, 1999)

(10) Malondialdehyde (MDA) was done by using the spectrophotometric Thiobarbituric acid (TBA) method (Buege and Aust, 1978)

(11) Urine Albumin Creatinine Ratio (UACR, Molly *et al.,* 2021) and urine Na^+/K^+ (Koo *et al,* 2018) were calculated

3.7.5 Glomerular Filtration Rate Formulas

The GFR formulae used in this study were;

(1) measured creatinine clearance (mCrCL),

(2) eGFR formulae used were (a) Cockcroft and Gault (CG) equation (1976), (b) National Kidney Foundation Chronic Kidney Disease-Epidemiology Collaboration 2021 creatinine equation (NKF CKD-EP! Cr 2021, Inker et al., 2021), (c) National Kidney Foundation Chronic Kidney Disease-Epidemiology Collaboration 2021 Cystatin C equation (NKF CKD-EP! cyst c 2021, Lesley et al., 2008), (d) Combined serum Creatinine and Cystatin C Chronic Kidney Disease-Epidemiology equation 2021 (NKF CKD-EP! cr-cystc equation, Inker et al., 2021) and (e) Modification of Diet in Renal Disease equation (MDRD, Levey et al., 2006).

3.7.6 Data Analysis

The Mean ± Sem of the data were calculated on excel worksheet and further statistical analysis were done with SPSS-29 which included Analysis of variance (ANOVA), Student's t-Test, regression analysis and correlations of parameters against age were done. The descriptive statistics of the demographic variables for subjects were expressed as mean and standard error of mean (Mean± Sem) for the age groups. The variables for male and female age groups were compared for statistical difference with ANOVA. A P-value less than 0.05 ($P < 0.05$) was accepted as statistically significant difference. Regression graphs and equations were plotted to observe the trend and calculate the gradient as rates of change in GFR versus the age groups and rates of change in GFR versus other parameters.

CHAPTER FOUR

RESULTS

Two hundred and seventy (270) subjects met the inclusion criteria (which comprised 135 males and 135 females) but 243 (121 males and 122 females) completed the procedures (i.e. were venipunctured and submitted their 12 hours urine collection).

The results of the data analysis are hereby presented.

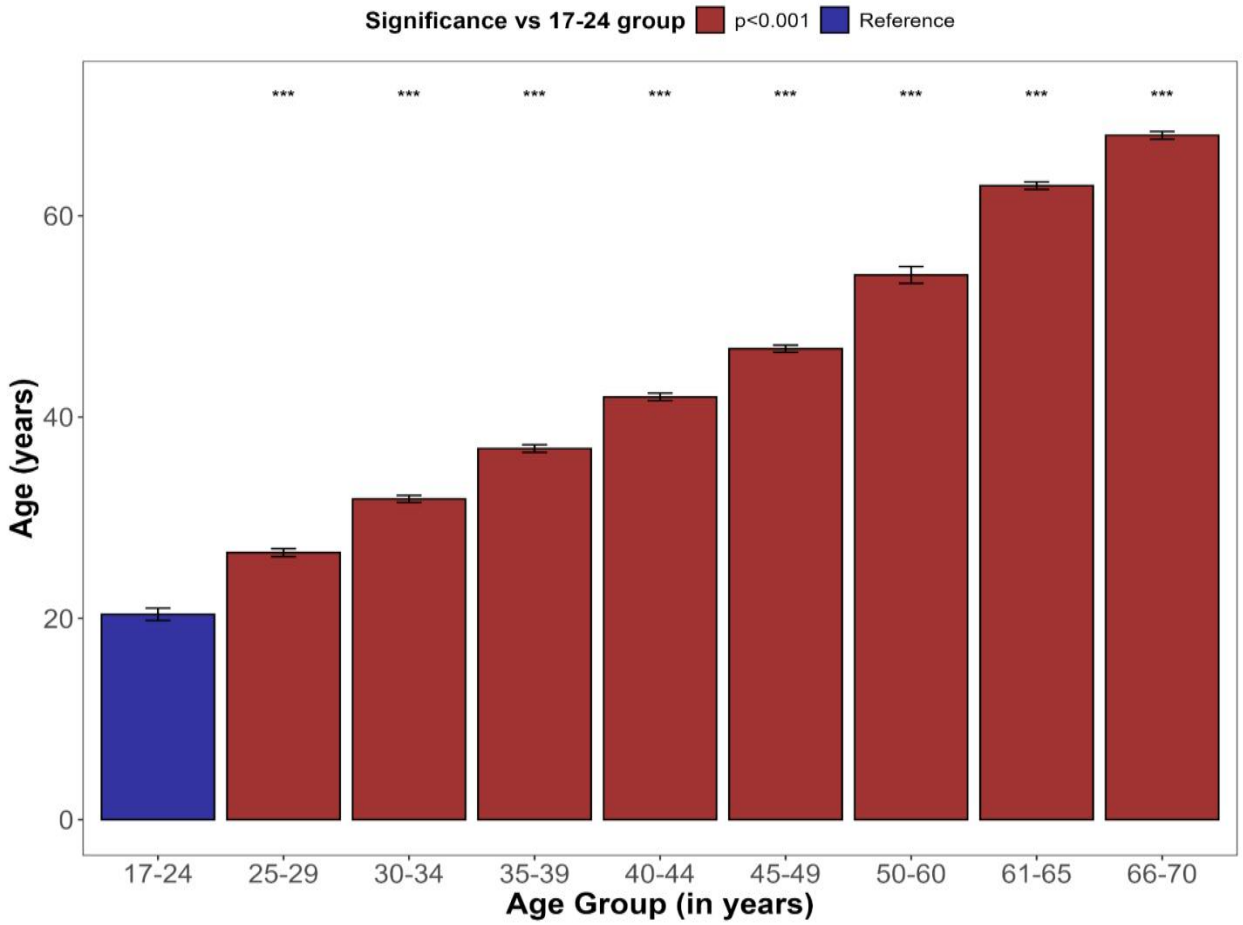

Fig 4.1: Male subjects age-group distribution for the study. Each Age-group is comprised of 15 subjects and their mean age (in years) was significantly different from each other (ANOVA, $P < 0.001$).

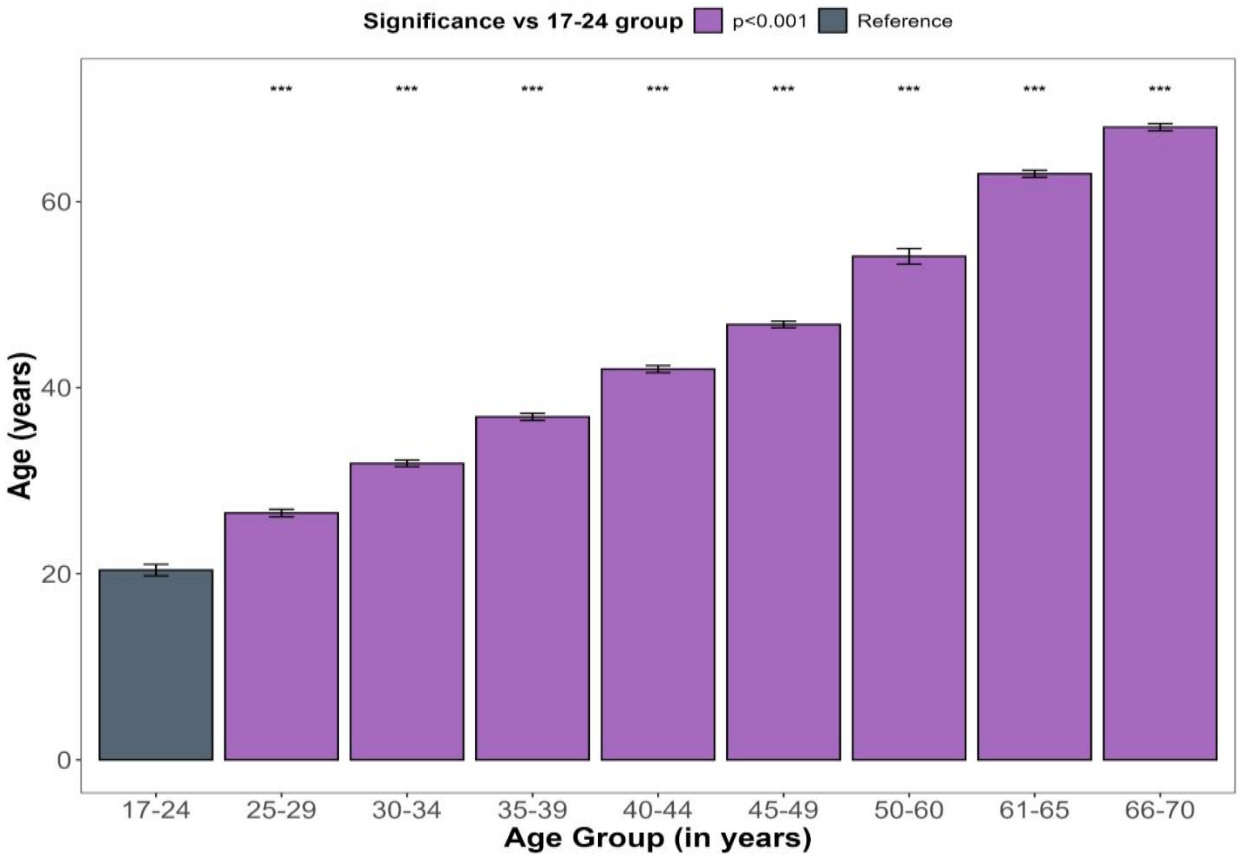

Fig 4.2: Female subjects age-group distribution for the study. Each Age-group was comprised of 15 subjects and their mean age (in years) was significantly different from each other (ANOVA), $P < 0.001$.

Table 4.1: Summary of descriptive statistics of some physiological parameters: Age, Weight, Height, BMI, PR, SBP, DBP and MAP

	Age (yrs)	Bwt (kg)	Ht (m)	BMI (kg/m^2)	PR (beats/min)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
Mean	38.35	67.78	1.73	22.65	75.92	123.79	74.97	91.13
Sem	0.99	0.97	0.01	0.31	0.68	1.16	0.77	0.86

This table shows the Mean ± Sem of data on Age, Body weight, Height, BMI, PR, SBP, DBP and MAP

Bwt = Body weight in kilogram

Ht = Height in meters

BMI = Body Mass Index in kilogram per meter ^{2}

PR = Pulse Rate in beats per minute

SBP = Systolic Blood Pressure in mmHg

DBP = Diastolic Blood Pressure in mmHg

MAP = Mean Arterial Blood Pressure in mmHg

mmHg = Millimeter Mercury

ANOVA AND REGRESSION GRAPHS FOR AGE RELATED CHANGES IN VARIOUS PARAMETERS (fig. 4.3 to 4.9).

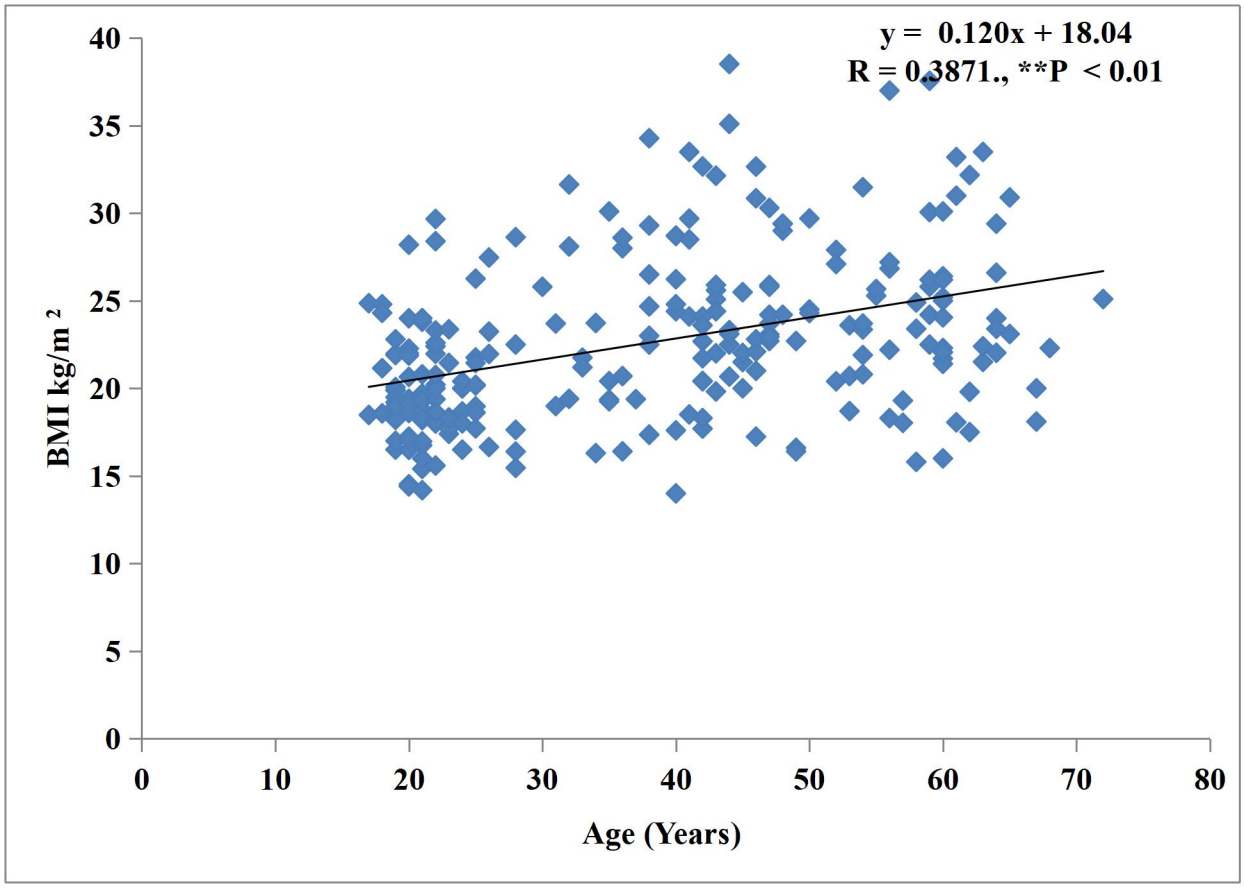

Fig 4.3: Linear regression of BMI vs. Age (in years) for all the subjects. The BMI increased annually and significantly with age ($P < 0.01$). Annual rate of increase was 0.120 kg/m ^{2} /yr.

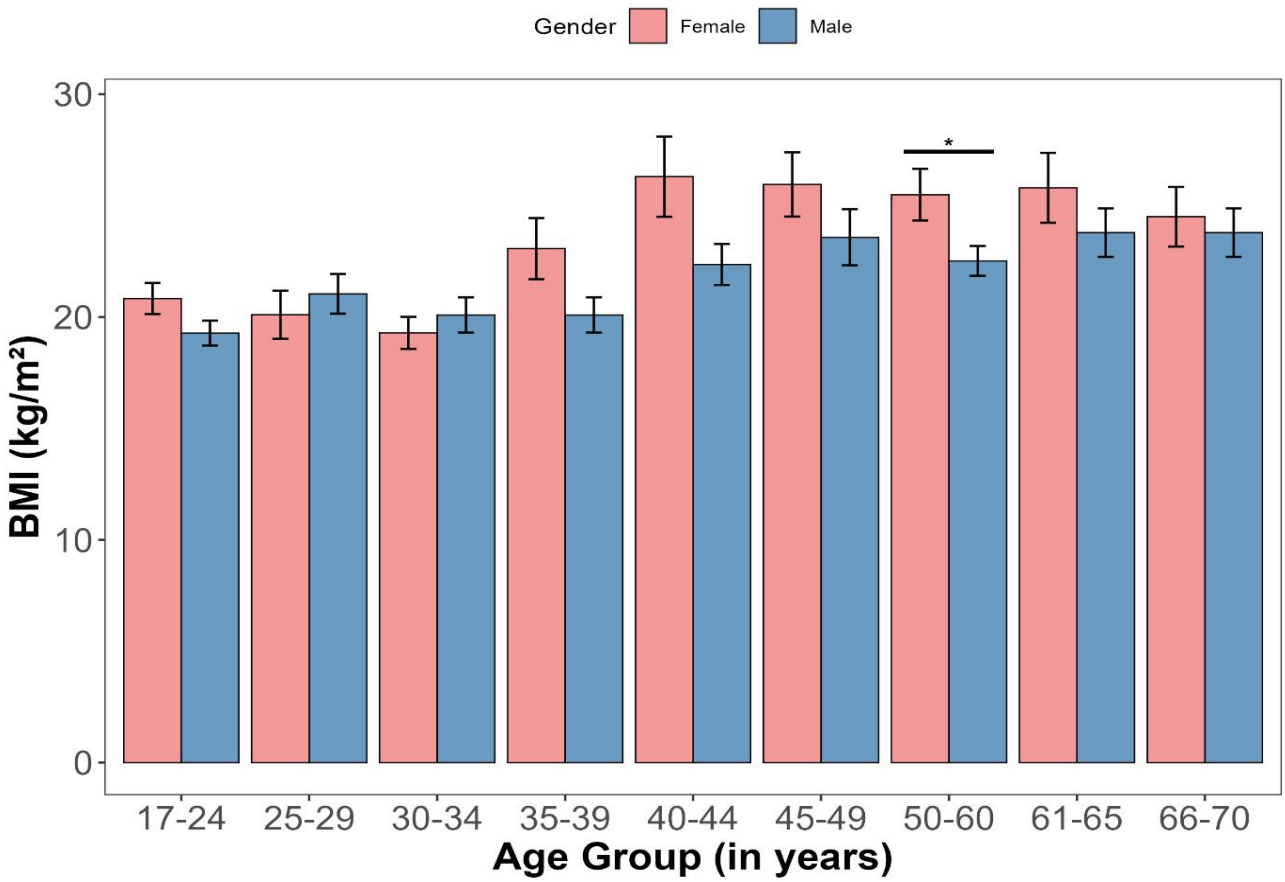

Fig. 4.4: Comparison of BMI in Female and Male Age groups (in years) with ANOVA. Generally Females had higher BMI than Males but significant ($P < 0.05$) in the 50-60 yr age-group.

BMI = Body Mass Index in kg/m^2

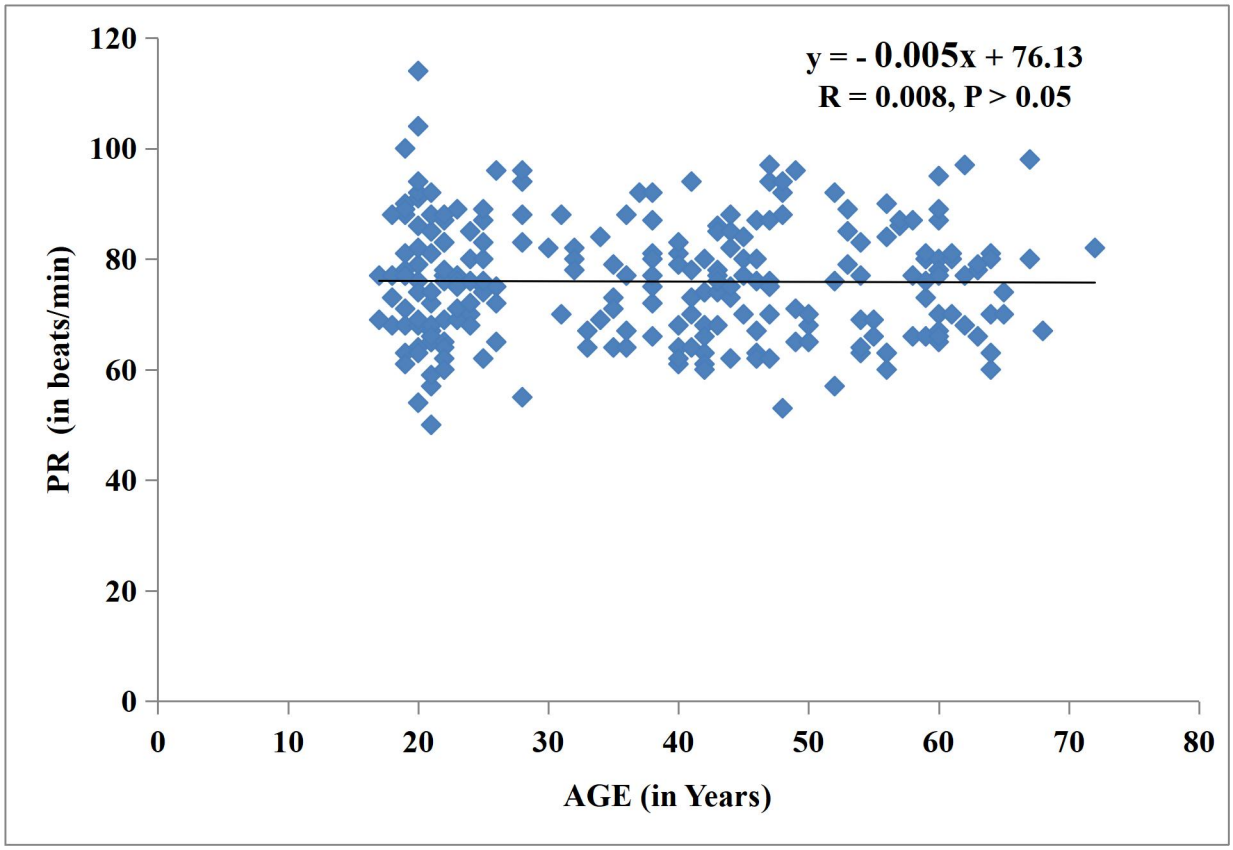

Fig. 4.5: Linear regression of Pulse Rate (PR in beats/min) vs. Age (in Years). There was no significant decline in PR (P > 0.05). Annual rate of decrease was - 0.005 beats /min /yr.

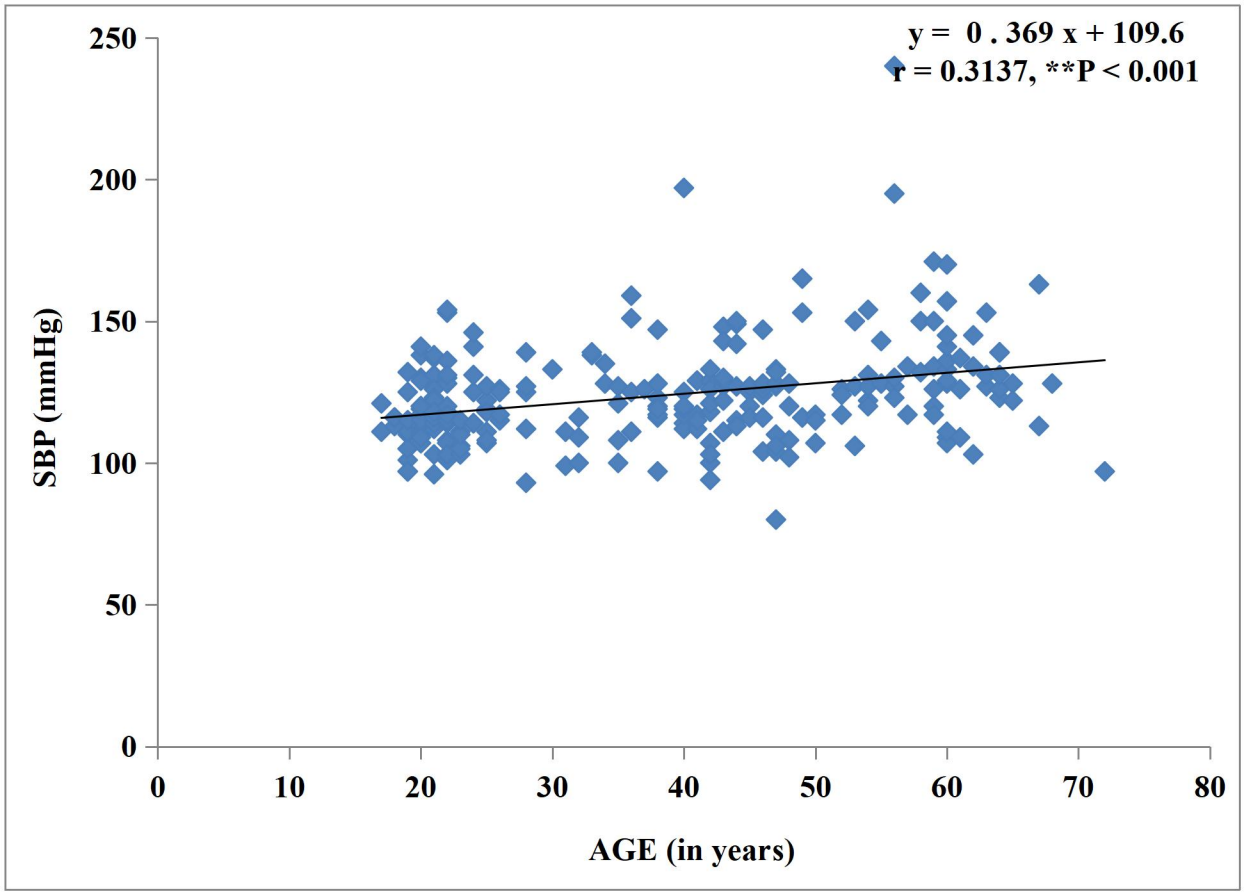

Fig. 4.6: Linear regression of Systolic Blood Pressure (SBP in mmHg) vs. Age (in years). There was significant increase in SBP ($P < 0.001$). The annual rate of increase in SBP was 0.369 mmHg/yr.

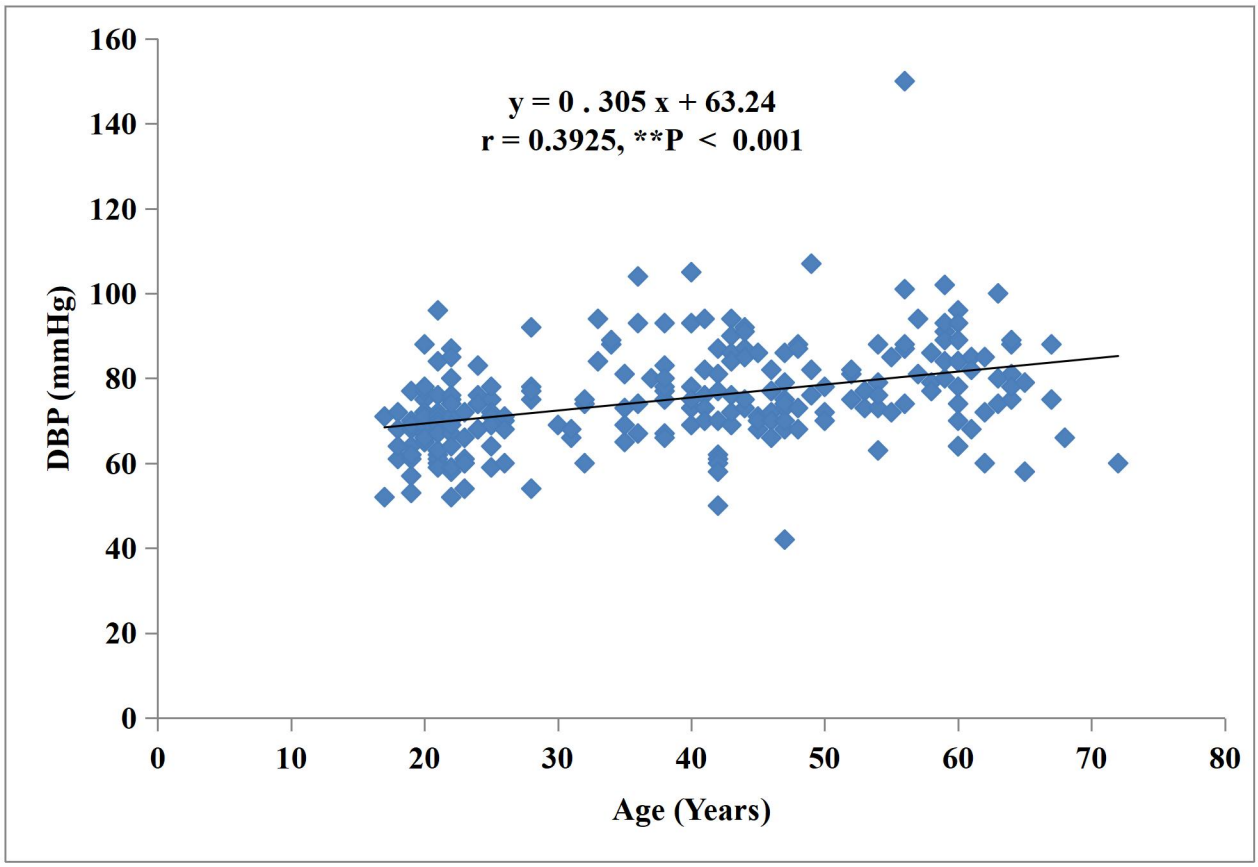

Fig. 4.7: Linear regression of Diastolic Blood Pressure (DBP in mmHg) vs. Age (in years). There was significant increase in DBP with age. The annual rate of increase in DBP was 0.305 mmHg / yr

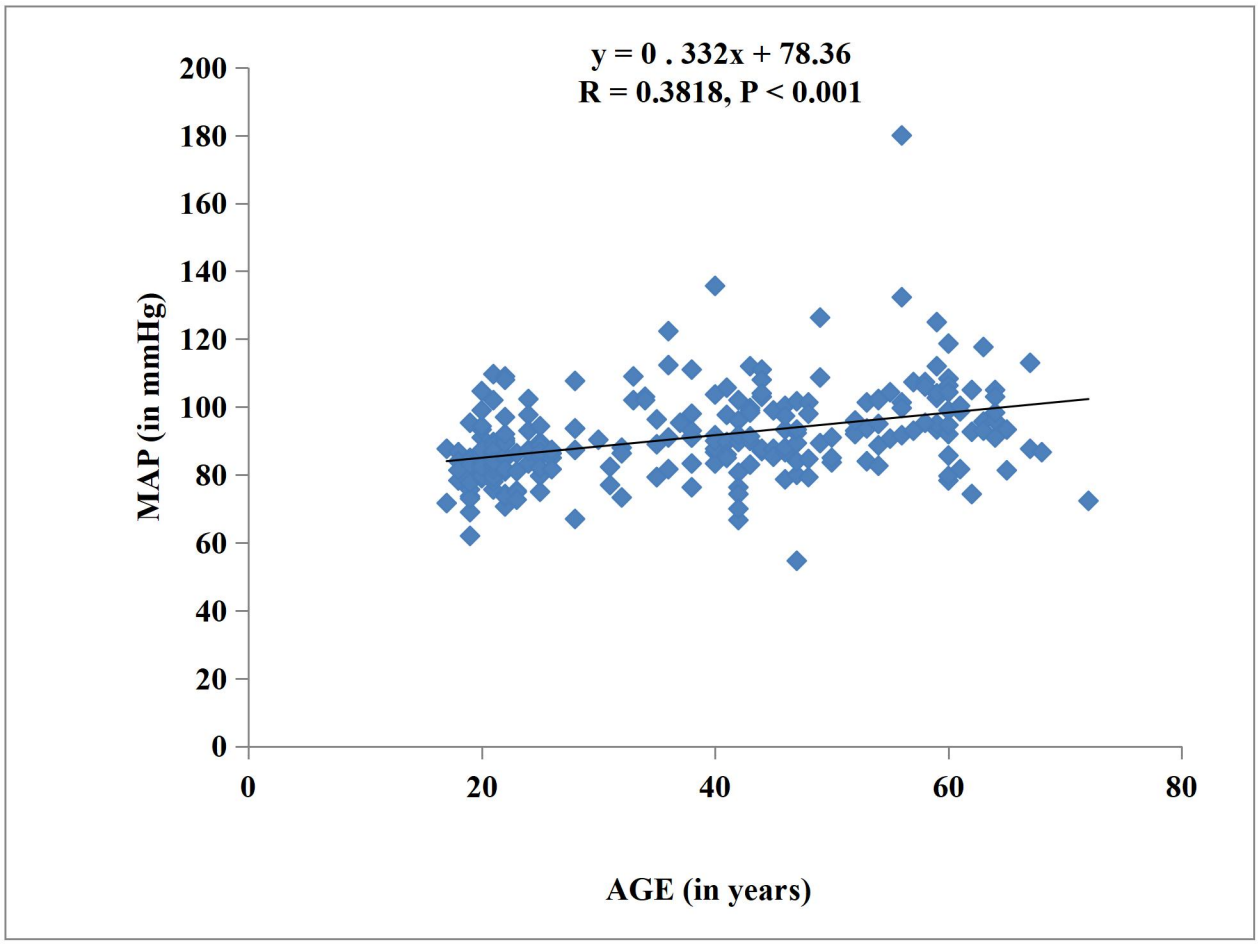

Fig. 4.8: Linear regression of Mean Arterial Pressure (MAP in mmHg) vs. Age (in years). MAP increased significantly with age ($P < 0.001$). The annual rate of increase in MAP was 0.332 mmHg /yr.

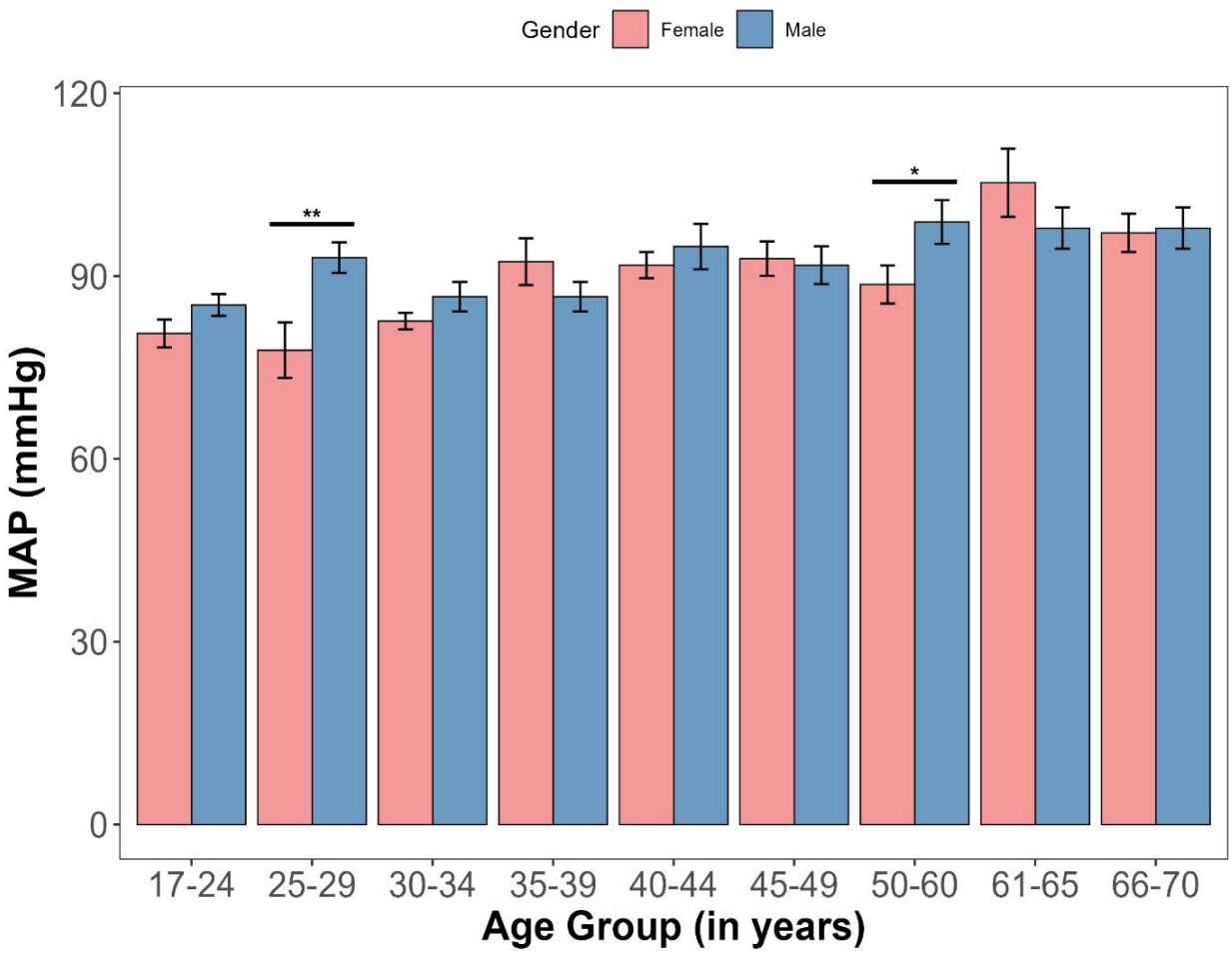

Fig. 4.9: Comparison of Mean Arterial Pressure among females and males. Increase in MAP was significantly greater in males than females in the 25-29 and 50-60 age-groups (i.e. 1st 30th and 2nd 30th age ranges, $P < 0.05$).

Table 4.2: Summary of descriptive statistics of Age and some hematological parameters

	Age (yrs)	HGB (g/dl)	HCT (%)	PLT (cells/mm ^{3})	PCT (< 0.5 ug/L)	ESR (mm/Hr)
Mean	38.37295	13.08279	40.29344	217.8361	0.307968	6.008658
± Sem	0.979716	0.125179	0.54395	8.221961	0.035089	0.279414

This table shows the Mean and Sem of Age, HGB, HCT, PLT, PCT and ESR

HGB = Blood Hemoglobin concentration in grams per deciliter

HCT = Blood Hematocrit or Packed cell volume in percentage of blood

PLT = Blood Platelet count in cells per cubic millimeter

PCT = Blood Procalcitonin concentration

ESR = Erythrocyte Sedimentation Rate in millimeter per Hour

REGRESSION GRAPHS FOR AGE AND SOME HEMATOLOGICAL PARAMETERS (Fig. 4.10 to 4.13)

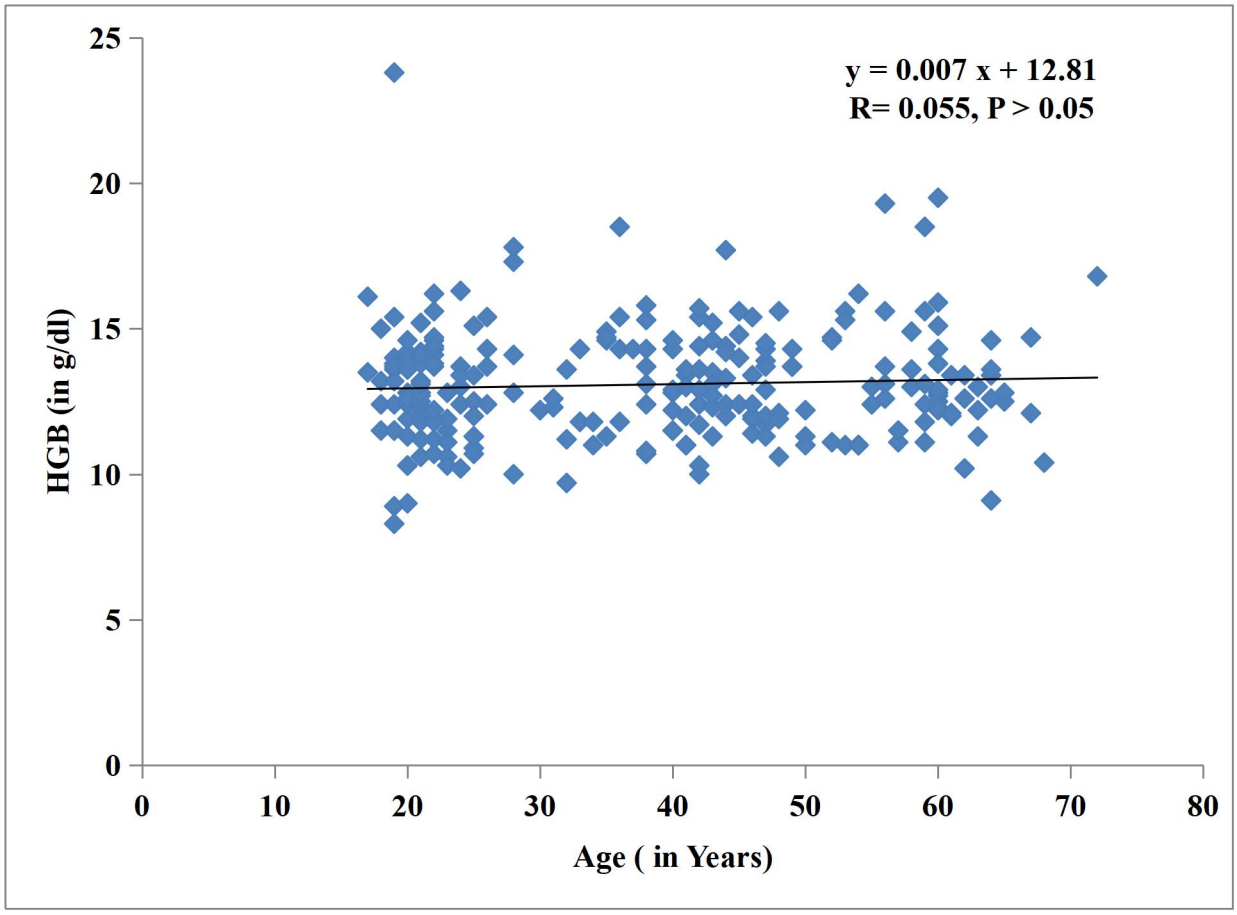

Fig. 4.10: Linear regression of Hemoglobin (HGB in g/dl) vs. Age (in years). There was no significant increase in HGB with Age. The annual increase in HGB was 0. 007 g /dl/yr.

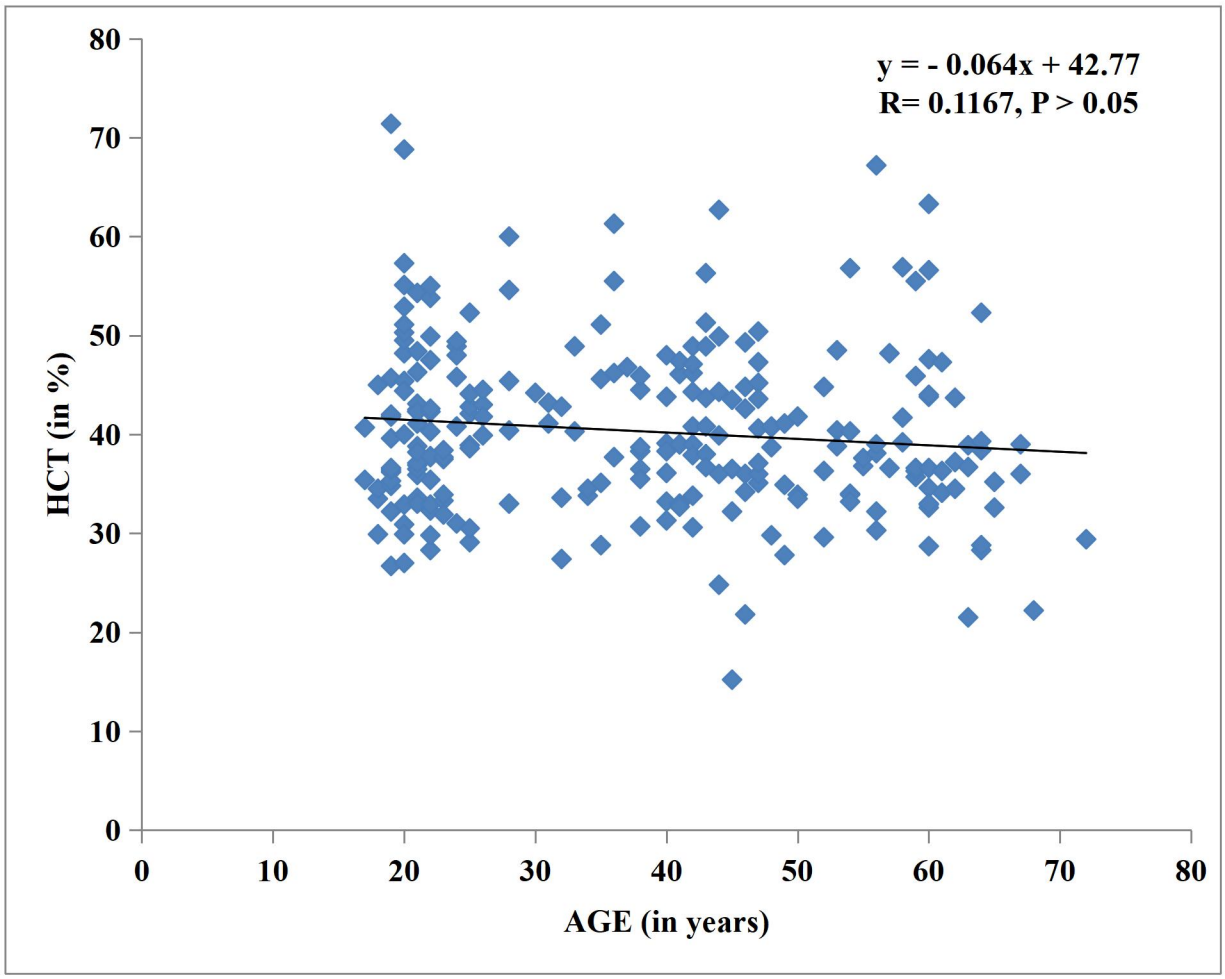

Fig. 4.11: Linear regression of Hematocrit vs. Age (HCT in years). There was no significant decrease in HCT with Age. The annual rate of decrease in HCT was 0 .064%/yr.

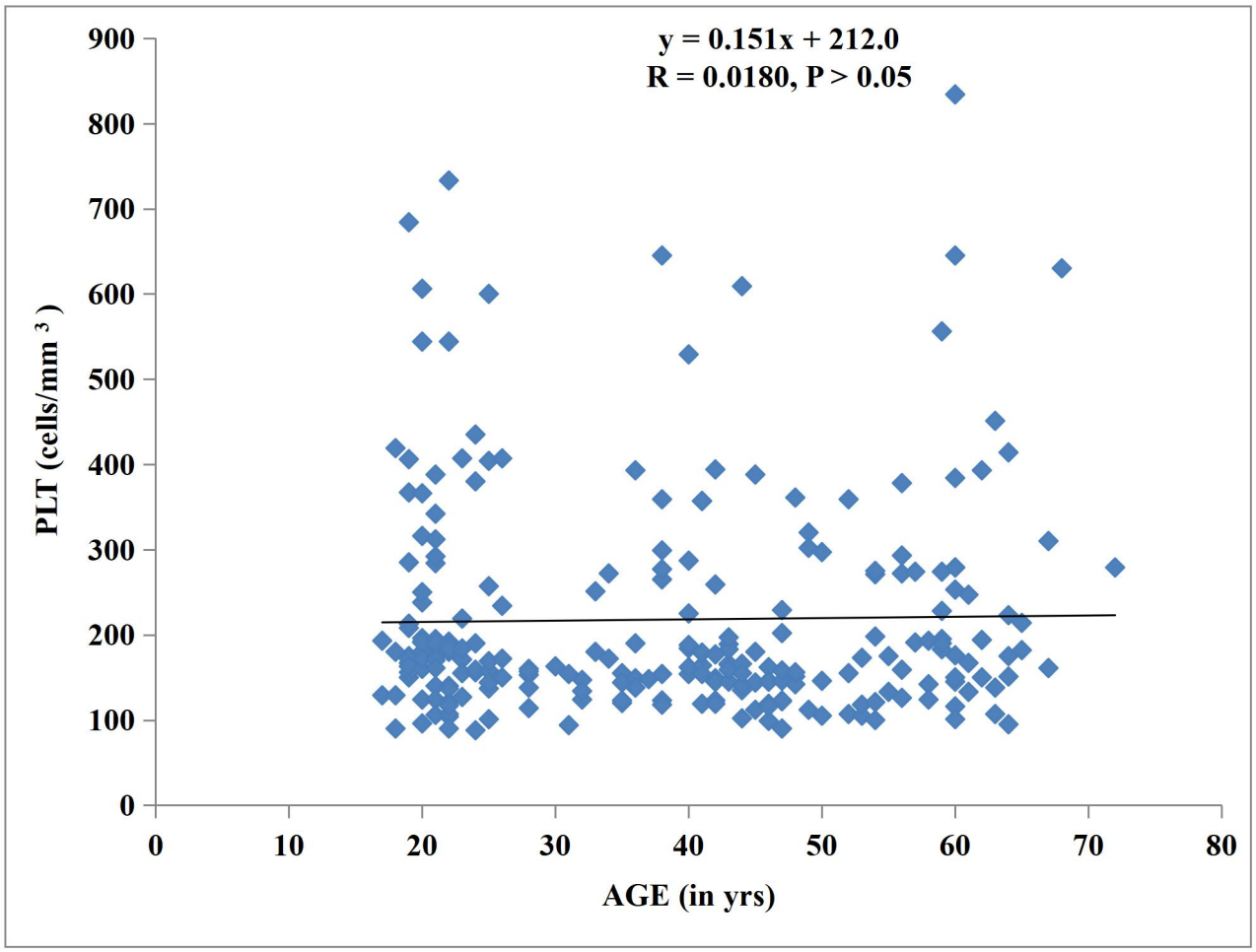

Fig. 4.12: Linear regression of Platelet count (PLT in cells/mm^3) vs. Age (in Years). There was no significant increase in PLT with Age. The annual rate of increase in PLT was 0.151 cells / mm ^{3}/yr.

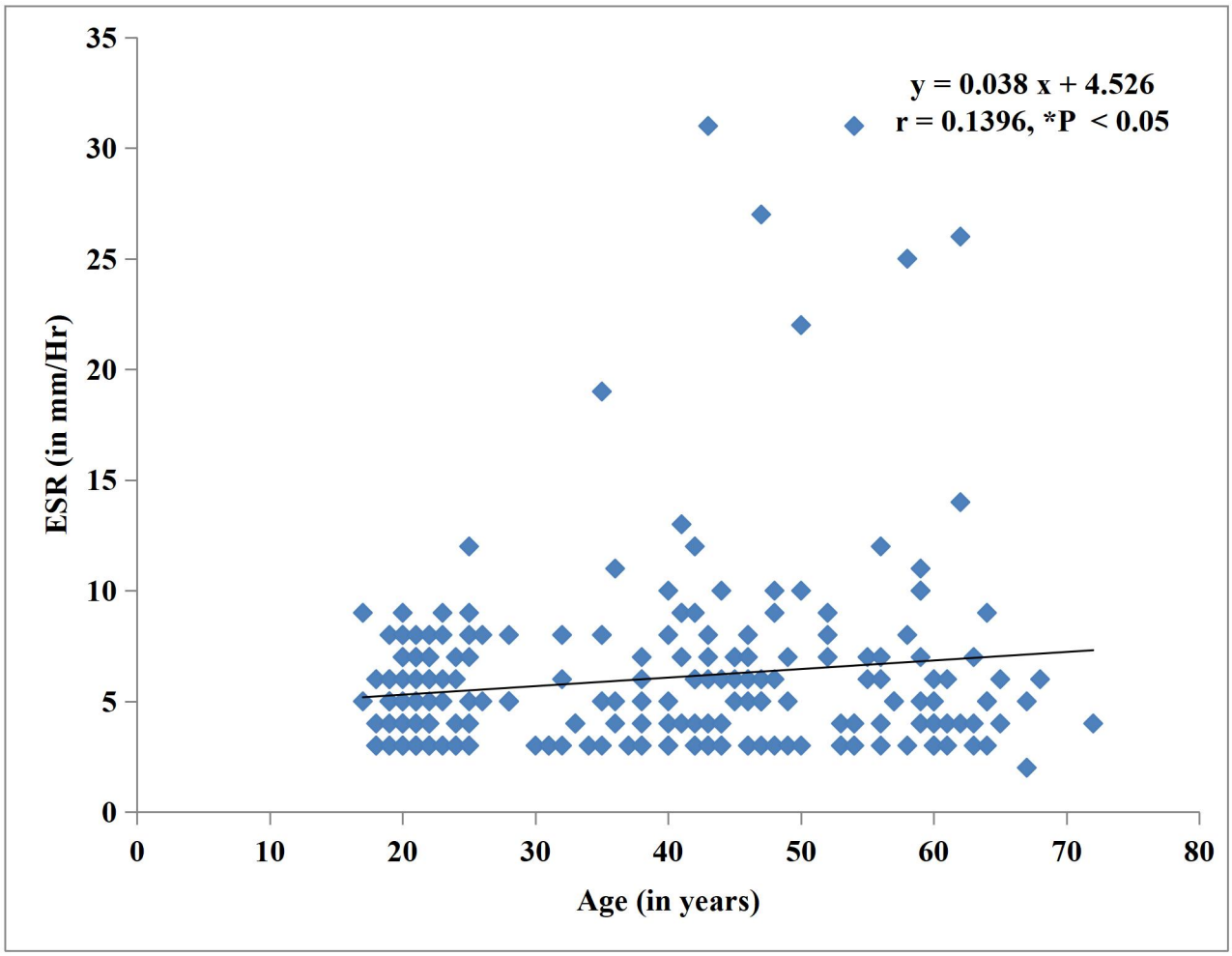

Fig. 4.13: Linear regression of Erythrocyte Sedimentation Rate vs. Age (in Years). There was significant increase in ESR with Age ($P < 0.05$). The annual rate of increase in ESR was 0.038 mm / Hr. /yr (Westergreen).

RESULTS FOR SOME KIDNEY FUNCTION TESTS

Table 4.3: Descriptive Statistics of Age and some Kidney Function Parameters

	Age (yrs)	UCr (mg/dl)	UCR (g/dl)	UrALB (mg/dl)	UrALB/ UCR ratio (mg/g)	SCr (mg.dl)	All CG (ml/min)
Mean	38.35	157.92	0.16	10.92	81.12	1.05	85.22
± Sem	0.99	3.77	0.00	0.37	3.38	0.02	1.69

UCr = urine creatinine in mg/dl

UCR = urine creatinine in g/dl

UrALB = urine Albumin excretion in mg/dl)

UrALB/CR = Urine Albumin: Creatinine ratio or UACR in mg/g

SCr = Serum Creatinine in mg/dl

All CG = Cockroft-Gault Creatinine clearance in ml/min/1.73m ^{2} for males and females

RESULTS FOR SOME KIDNEY FUNCTION TESTS (continued)

Table 4.4: Descriptive Statistics of Age and some Kidney Function Parameters (continued)

	All CKD-EP!Cr (ml/min)	All MDRD (ml/min)	UCr (mg/dl)	Ur flow rate (ml/min)	All CrCl (ml/min)	Urine Na+/K+ ratio
Mean	82.95	93.44	157.79	0.83	124.86	4.29
± Sem	1.27	1.01	3.71	0.03	5.09	0.19

All CKD-EP!Cr = NKF CKD-EP! Creatinine eGFR equation, ml/min/1.73m ^{2} for males and females

All MDRD = Modification of Diet in Renal Disease eGFR equation in ml/min/1.73m ^{2} for males and females

All UCr = Urine Creatinine, mg/dl)

All Urine flow rate ml/min for males and females

All SCr = Serum Creatinine in mg/dl for males and females

All mCrCl = measured Creatinine Clearance in ml/min/1.73m ^{2} or mGFR for males and Females

All Urine Na^+/K^+ ratio = Sodium : Potassium ratio for males and females

Anova and Regression graphs for Age and Renal Function Parameters (Fig. 4.14 to 4.25)

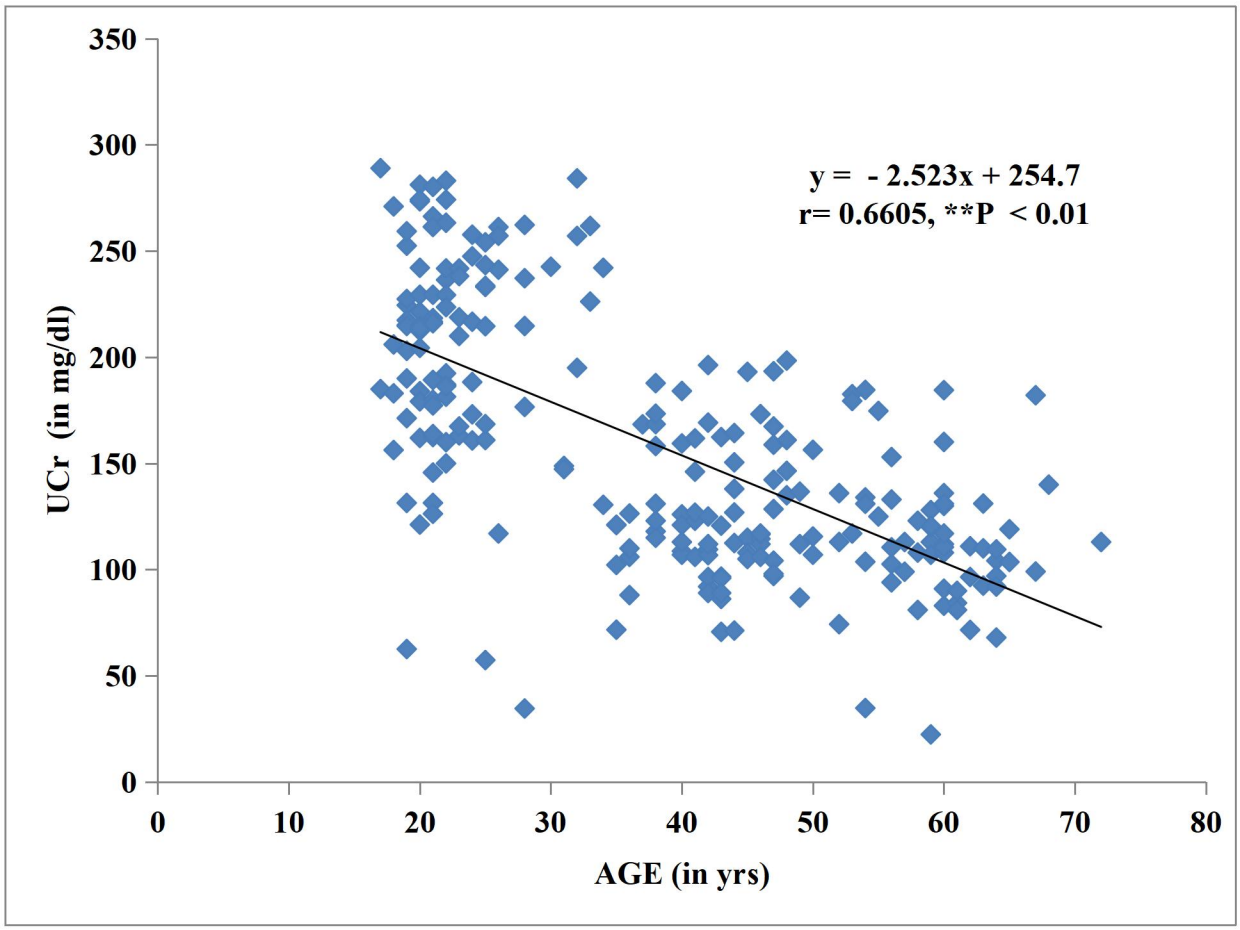

Fig. 4.14: Linear regression of Urine Creatinine (in mg/dl) vs. Age (in years). There was significant reduction in Urine Creatinine as age increased ($P < 0.01$). Urine creatinine declined at the rate of 2.523 mg / dl /yr.

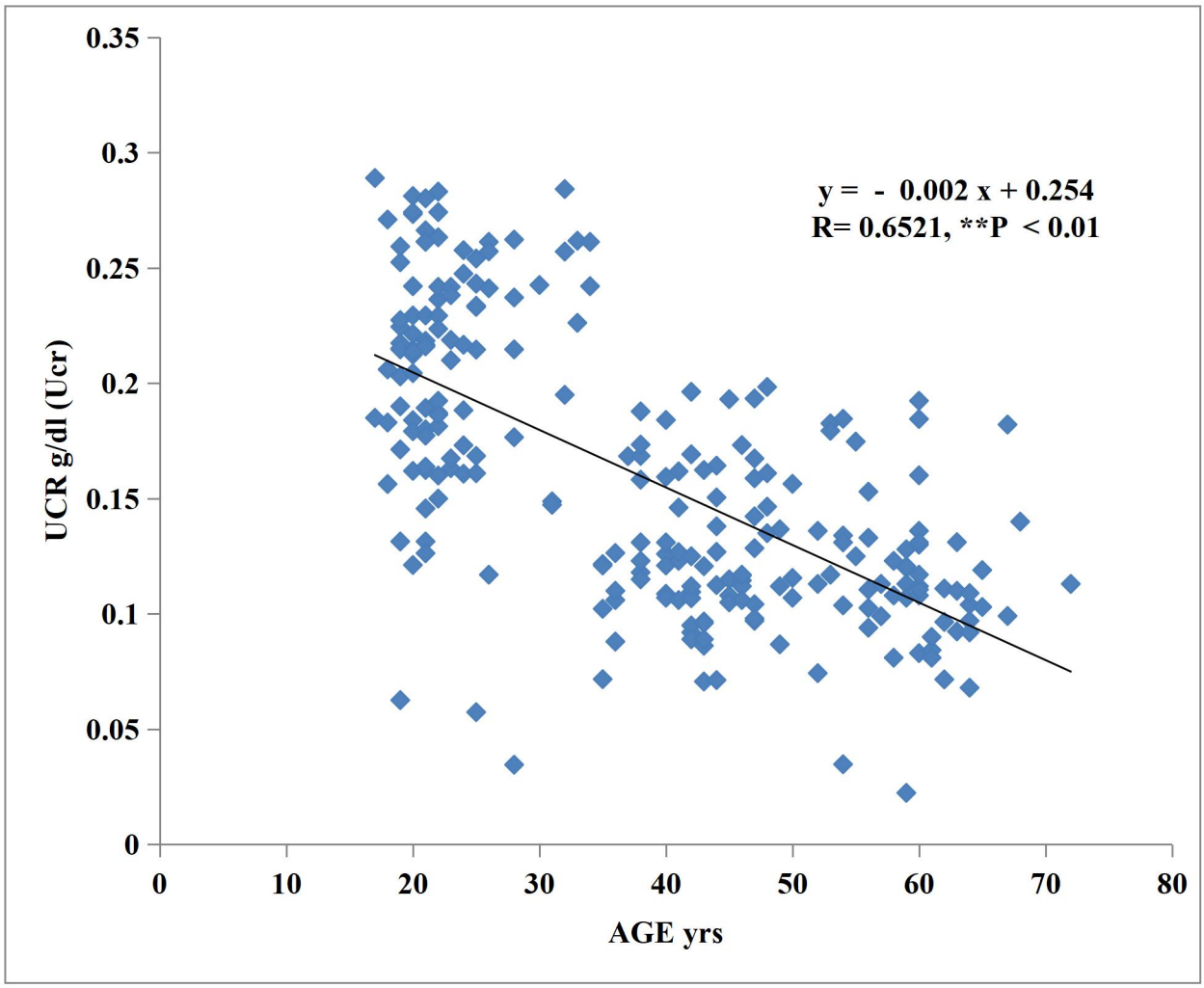

Fig. 4.15: Linear regression of Urine Creatinine (in g/dl) vs. Age (in years). There was significant decline in Urine Creatinine as age increased (P < 0.01). It declined at the rate of 0.002g/dl/yr.

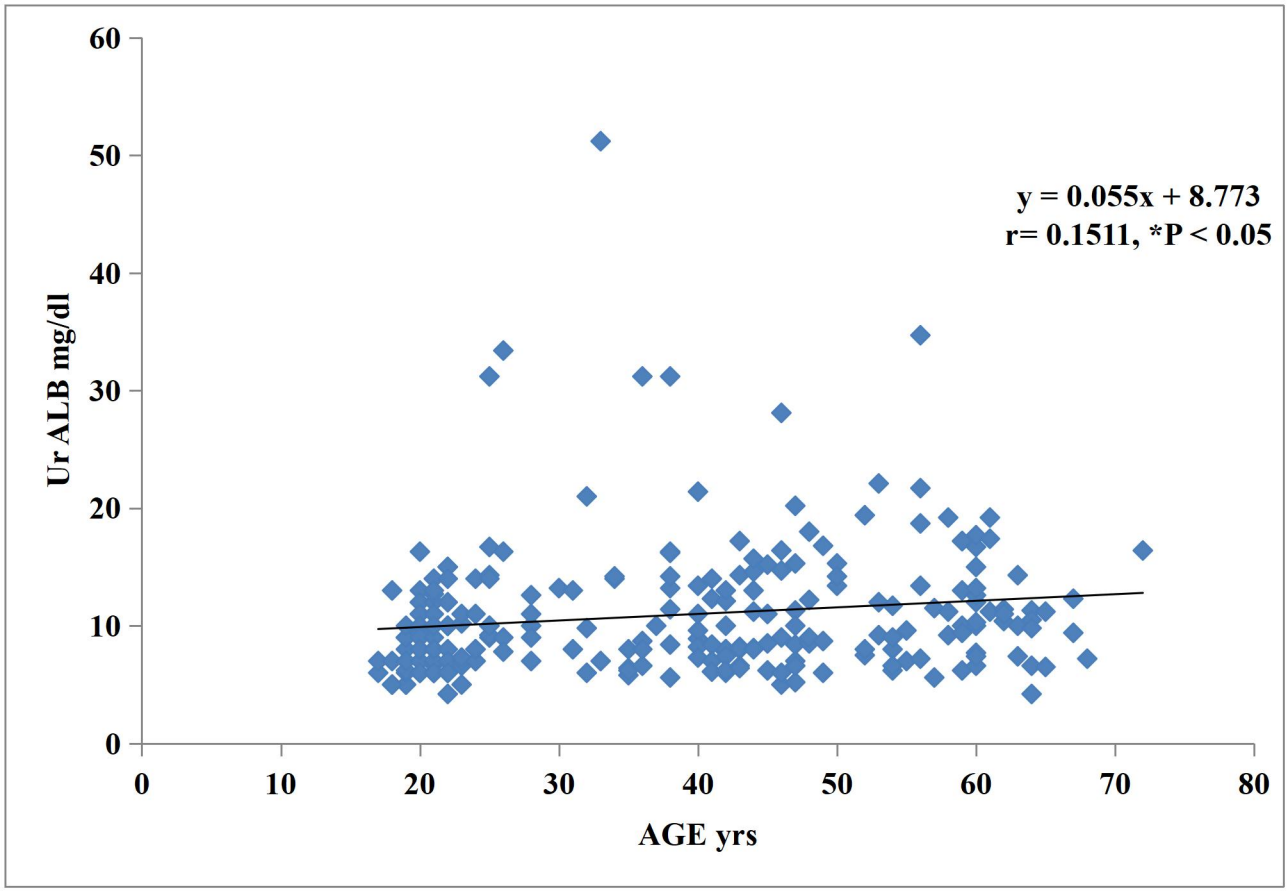

Fig. 4.16: Linear regression of UrALB excretion (in mg/dl) vs. Age (in years). There was significant increase in Urine Albumin excretion in mg/dl as age increased ($P < 0.05$). The rate of increase was 0.055 mg/dl/yr.

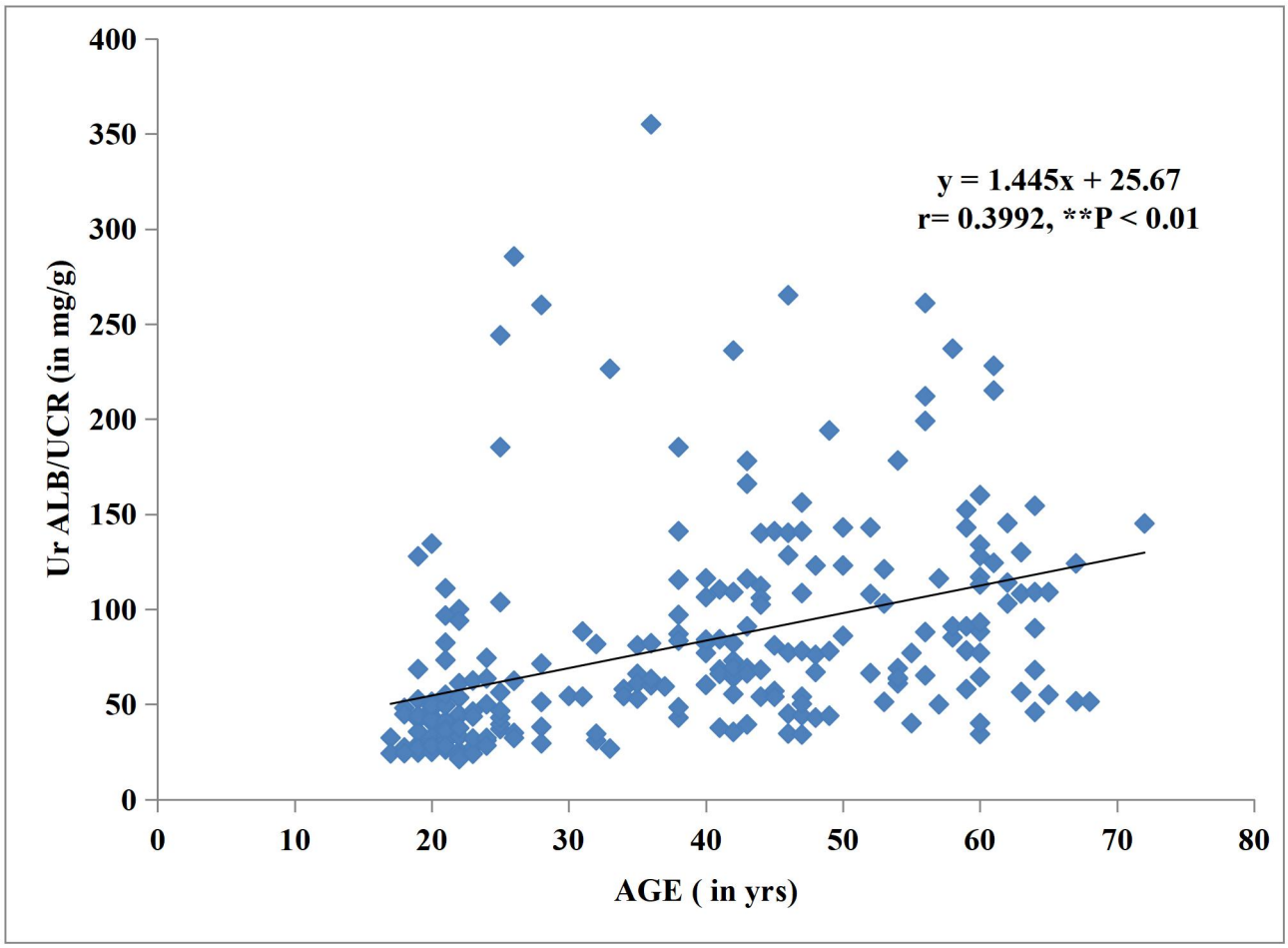

Fig. 4.17: Linear regression of Urine ALB/UCR vs. Age (Years). There was significant increase in urine Albumin: Creatinine ratio as age increased ($P < 0.01$). The rate of increase was 1.445 mg/g/yr.

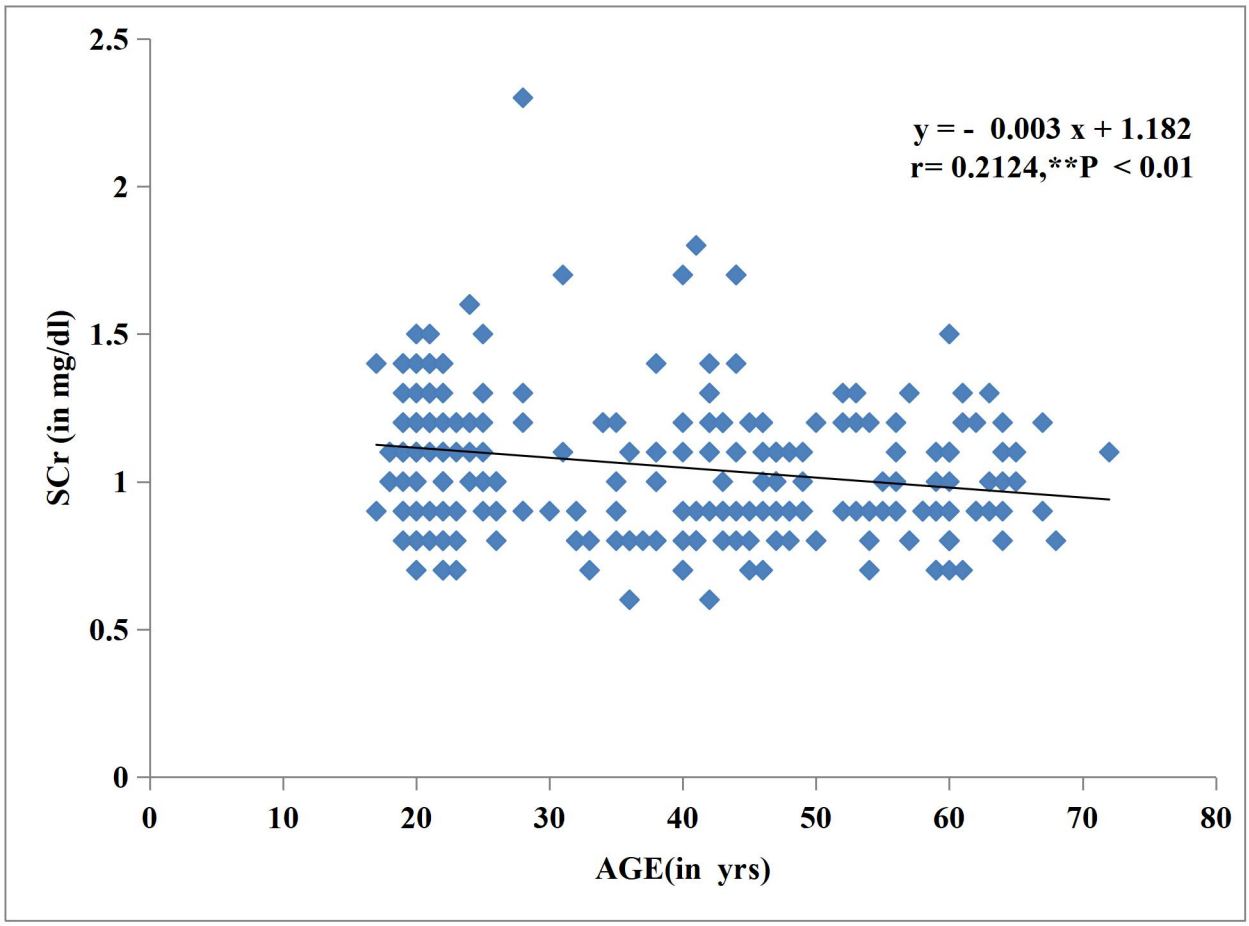

Fig. 4.18: Linear regression of Serum Creatinine on Age (years). There was significant decrease in Serum Creatinine (in mg/dl) as age of subjects increased ($P < 0.01$). The rate of decrease was 0.003 mg/dl/yr.

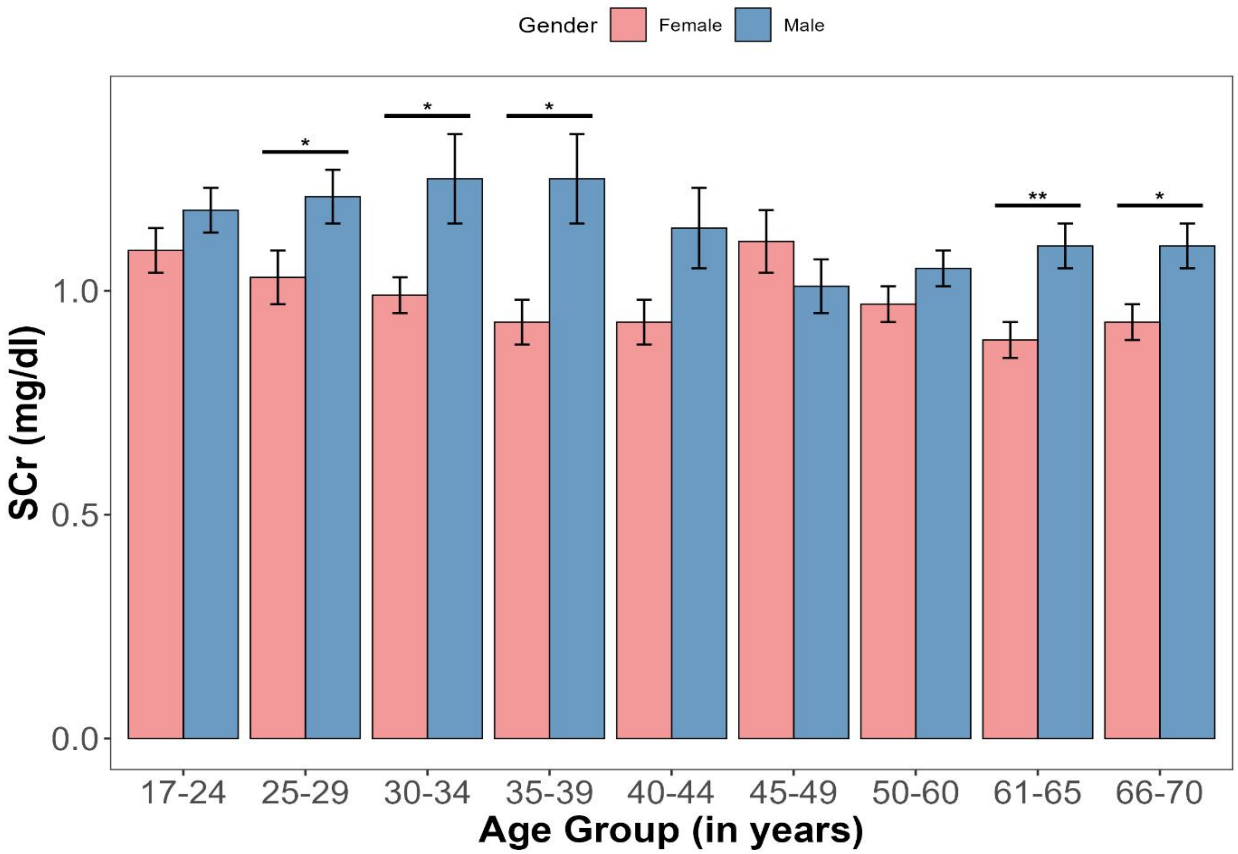

Fig. 4.19: Shows ANOVA presentation of Serum Creatinine for male and female subjects Age group categories (in years). Male subjects had significantly higher Serum Creatinine (in mg/dl) than females for most of the Age group categories ($P < 0.01$).

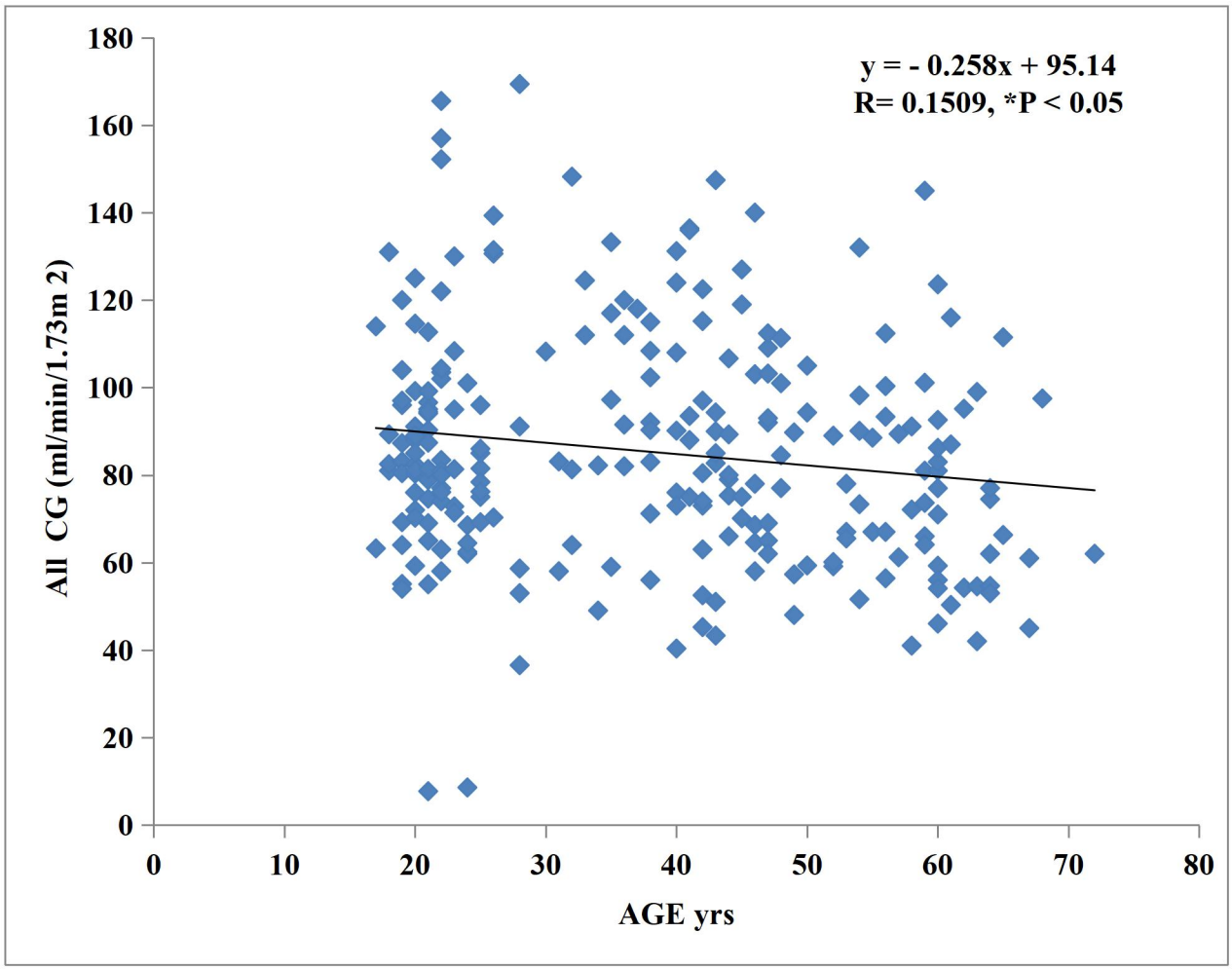

Fig. 4.20: Linear regression of All Cockroft-Gault eGFR vs. Age (in years) for male and female subjects. There was significant decline in eGFR ($P < 0.05$). The rate of decrease was 0.258 ml/min/1.73m^2 /yr for all the subjects.

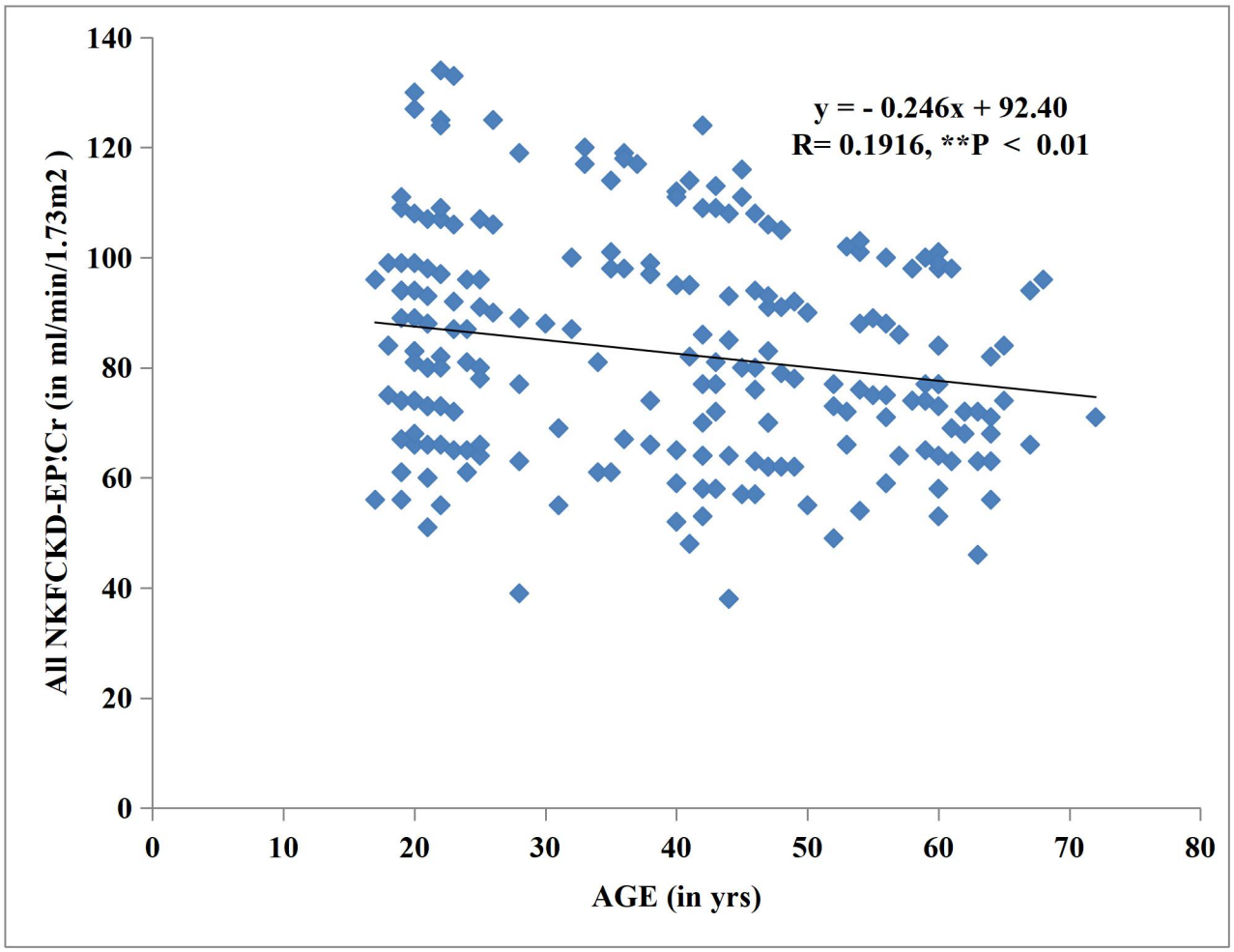

Fig. 4.21: Linear regression of All NKF CKD-EP! Cr 2021 eGFR (ml/min/1.73m ^{2}) vs. Age (in years) for all male and female subjects. There was significant decline in eGFR ($P < 0.01$). The rate of decline was 0.246 ml/min/1.73m^2 /yr for all the subjects.

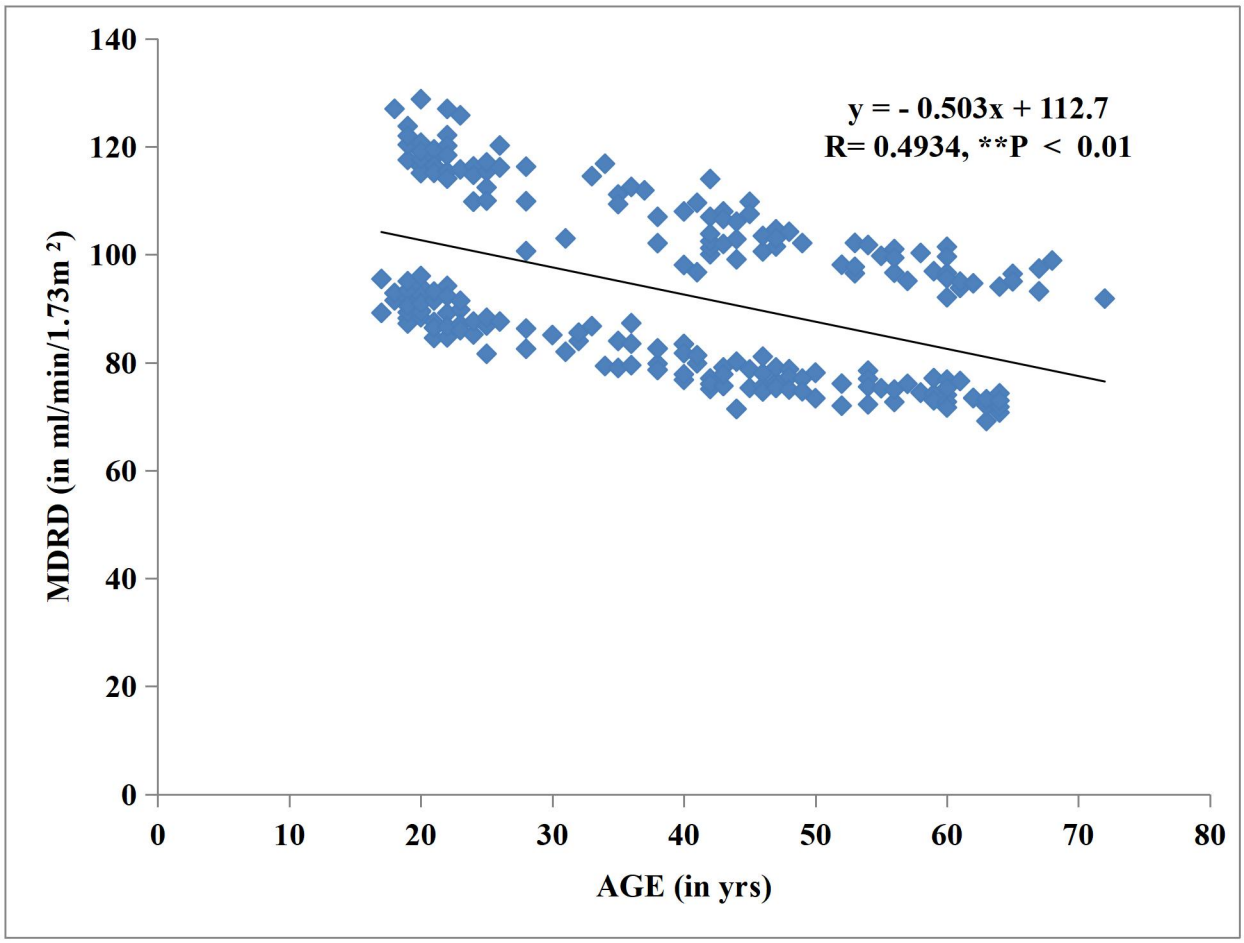

Fig. 4.22: Linear regression of All MDRD eGFR on Age (Years) for male and female subjects. There was significant annual decline in eGFR ($P < 0.01$). The eGFR declined at the rate of 0.503 ml/min/1.73m^2/yr.

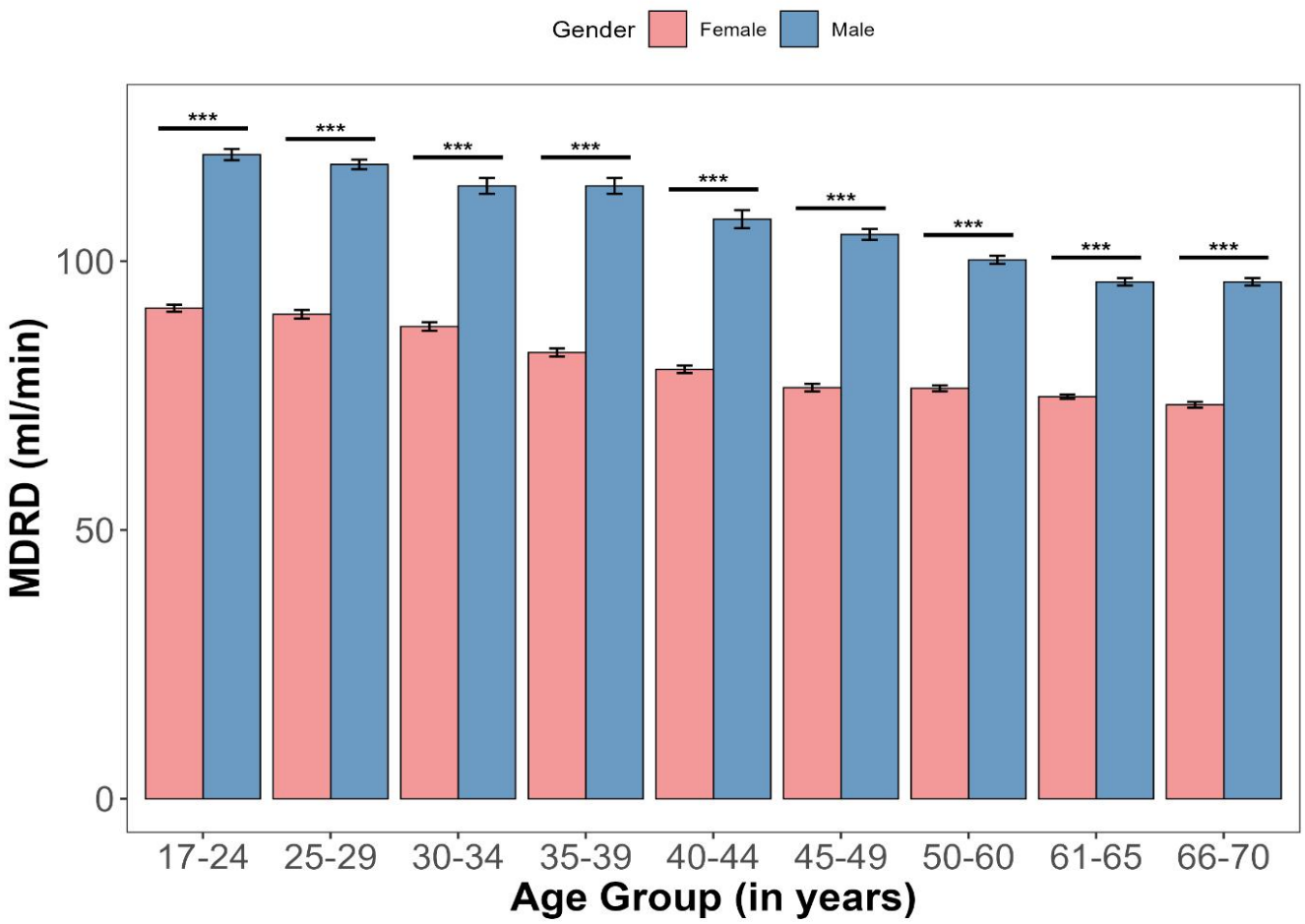

Fig. 4.23: Shows ANOVA presentation of All MDRD eGFR vs. Age (Years) for male and female subjects. This showed significant difference in eGFR for male and female subjects for various age groups in years ($P < 0.01$).

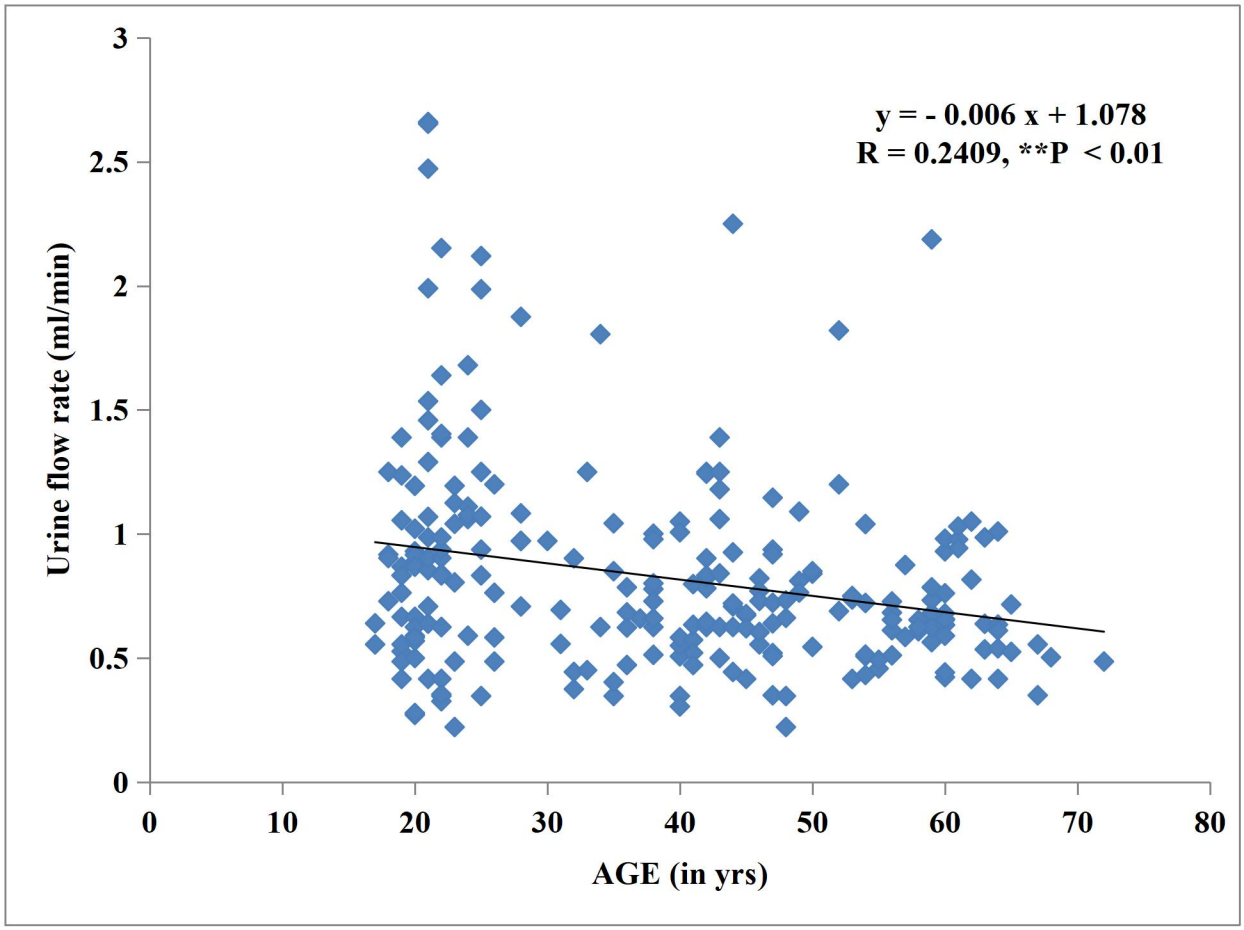

Fig. 4.24: Linear regression of Urine flow rate of all male and female subjects (in ml/min) vs. Age (in years). There was significant annual decrease in Urine flow rate as age of subjects ($P < 0.01$). The decline in urine flow rate was 0.006 ml/min/yr.

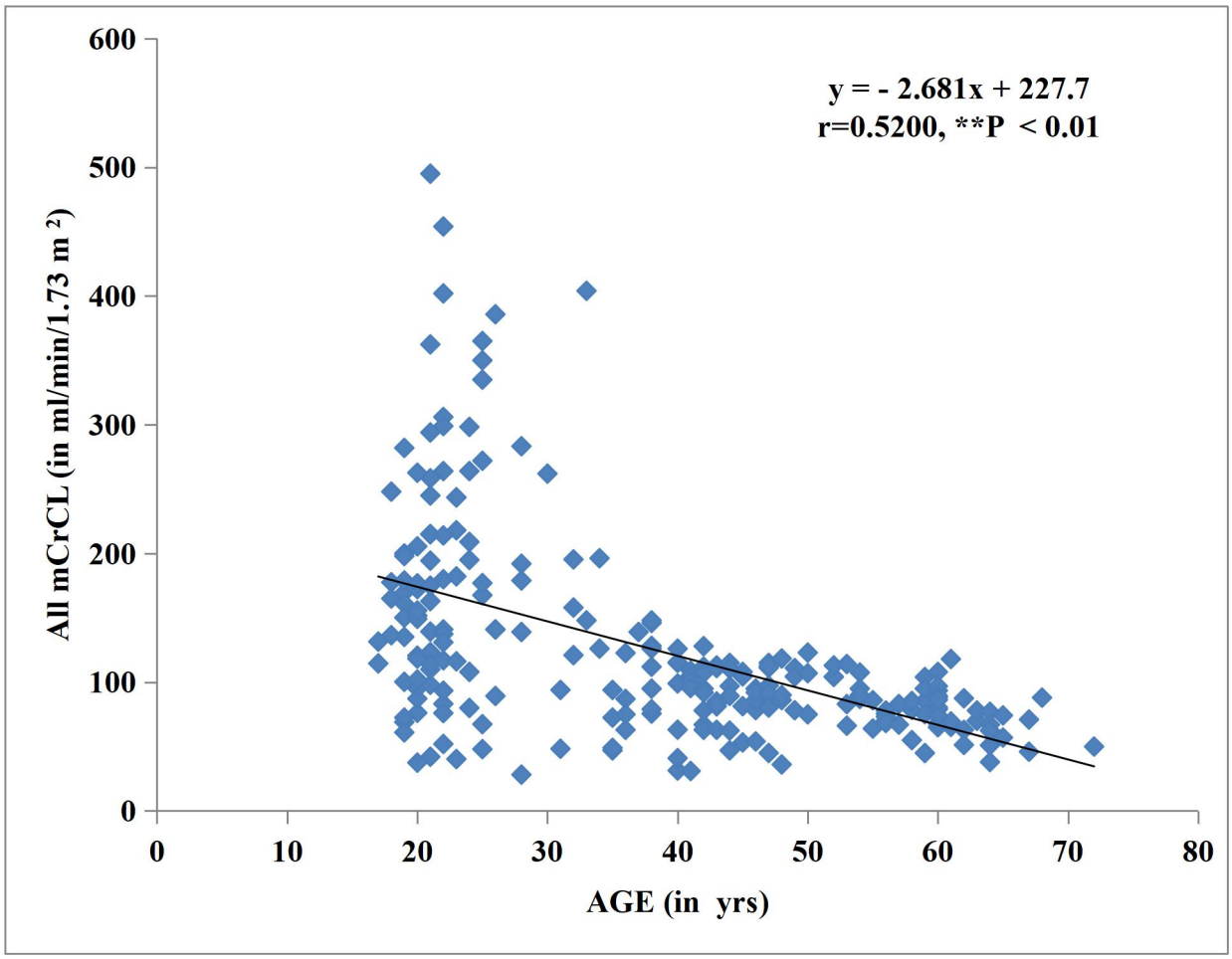

Fig. 4.25: Linear regression of All measured Creatinine clearance (in ml/min/1.73 m ^{2}) vs. Age (in years). There was significant decline in mGFR as age increased ($P < 0.01$). The annual rate of decline was 2.681 ml/min/1.73m ^{2}/yr.

Table 4.5: Descriptive statistics for Age and Cystatin C based Parameters

	Age (yrs)	Se Cyst C (nmol/l)	Serum Cystatin C (mg/l)	eGFR Simple Cyst C eq. (ml/min)	eGFR Cyst C alone eq. (ml/min/1.73 m ^{2})	Se Cr (mg/dl)	eGFR for combined NKF CKD-EP! Cyst C + Cr (ml/min/1.73 m ^{2})
Mean	45.11	90.38	1.21	104.47	72.90	1.03	79.62
± Sem	1.51	5.59	0.07	6.08	3.88	0.03	2.64

Se Cyst C = Serum Cystatin C in nmol/l

Serum Cystatin C = Serum Cystatin C in mg/l

eGFR Simple Cystatin C eq = estimated Glomerular Filtration Simple Cystatin C equation in ml/min/1.73 m ^{2}

Se Cr = Serum Creatinine

Combined NKF CKD -EP! cyst c + scr eq. = Combined National Kidney Foundation Chronic kidney disease Epidemiology Cystatin C and Creatinine equation

Regression Graphs for Cystatin C based eGFR (fig. 4.26 to 4.31)

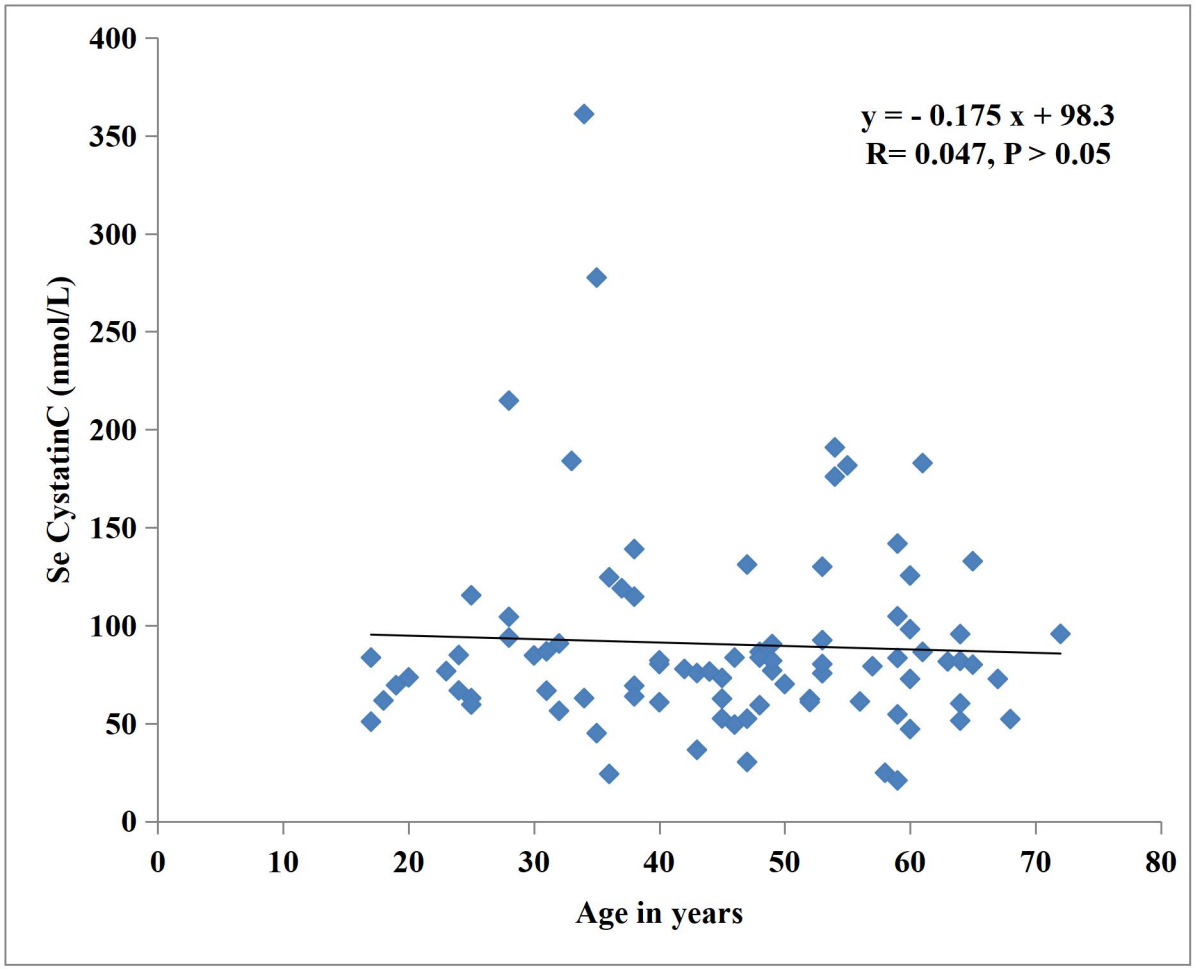

Fig. 4.26: Linear regression of Serum Cystatin C (in nmol/l) for male and female subjects vs. Age (in years). The annual rate of decline in Serum Cystatin C was not significant (P > 0.05).

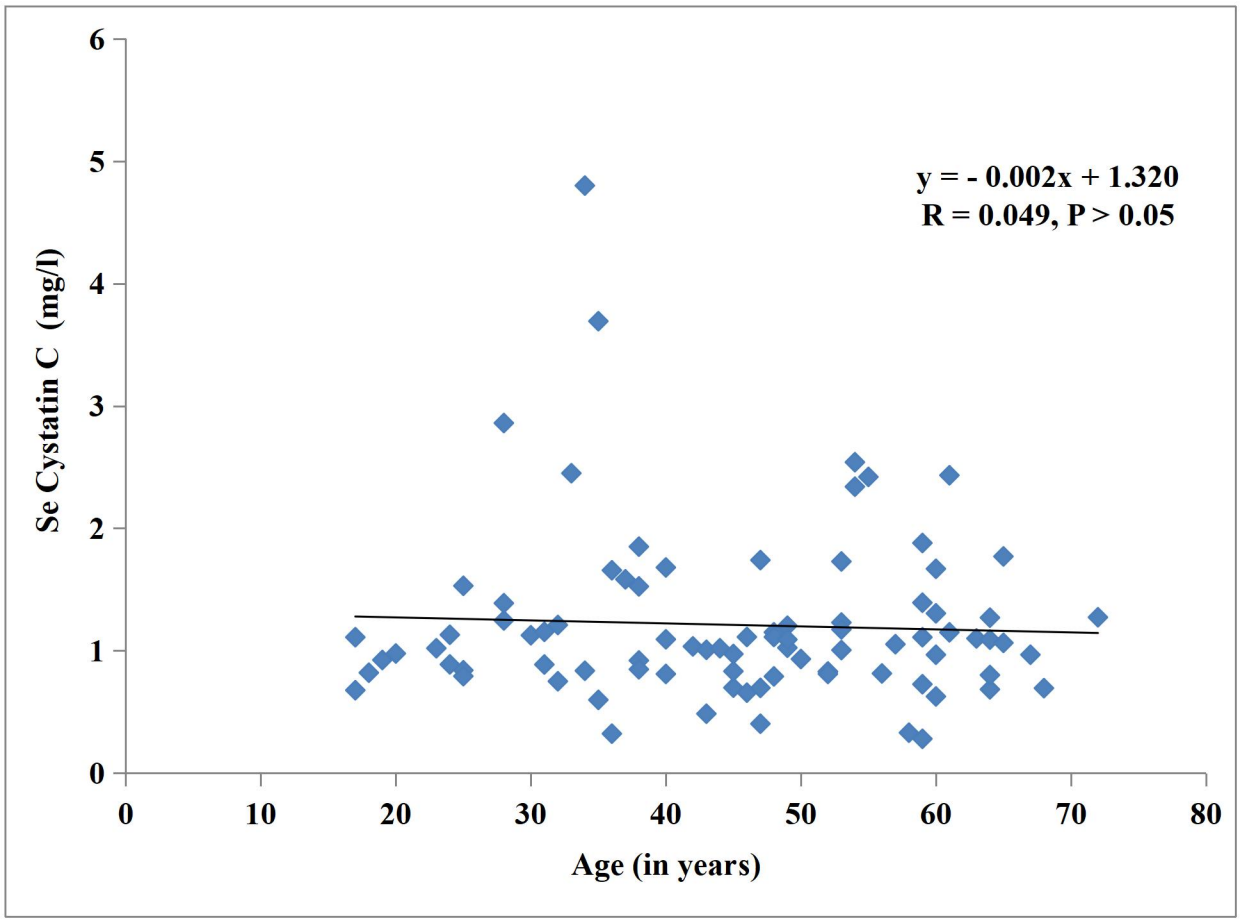

Fig. 4.27: Linear regression of Serum Cystatin C (in mg/l) for male and female subjects vs. Age (in years). The annual rate of decline in Serum Cystatin C was not significant (P > 0.05).

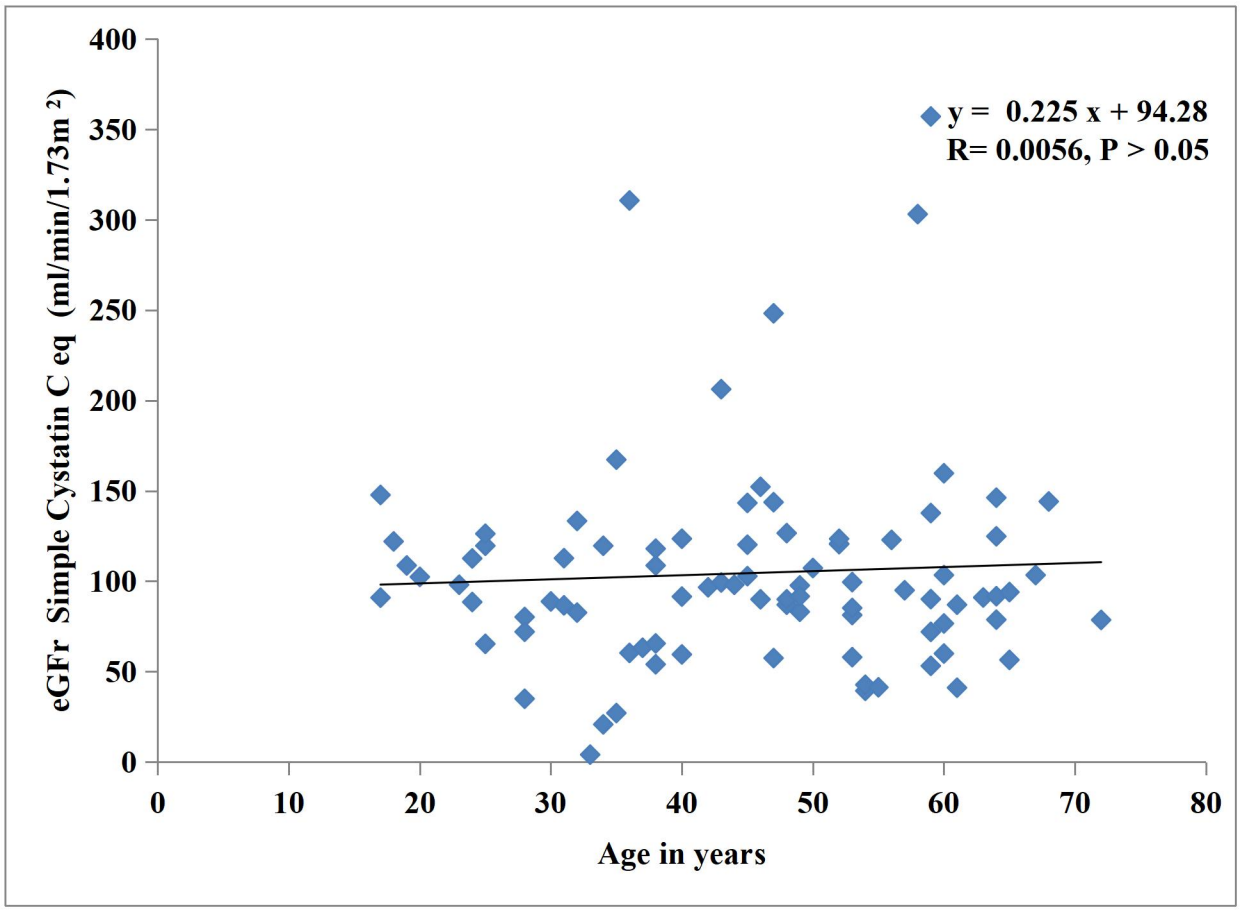

Fig. 4.28: Linear regression of Simple Cystatin C eGFR equation (in ml/min/1.73m ^{2}) vs. Age (in years). There was annual increase in GFR which was not significant (P > 0.05).

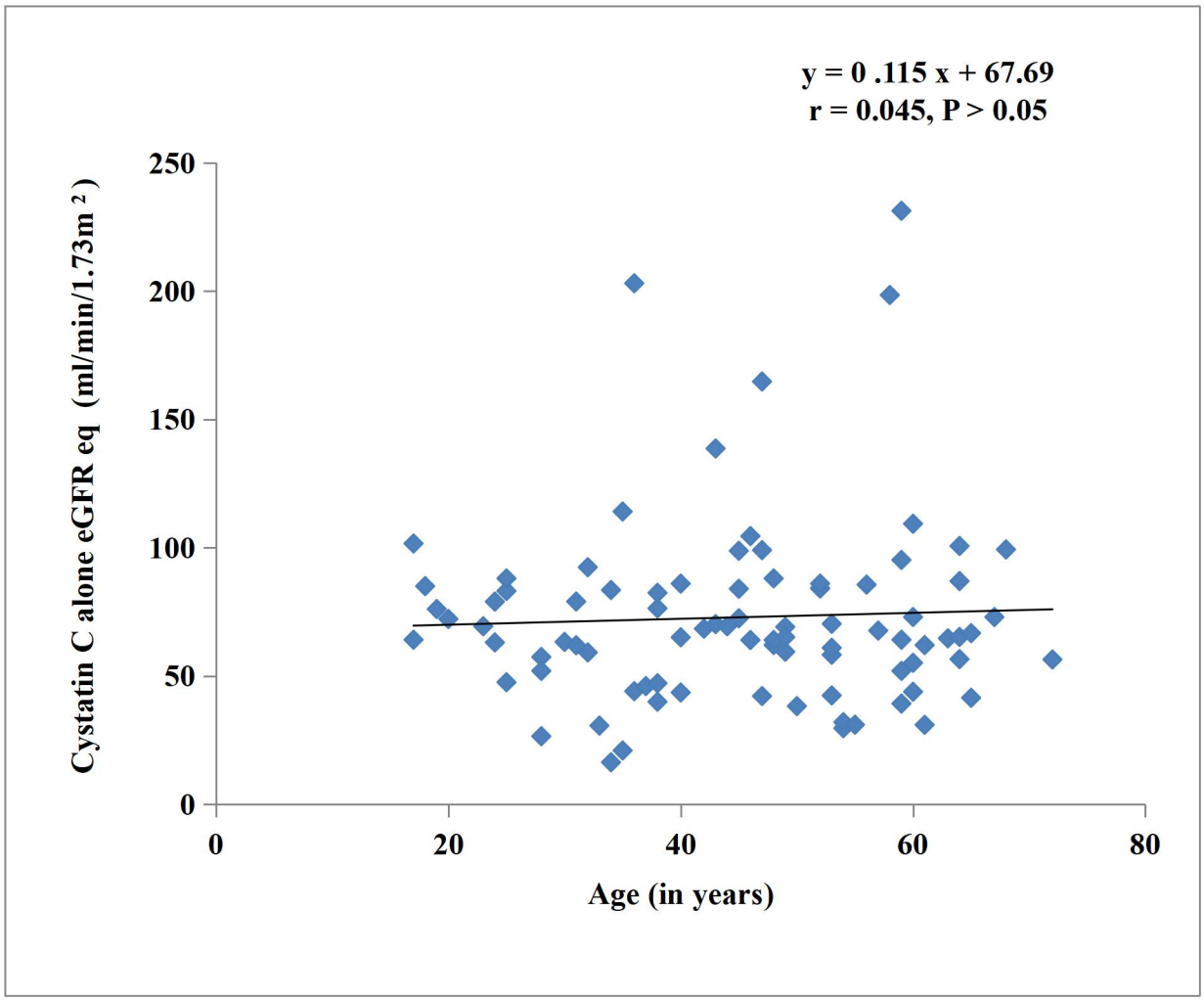

Fig 4.29: Linear regression of Cystatin C alone eGFR vs. Age (in years). There was no significant annual increase or decline in eGFR (P > 0.05).

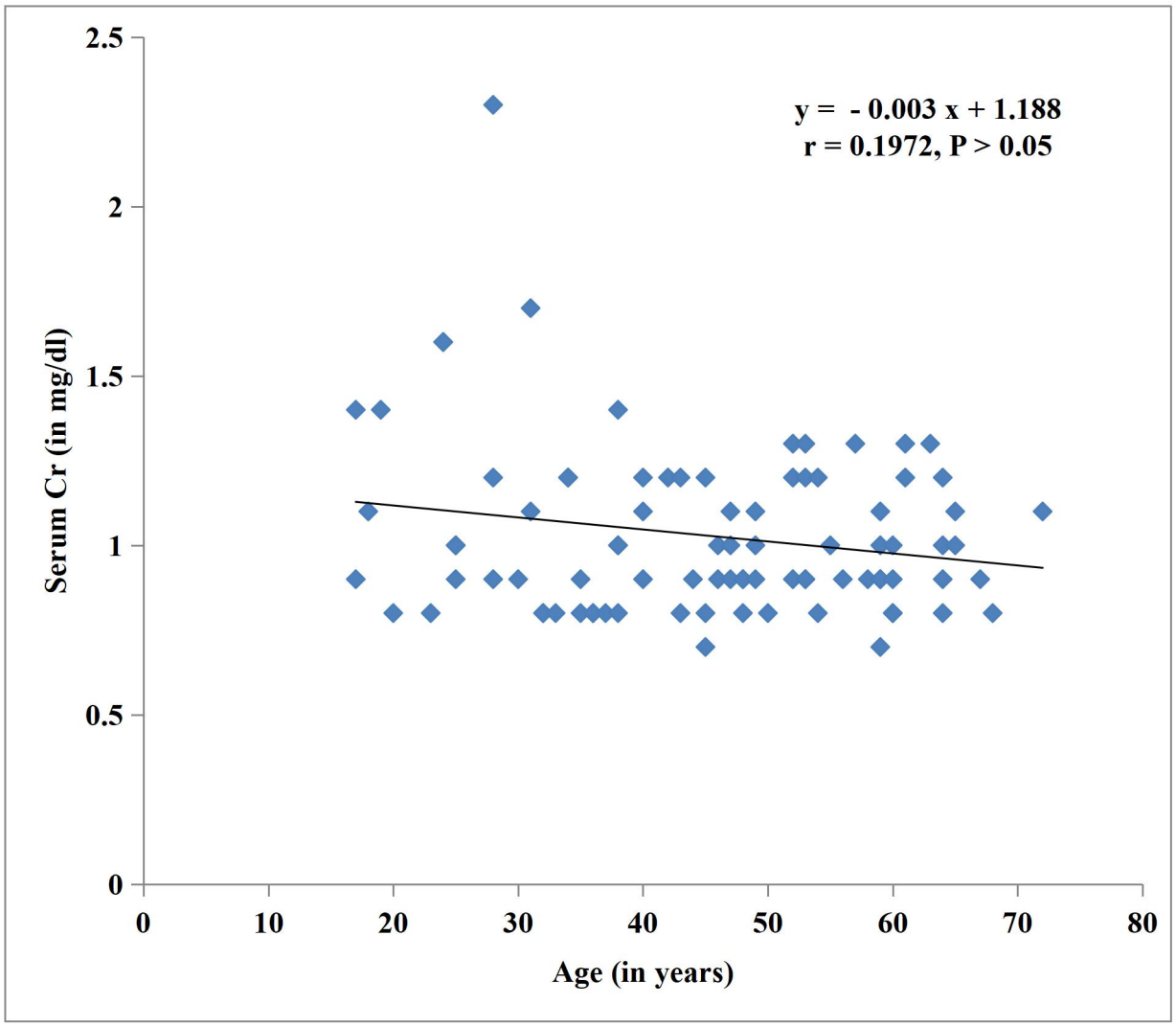

Fig 4.30: Linear regression of Serum Creatinine for male and female subjects vs. Age (in years). The annual decline in Serum Cr was not significant (P > 0.05).

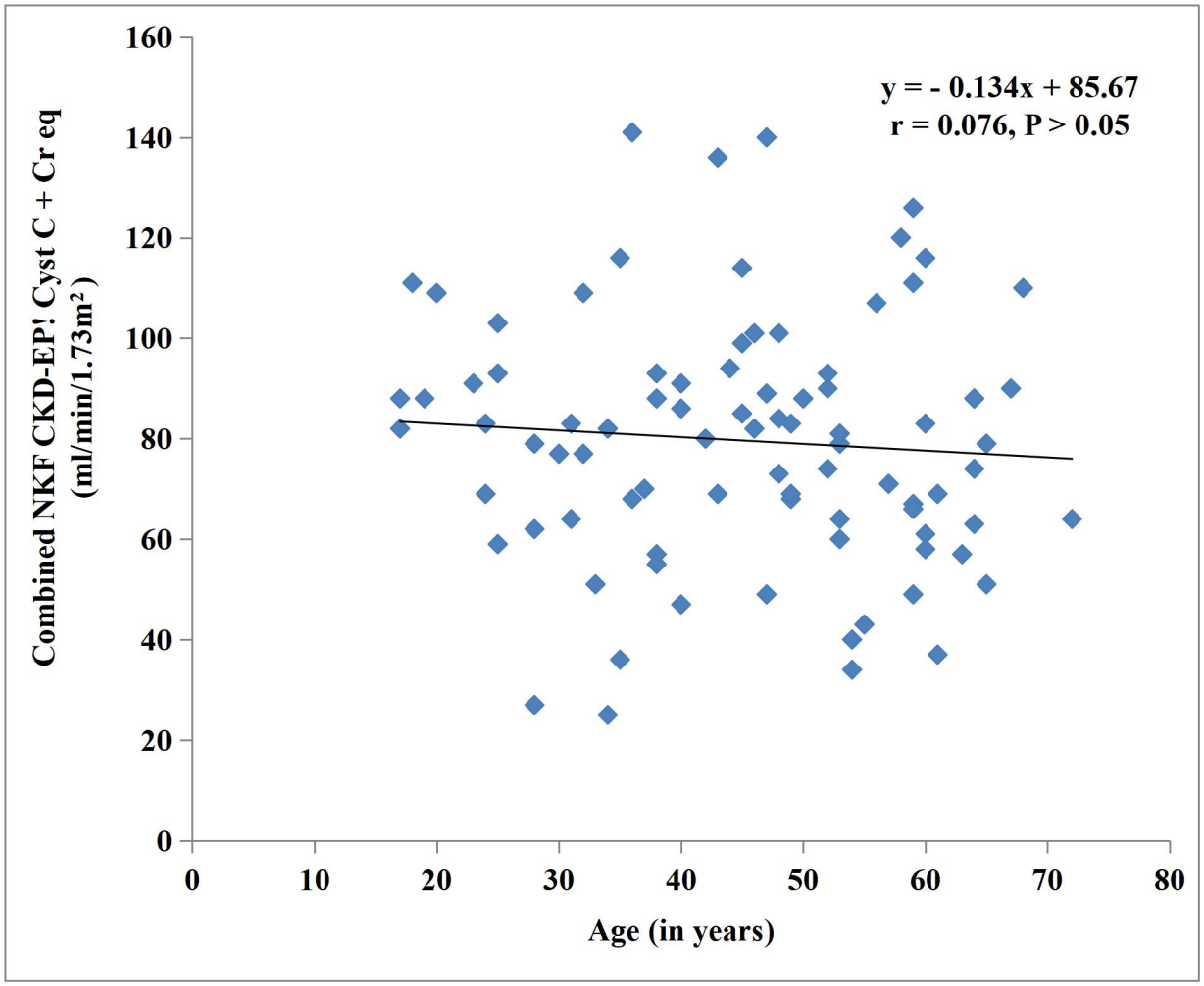

Fig. 4.31: Linear regression of combined NKF CKD-EP! CystatinC+ creatinine equation vs. Age (in years) for 88 male and female subjects. The decline in eGFR was not significant.

Table 4.6: Showing Descriptive analysis of Age, MDRD and Urine Peptidomic molecules for 88 male and female subjects

	Age (in years)	MDRD eGFR (ml/min/1.73m^2)	Hua-1m (ng/ml)	MCP-1 (ng/ml)
Mean	34.23	97.53	0.88	2.23
± Sem	1.51	1.66	0.0056	0.05

This table shows the Mean ± Sem of parameters for

MDRD eGFR = Modification of Diet in Renal Disease equation in ml/min/1.73 m ^{2}

MCP-1 = Urine Monocyte Chemoattractant protein in ng/ml

Hua-1m = Human urine Alpha-1-microglobulin in ng/ml

Regression Graphs for Age, Urine Peptidomic Molecules and MDRD eGFR equation (fig. 4.32 to 4.39)

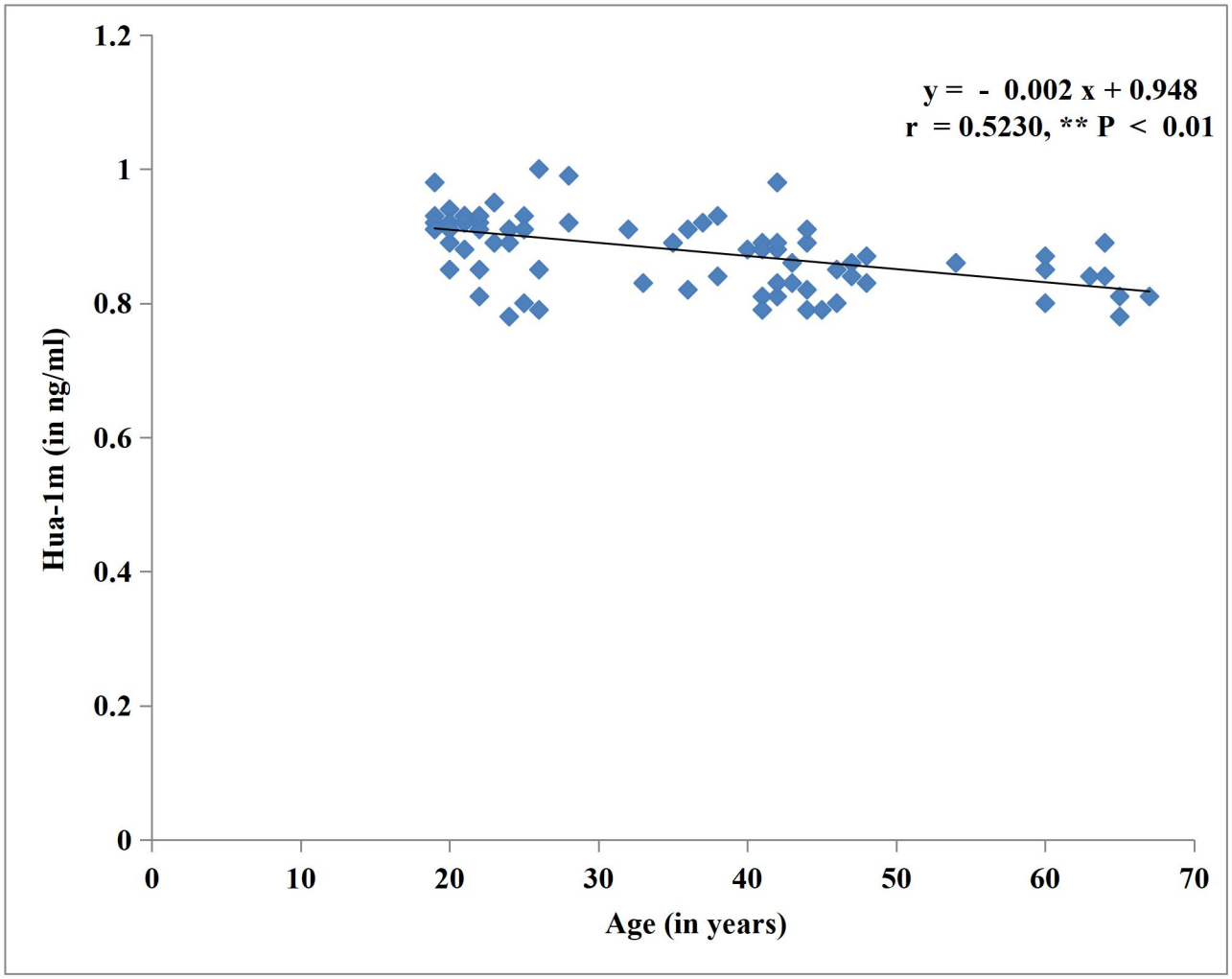

Fig. 4.32: Linear regression of Human Urine alpha -1microglobulin (Hua-1m in ng/ml) vs. Age (in years) for male and female subjects. There was significant annual decline in Hua-1m ($P < 0.01$).

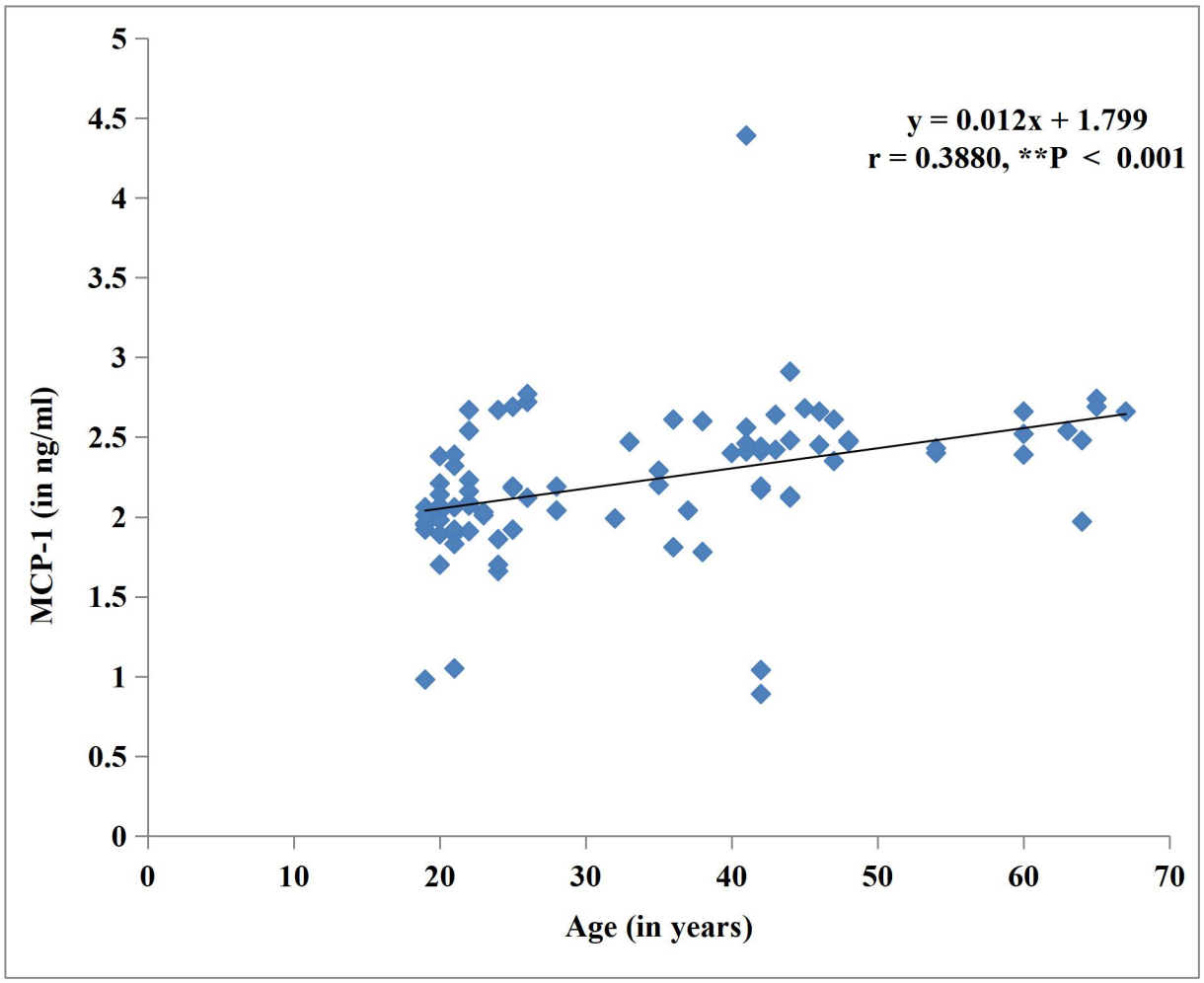

Fig. 4.33: Showing Linear regression of urine Monocyte Chemoattractant protein-1 (MCP-1 in ng/ml) vs. Age (in years) for male and female subjects. There was significant increase in urine Monocyte Chemoattractant protein-1 as age of the subjects increased ($P < 0.001$).

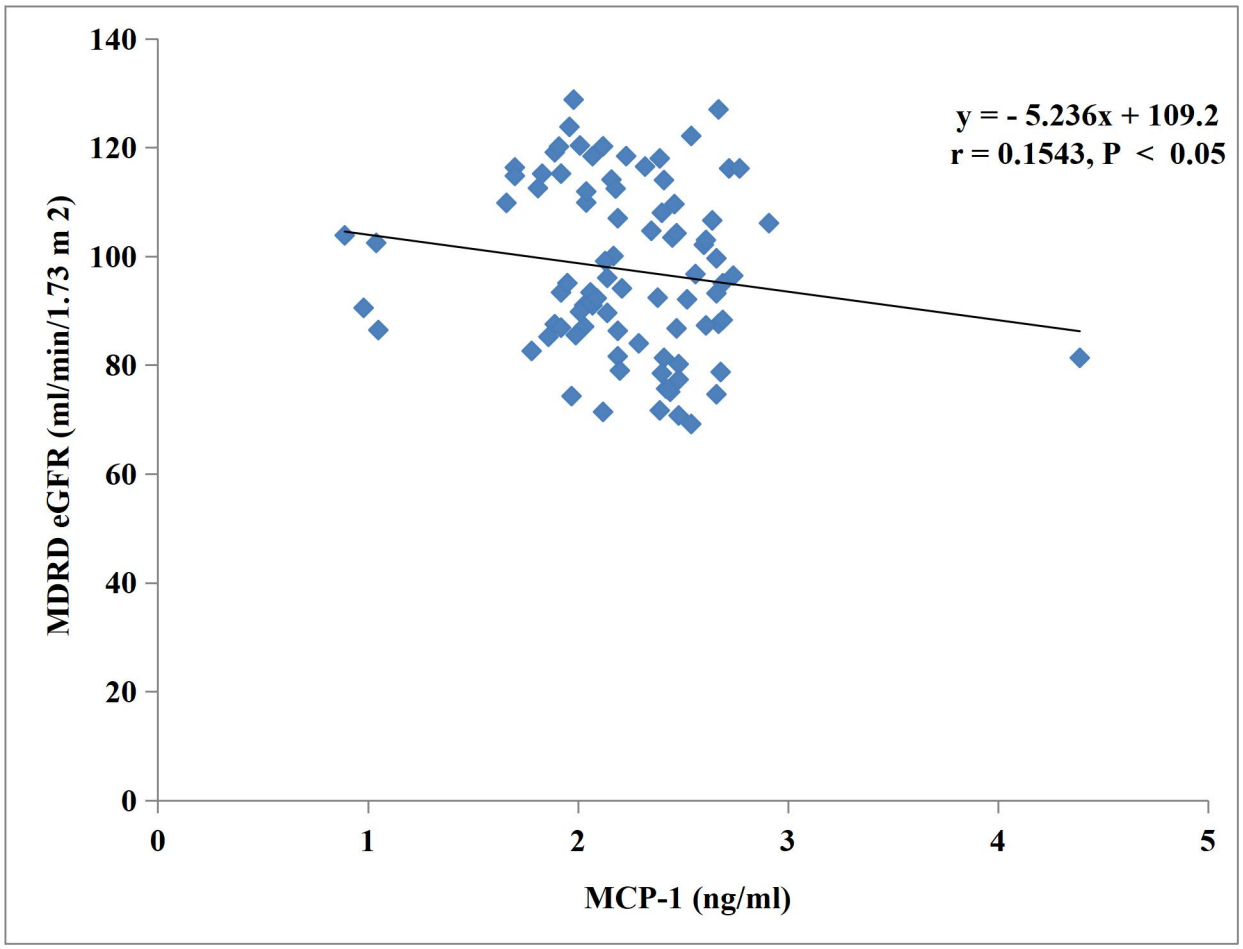

Fig. 4.34: Linear regression of MDRD eGFR vs. MCP-1. There was significant effect of MCP-1 on annual decline in MDRD eGFR ($P < 0.05$). The rate of decline was 5.24 ml/min/yr.

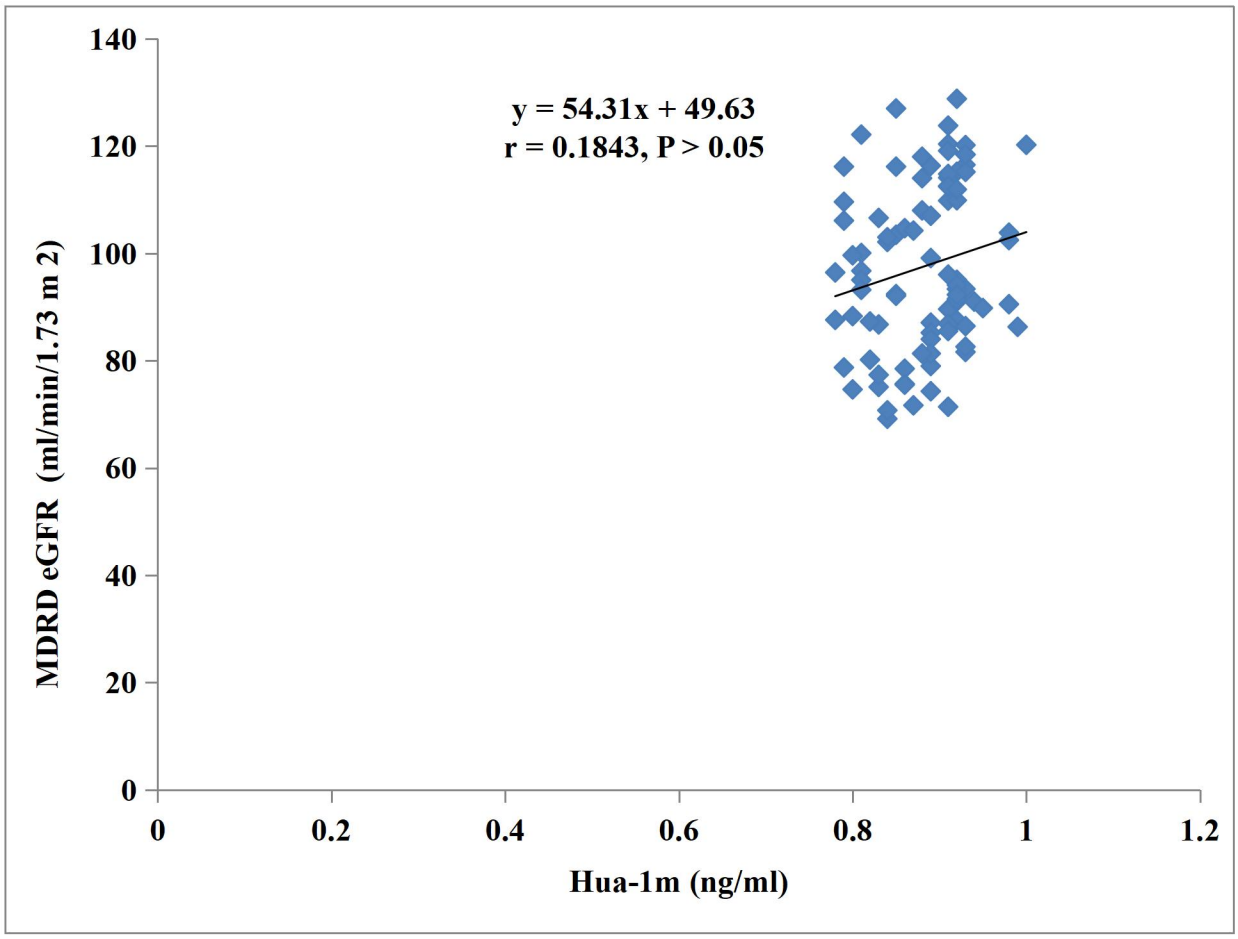

Fig 4.35: Showing linear regression of MDRD eGFR on Human urine Alpha-1-microglobulin (Hua-1m in ng/ml). There was no significant relationship between Hua-1m on MDRD eGFR ($P > 0.05$).

Table 4.7: Showing summary of Descriptive analysis of Age, Urine Cr, Urine Albumin and Urine Albumin/UCR ratio for all male and female subjects

	Age (yrs)	UCr (mg/dl)	UCR (g/dl)	UrALB (mg/dl)	UrALB/UCR ratio (mg/g)
Mean	38.35	157.92	0.16	10.92	81.12
± Sem	0.99	3.77	0.00	0.37	3.58

UCr = Urine creatinine in mg/dl

UCR = Urine creatinine in g/dl

UrALB = Urine Albumin excretion in mg/dl

UrALB/UCR = UrALB/UCR ratio in mg/g or Urine Albumin: Creatinine Ratio (ACR)

Regression Graphs of Age, Urine Albumin excretion and Urine Albumin: Creatinine ratio for males and female subjects (fig. 4.36 to 4.39)

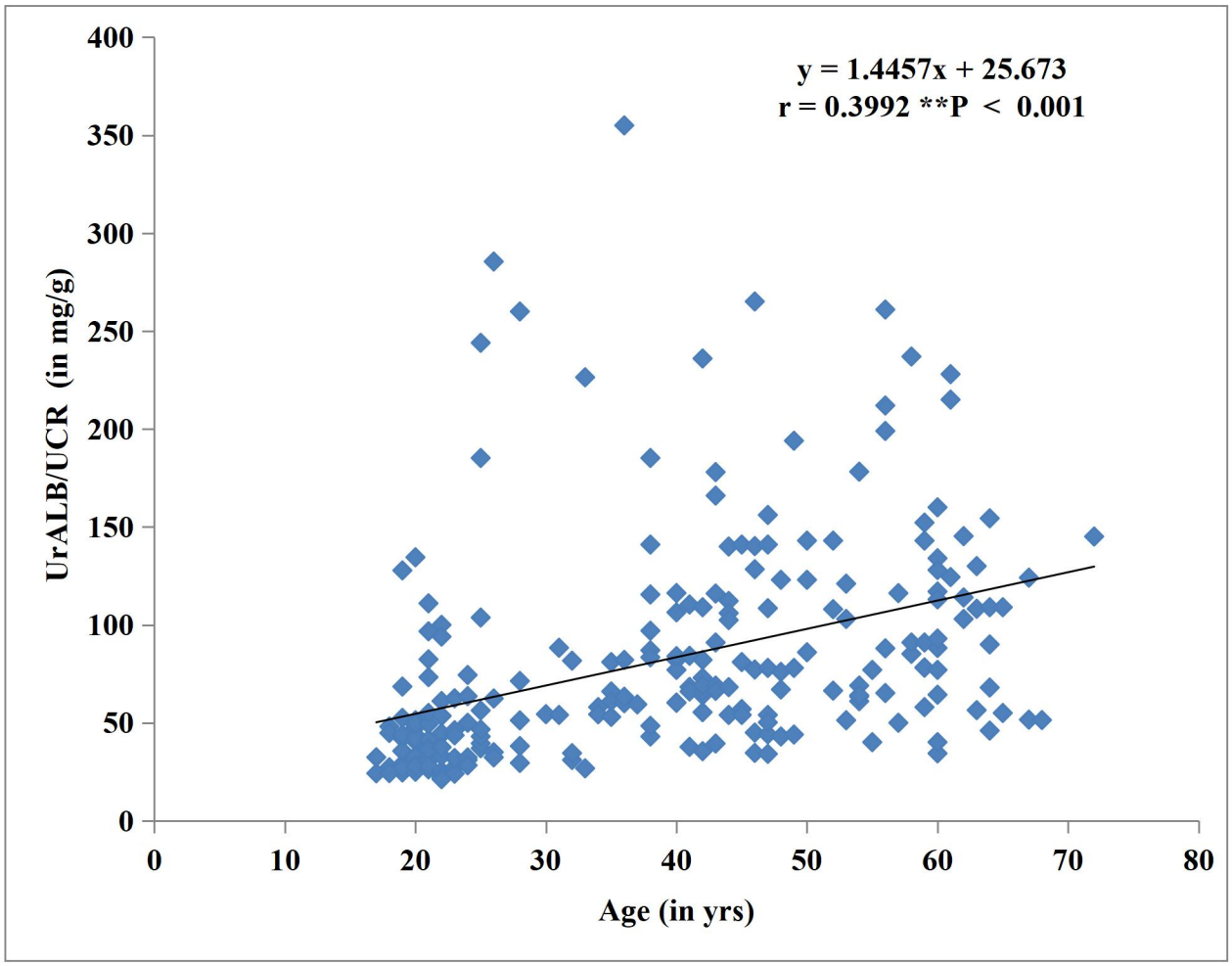

Fig. 4.36: Linear regression of all Urine Albumin: Creatinine ratio vs. Age (in years) for male and female subjects. There was significant increase in annual UrALB/CR ratio or ACR with age ($P < 0.001$). The annual rate of increase in UACR was 1.446 mg/g/yr.

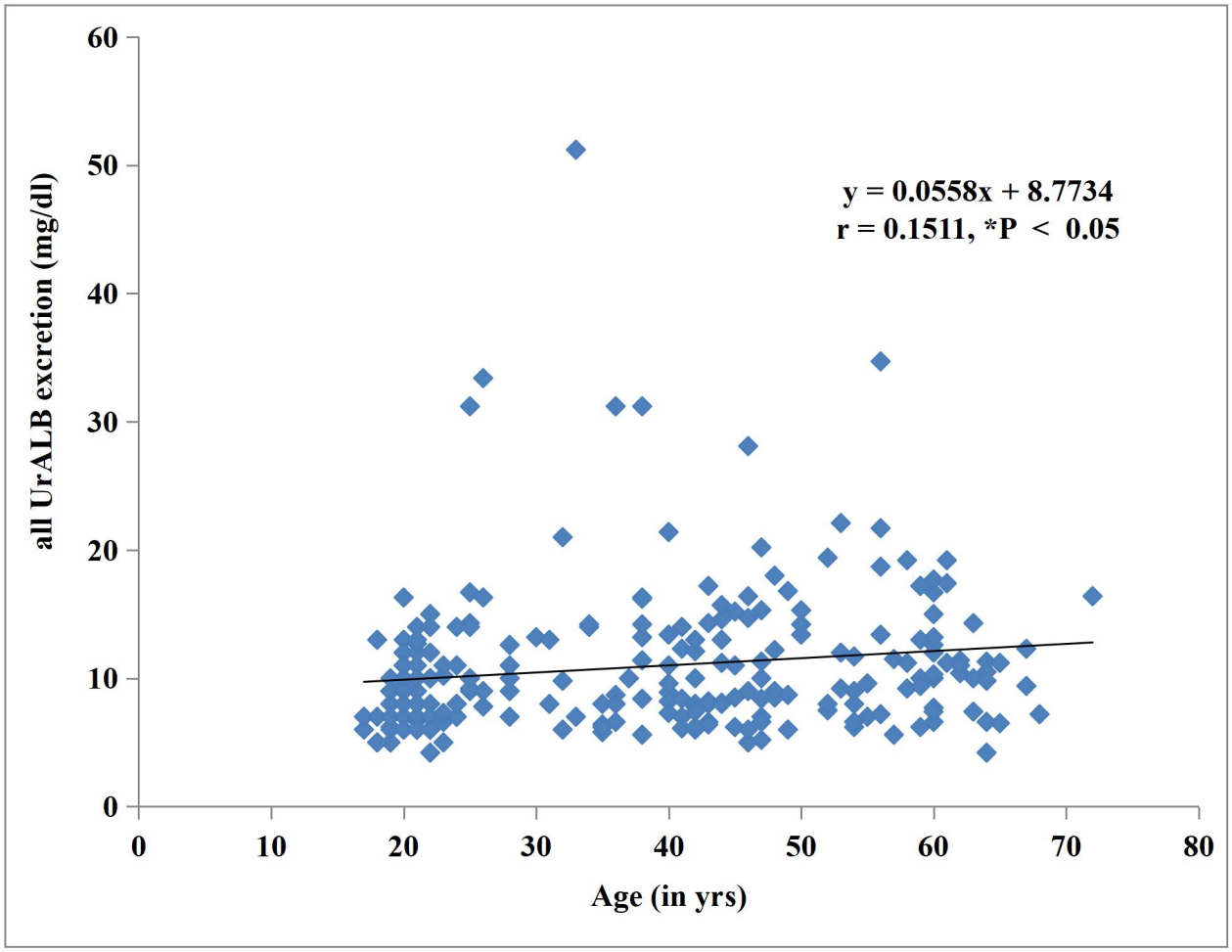

Fig 4.37: Linear regression of all urine Albumin excretion vs. Age for the subjects. There was significant annual increase in urine albumin excretion ($P < 0.05$) at the rate of 0.056 mg/dl/yr.

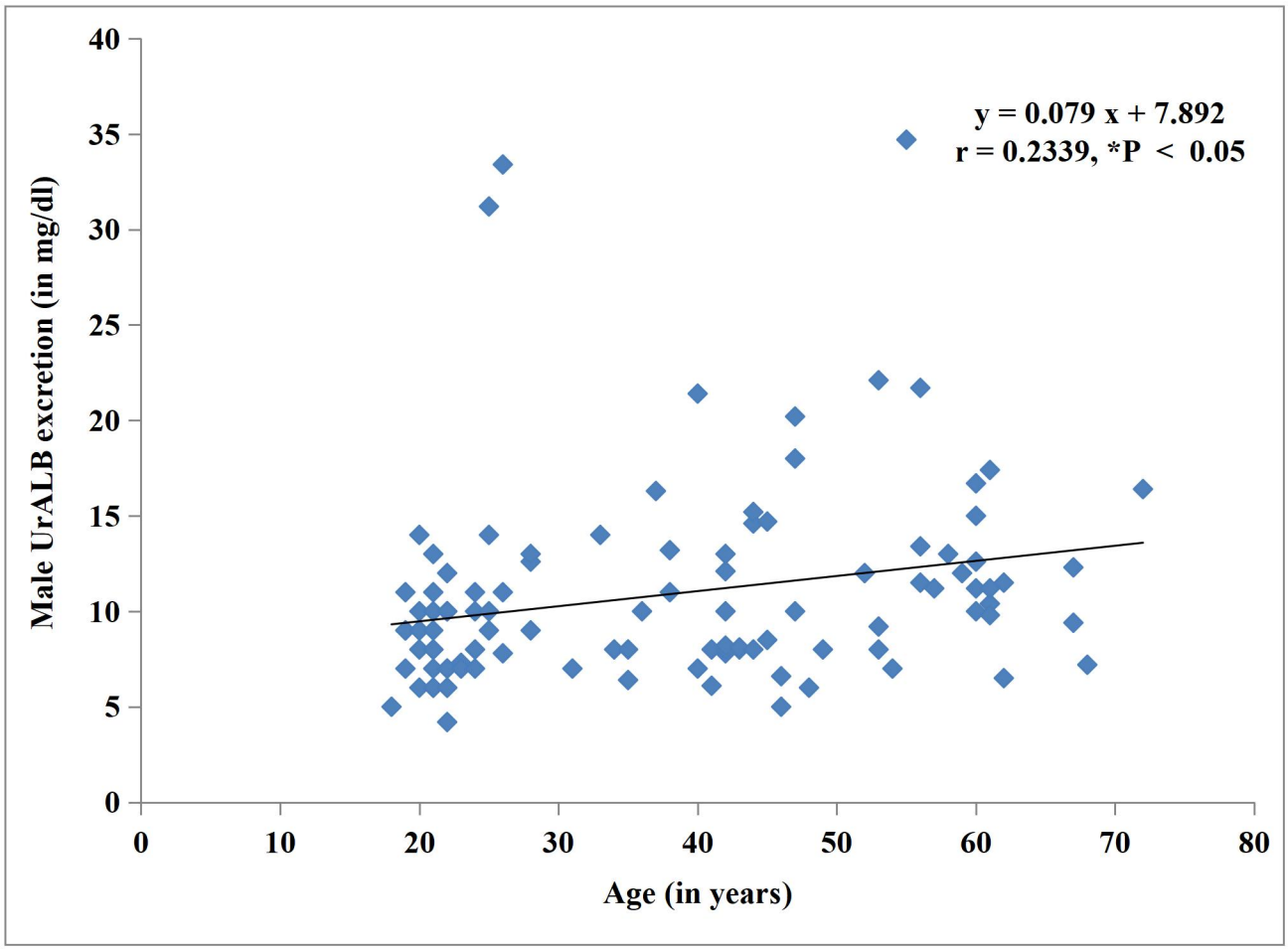

Fig. 4.38: Linear regression of UrALB excretion vs. Age (in years) for all Male subjects. There was significant increase in urine albumin excretion ($P < 0.05$) at the rate of 0.079 mg/dl/yr.

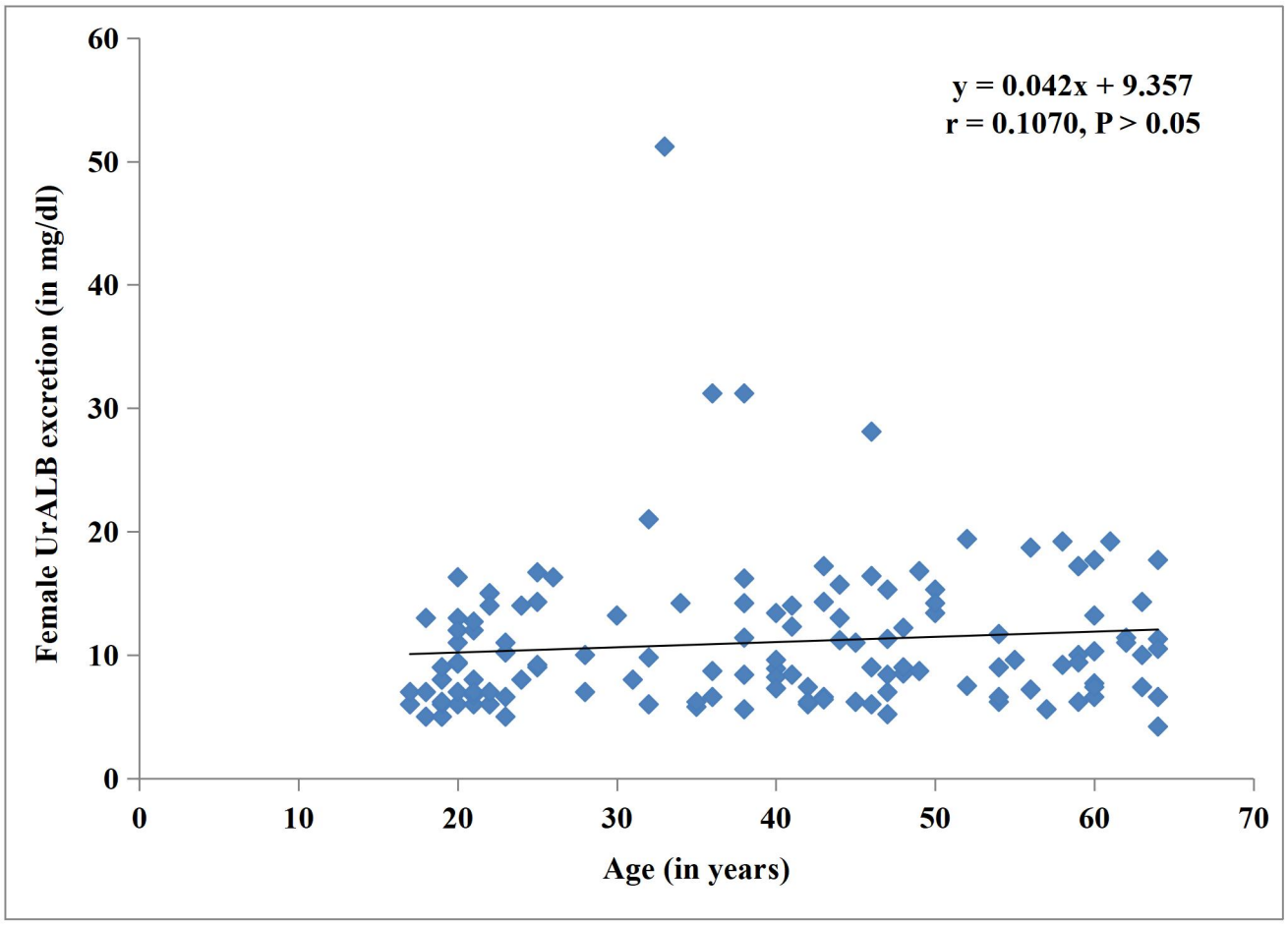

Fig 4.39: Linear regression of UrALB excretion vs. Age (in years) for all Female subjects. The annual increase in urine albumin excretion was not significant (P > 0.05).

Table 4.8: Showing summary of descriptive analysis of Age, MDRD, mCrCl, MDA and TAC for Male and Female subjects

	AGE (yrs)	MDRD eGFR (ml/min/1.73 m ^{2})	mCrCl (ml/min/1.73 m ^{2})	MDA (mol/gm Protein)	TAC (ug/ml)
Mean	42.26	95.65	221.38	0.21	2077.86
± Sem	1.55	1.59	18.12	0.01	1937.65

MDRD = Modification of diet in Renal Disease estimated Glomerular filtration equation

mCrCl = measured creatinine clearance in ml/min/1.73 m ^{2}

MDA = Serum Malondialdehyde in mol/gm Protein

TAC = Serum Total antioxidant capacity in ug/ml

Regression Graphs for Age, MDA, TAC, MDRD eGFR and mCrCl (fig. 4.40 to 4.44)

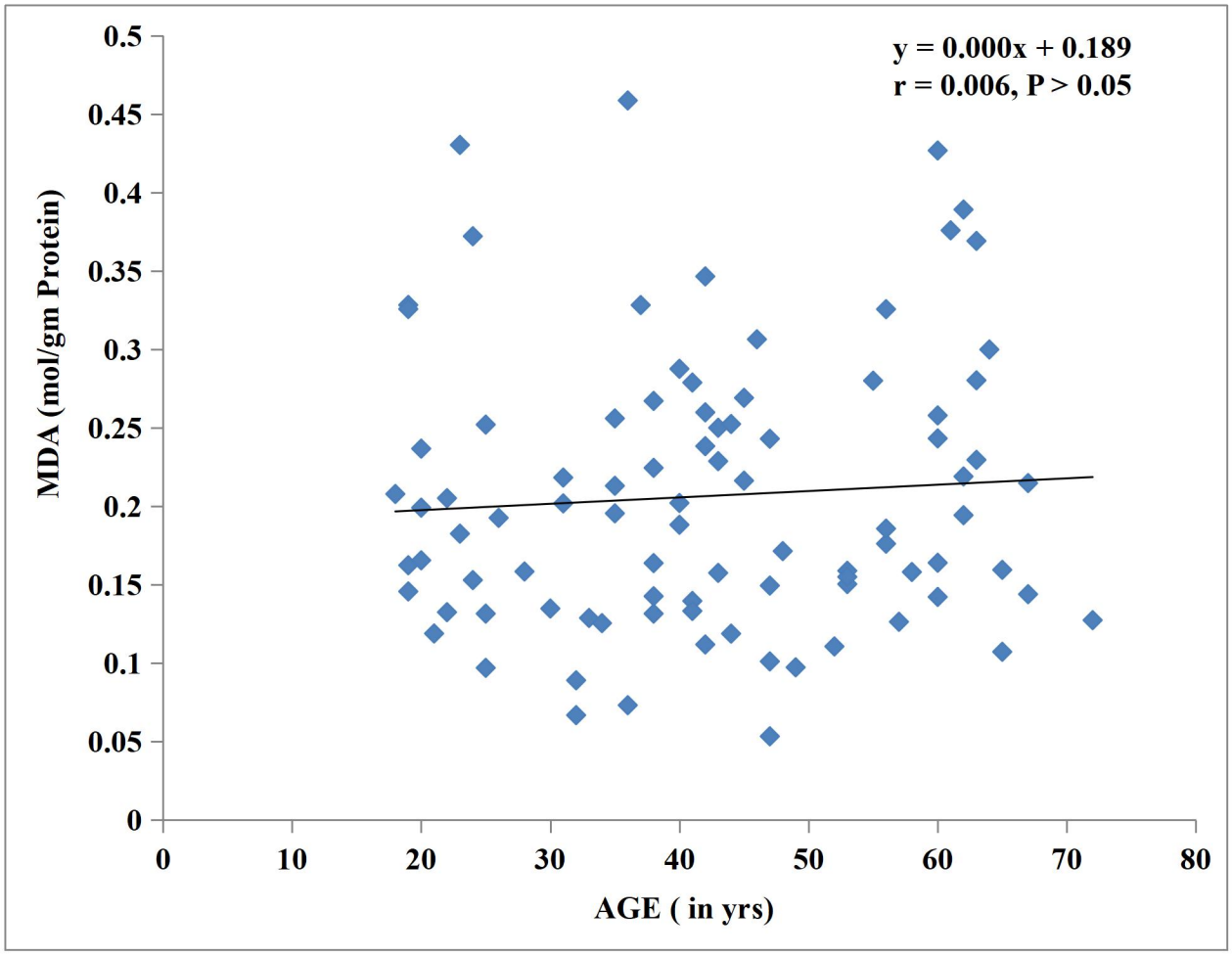

Fig. 4.40: Linear regression of serum Malondialdehyde (in mol/g protein) vs. Age (in years). There was no significant increase in MDA as age increased (P > 0.05).

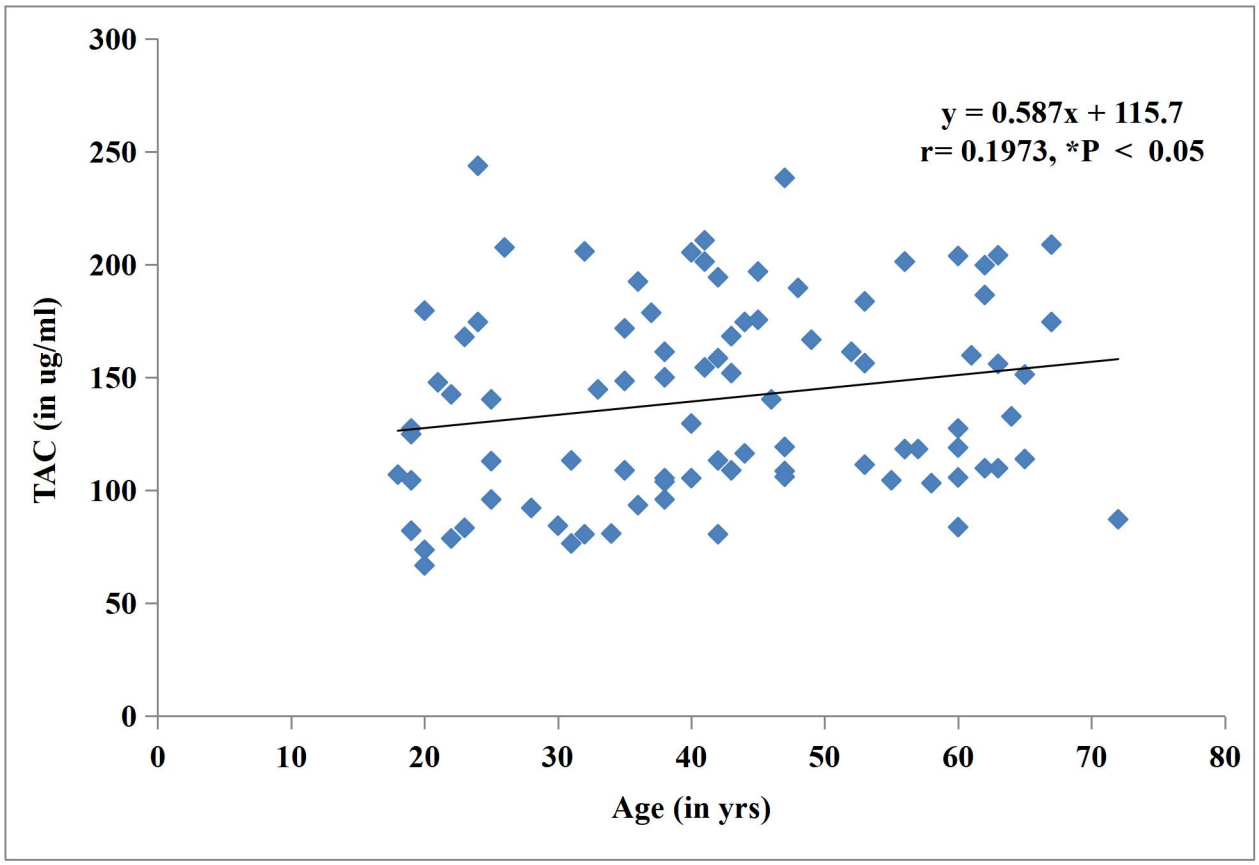

Fig. 4.41: Linear regression of Total Antioxidant Capacity (TAC) vs. Age (in years). There was significant Age dependent increase in TAC ($P < 0.05$).

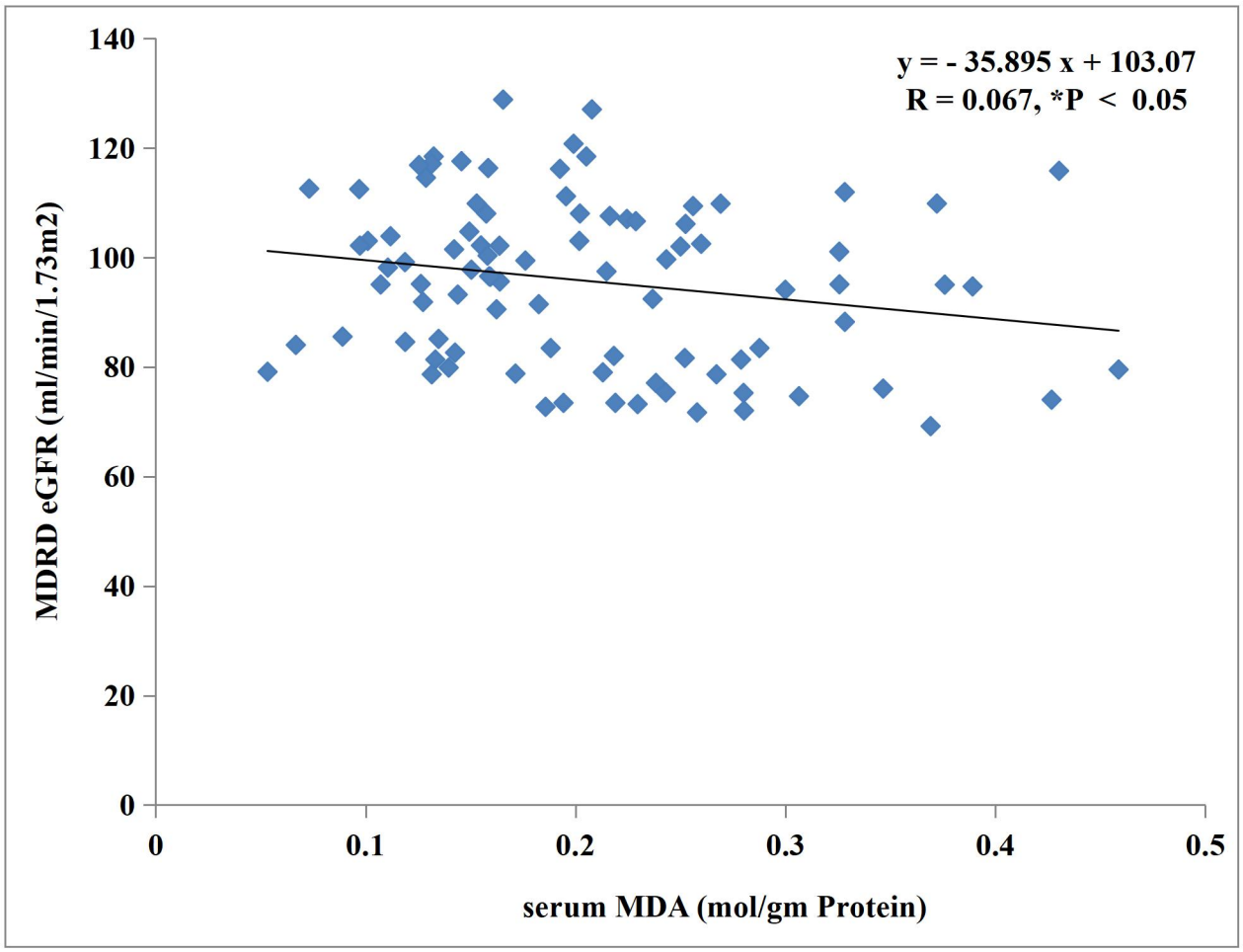

Fig. 4.42: Linear regression of MDRD eGFR vs. serum Malondialdehyde (MDA) for males and females. There was significant decrease in MDRD eGFR as serum MDA increased ($P < 0.05$).

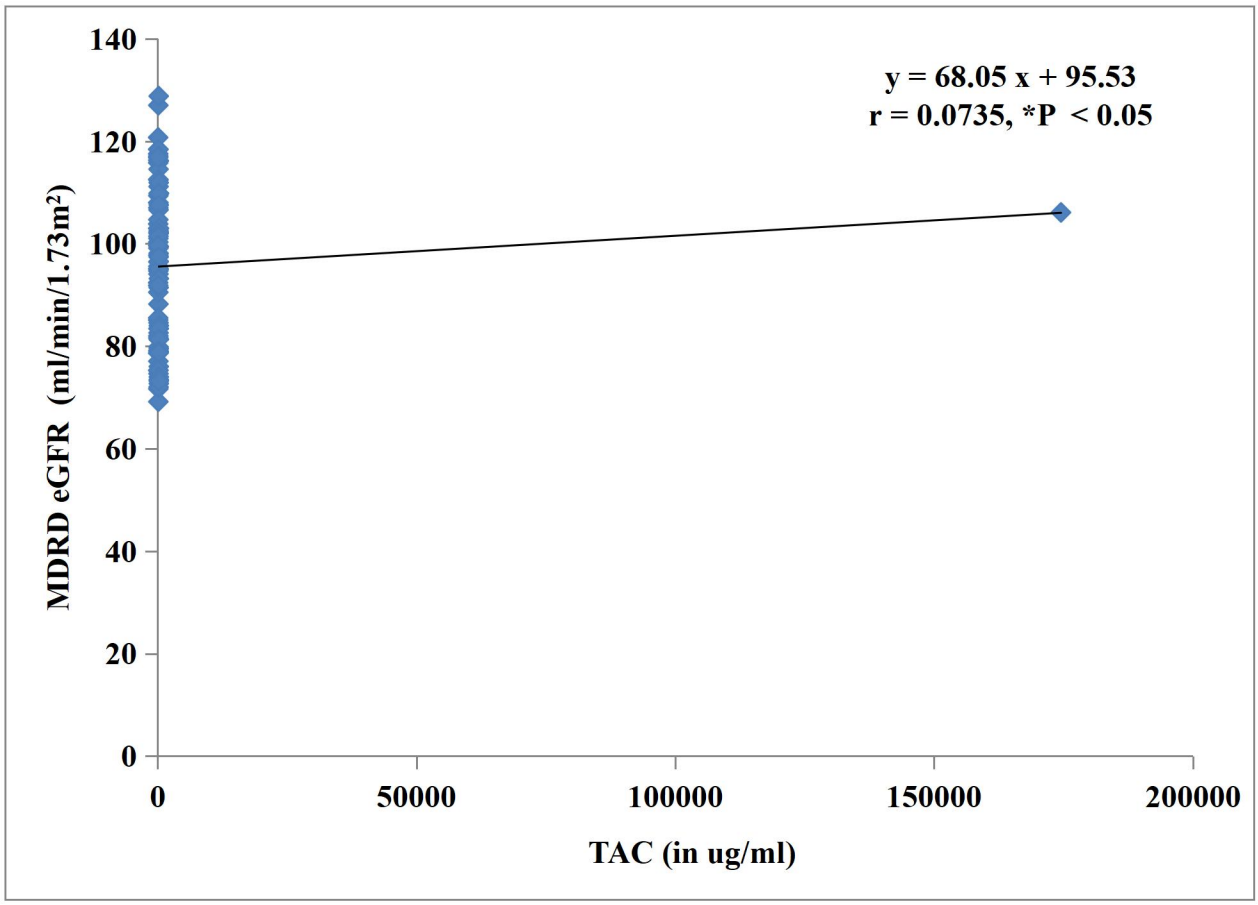

Fig. 4.43: Linear regression of MDRD eGFR vs. TAC for male and female subjects. There was significant increase in MDRD eGFR with increase in TAC ($P < 0.05$).

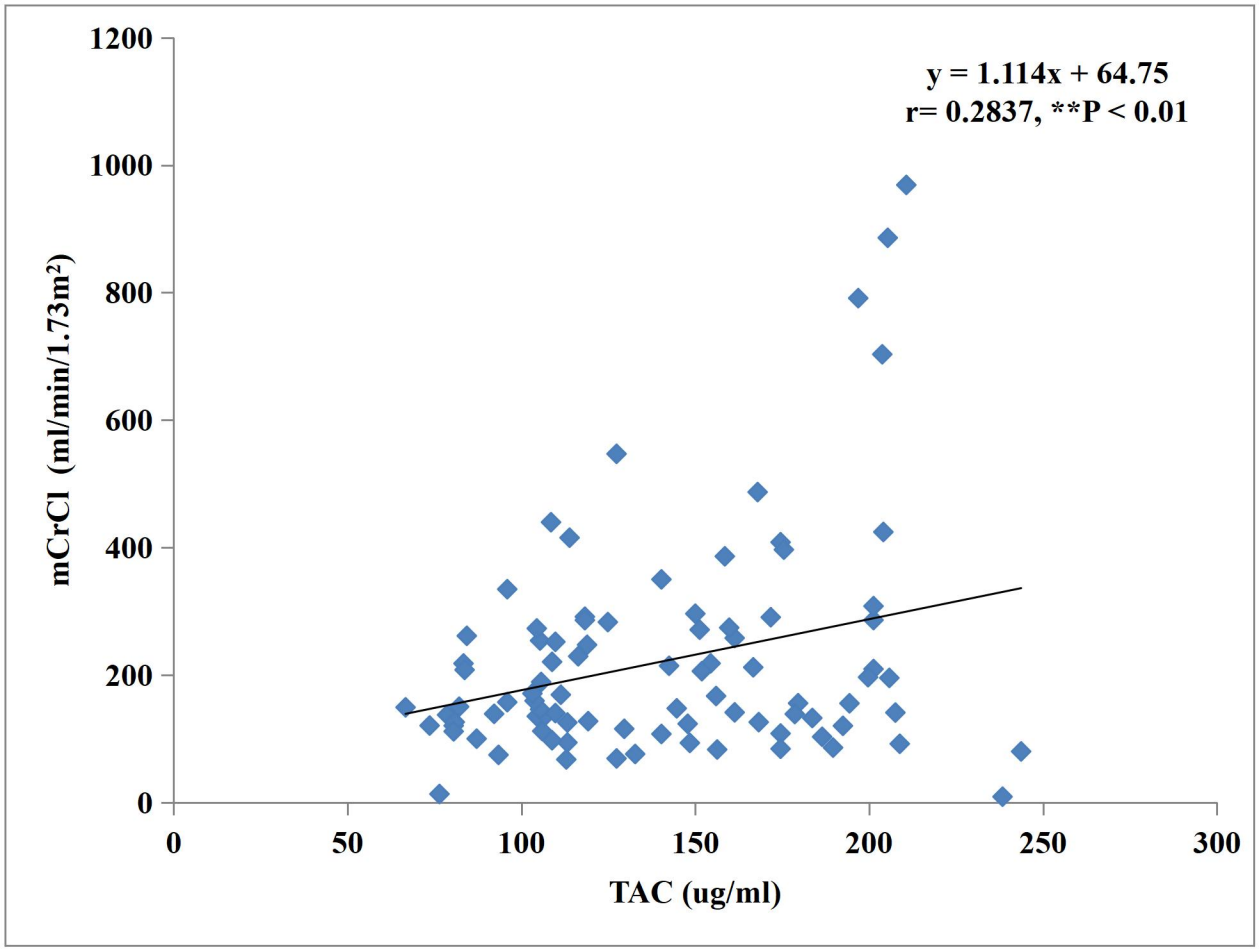

Fig. 4.44: Linear regression of mCrCL (as mGFR in ml/min/1.73m ^{2}) vs. TAC (in ug/ml) for males and females. There was significant increase in mCrCl as Total Antioxidant Capacity increased ($P < 0.01$). The rate of increase was 1.114ml/min/1.73m^2/ug.

Table 4.9: Showing summary of descriptive statistics of Age and Urine Sodium to Potassium ratio for Male and Female the subjects

	All Urine Na+/K+ Ratio		Female Urine Na+/K+ ratio		Male Urine Na+/K+ ratio	
	AGE	ratio	AGE	ratio	AGE	ratio
Mean	38.35	4.29	38.92	4.07	37.78	4.45
± Sem	0.99	0.19	1.30	0.24	1.51	0.29

Urine Na+/K+ Ratio = Urine Sodium to Potassium ratio

Regression Graphs of Age and Urine Sodium to Potassium ratio for Male and Female subjects (fig. 4.45 to 4.47)

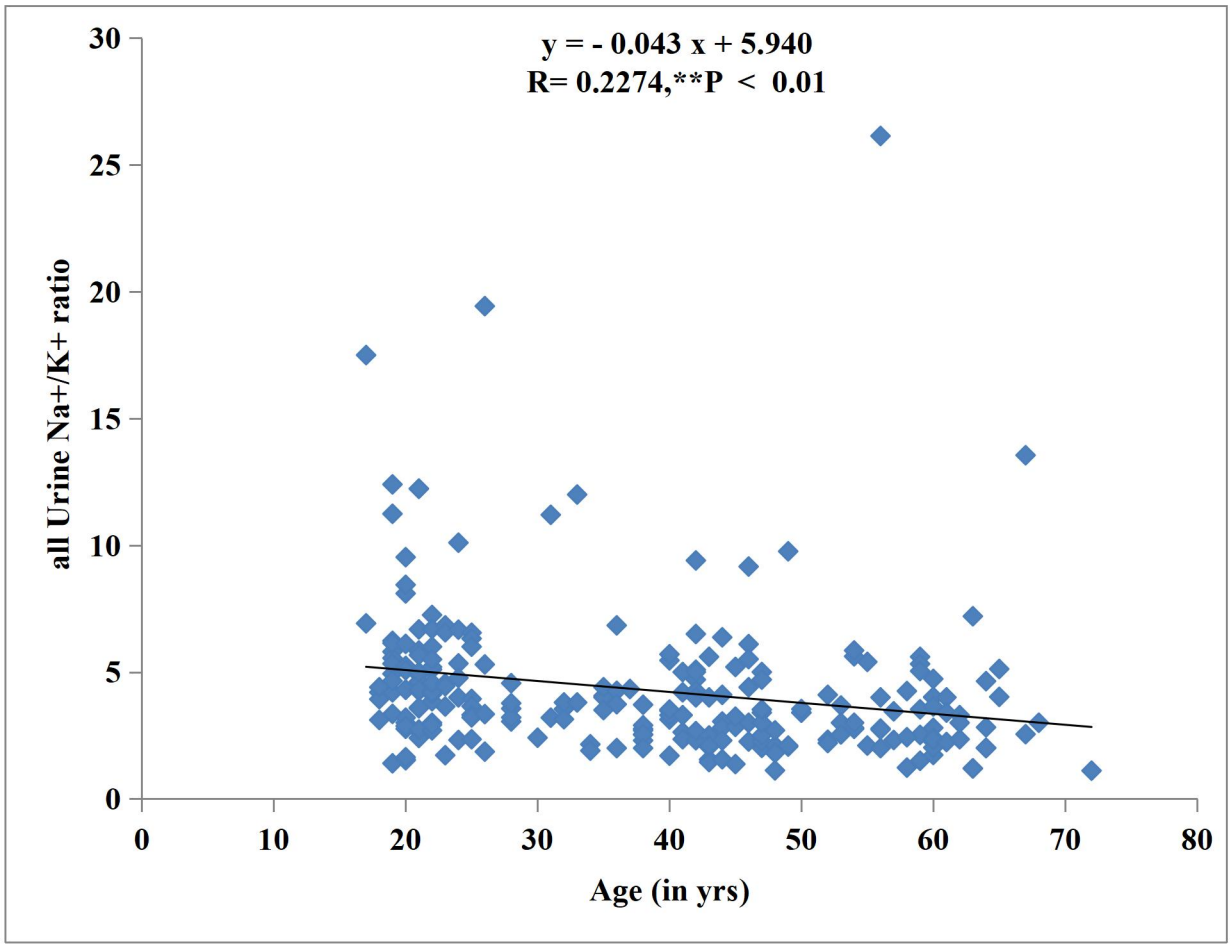

Fig. 4.45: Linear regression of Urine Na/K ratio on Age (in years) for Male and Female subjects. There was significantly lower Urine Na+/K+ ratio as age of the subjects increased ($P < 0.01$). The rate ratio of decrease in sodium over potassium was 0.043 per year.

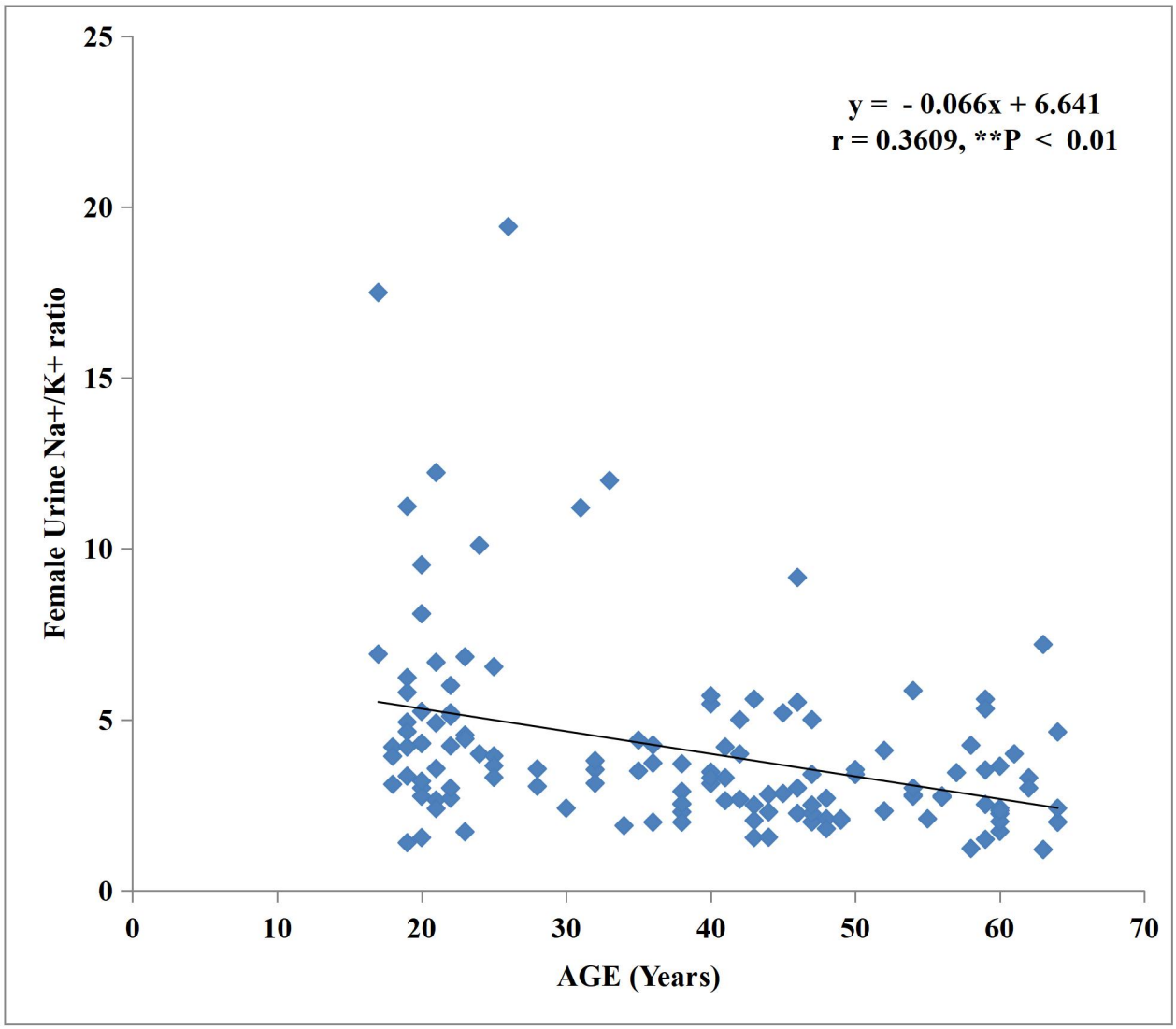

Fig. 4.46: Linear regression of Urine Na+/K+ ratio on Age (in years) for Female subjects. The urine Na+/K+ ratio was decreased significantly as age of Female subjects increased ($P < 0.01$). There was significantly lower sodium excretion than potassium. The rate of decrease in sodium to potassium excretion was 0.066 per year.

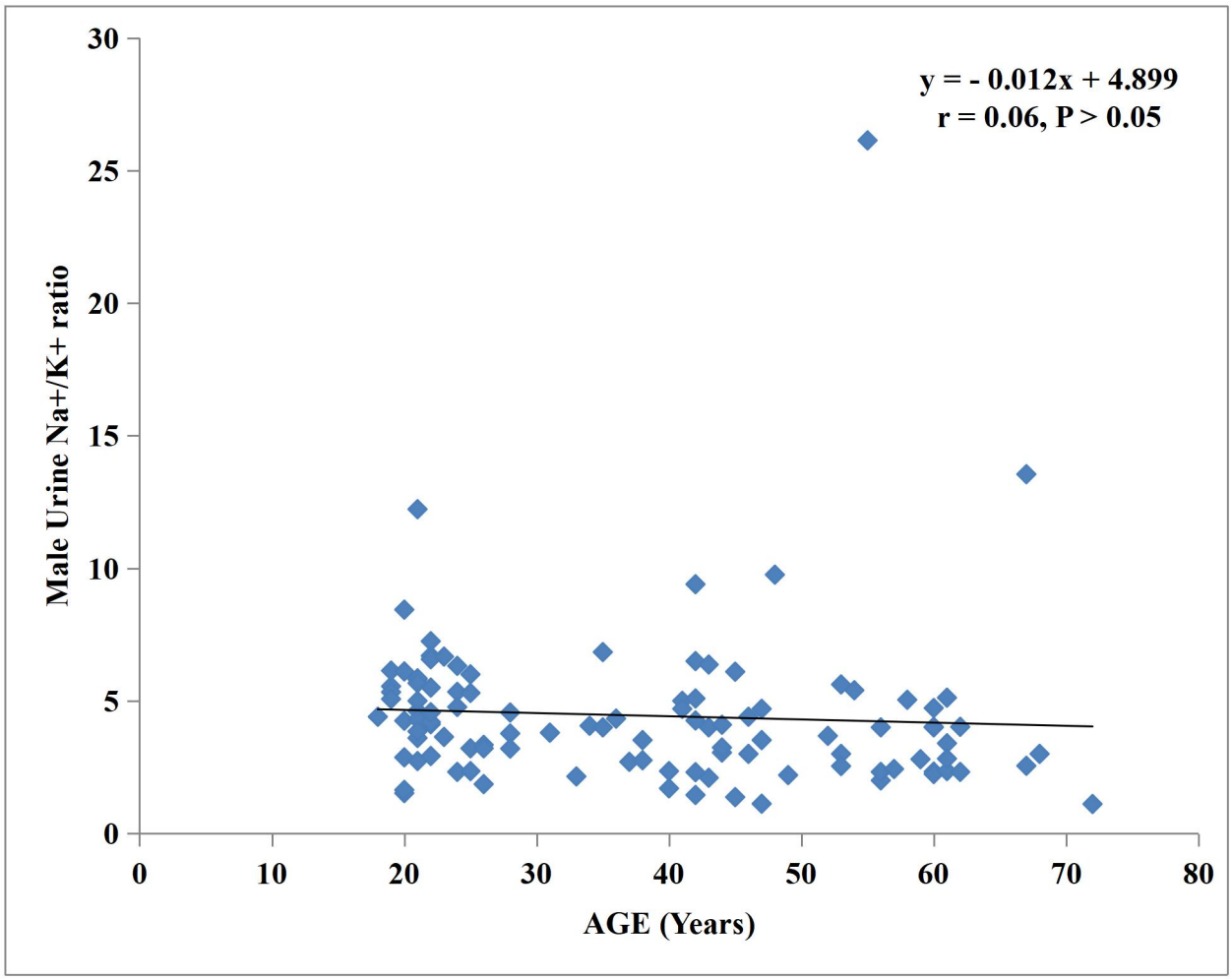

Fig. 4.47: Linear regression of Urine Na+/K+ ratio on Age (in years) for Male subjects. The decrease in urine Na+/K+ ratio was not significant as age of Male subjects increased ($P > 0.05$). There was relatively less sodium excretion than potassium. The rate of decrease in sodium /potassium excretion was 0.012 per year.

Table 4.10: T-test Comparison of Renal Parameters in Non-Hypertensive and Hypertensive Subjects

Parameters	**Non-Hypertensive and Hypertensive subjects**	**N**	**Mean ± Sem**	**T-value**	**P-Value**
MAP (mmHg)	Non-Hypertensive (78.93%)	191	86.18±0.59	-15.94	**0.001
	Hypertensive (21.074%)	51	109.67±1.8		
UCR (mg/dl)	Non-Hypertensive	191	162.93±4.24	2.60	**0.01
	Hypertensive	51	139.16±7.77		
UCr (g/dl)	Non-Hypertensive	191	0.16±0.00	2.05	*0.04
	Hypertensive	51	0.14±0.01		
Ur ALB (mg/dl)	Non-Hypertensive	191	10.51±0.36	-2.16	*0.03
	Hypertensive	51	12.43±1.05		
Ur ALB/UCr ratio	Non-Hypertensive	191	76.57±3.74	-2.49	**0.01
	Hypertensive	51	98.14±9.29		
CG (ml/min)	Non-Hypertensive	191	85.66±1.8	0.50	0.62 NS
	Hypertensive	51	83.57±4.38		
NKF CKD-EP!Cr (ml/min/1.73 m ^{2})	Non-Hypertensive	191	83.82±1.42	1.33	0.19 NS
	Hypertensive	51	79.69±2.81		
MDRD (ml/min/1.73 m ^{2})	Non-Hypertensive	191	94.5±1.12	2.05	*0.04
	Hypertensive	51	89.46±2.21		
Ur flow rate (ml/min)	Non-Hypertensive	191	0.81±0.03	-1.09	0.28 NS
	Hypertensive	51	0.88±0.07		
CrCl (ml/min/1.73 m ^{2})	Non-Hypertensive	191	127.6±5.62	1.04	0.30 NS
	Hypertensive	51	114.61±11.91		
Urine Na+/K+ ratio	Non-Hypertensive	191	4.43±0.22	1.51	0.13NS
	Hypertensive	51	3.74±0.28		

Note: **P<0.01- Highly Significant, * P<0.05- Significant P>0.05- Not significant (NS)

MAP (mmHg) = Mean Arterial Blood Pressure in millimeter mercury

UCr (mg/dl)	=	Urine Creatinine in milligrams per deciliter
UCR (g/dl)	=	Urine Creatinine in grams per deciliter
Ur ALB (mg/dl)	=	Urine Albumin in milligrams per deciliter
Ur ALB/UCr ratio (mg/g)	=	Urine Albumin Creatinine ratio in milligram per gram
CG (ml/min/1.73 m ^{2})	=	Cockroft-Gault eGFR in milliliters per minute per 1.73 m ^{2} of body surface area
NKF CKD-EP!Cr eq. (ml/min/1.73 m ^{2})	=	National Kidney Foundation Chronic Kidney Disease Epidemiology eGFR equation 2021
MDRD (ml/min/1.73 m ^{2})	=	Modification of Diet in Renal Disease eGFR equation
Ur flow rate (ml/min)	=	Urine flow rate in milliliter per minute
mCrCl (ml/min/1.73 m ^{2})	=	measured Creatinine Clearance in milliliter per minute per 1.73m ^{2} of body surface area
Urine Na+/K+ ratio	=	Urine Sodium Potassium ratio

Graphical presentation of the T-test comparison for Non-Hypertensive and Hypertensive Subjects shown in table 15 above (from fig. 4.48 to 4.56)

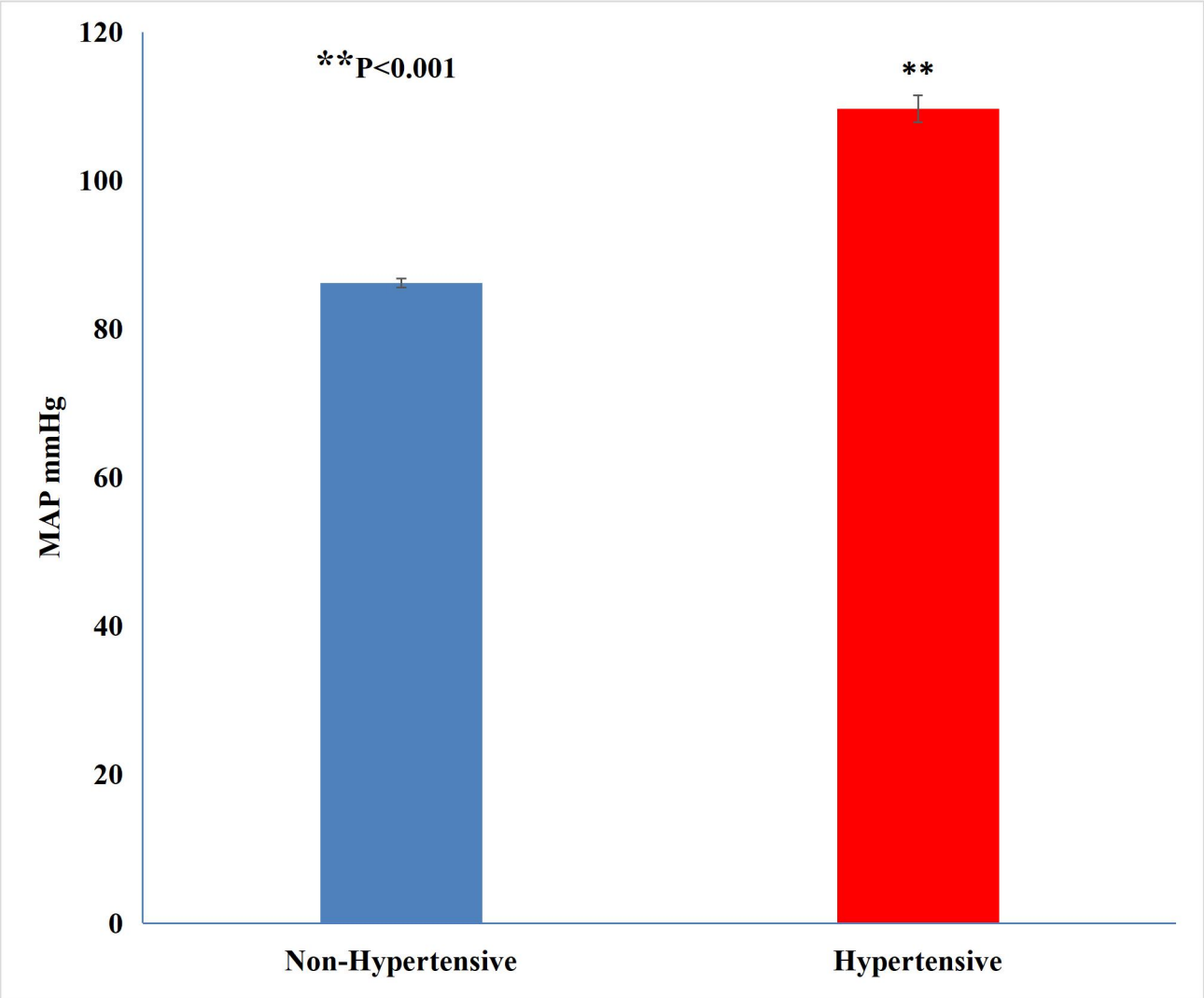

Fig. 4.48: Bar Chat graph of Mean Arterial Blood Pressure for all male and female Hypertensive (red, MAP ≥ 100mmHg) and non-Hypertensive (blue, MAP < 100 mmHg) subjects. The Hypertensive subjects have significantly higher Mean Arterial Blood (in mmHg) Pressure than Non-Hypertensive subjects ($P < 0.001$).

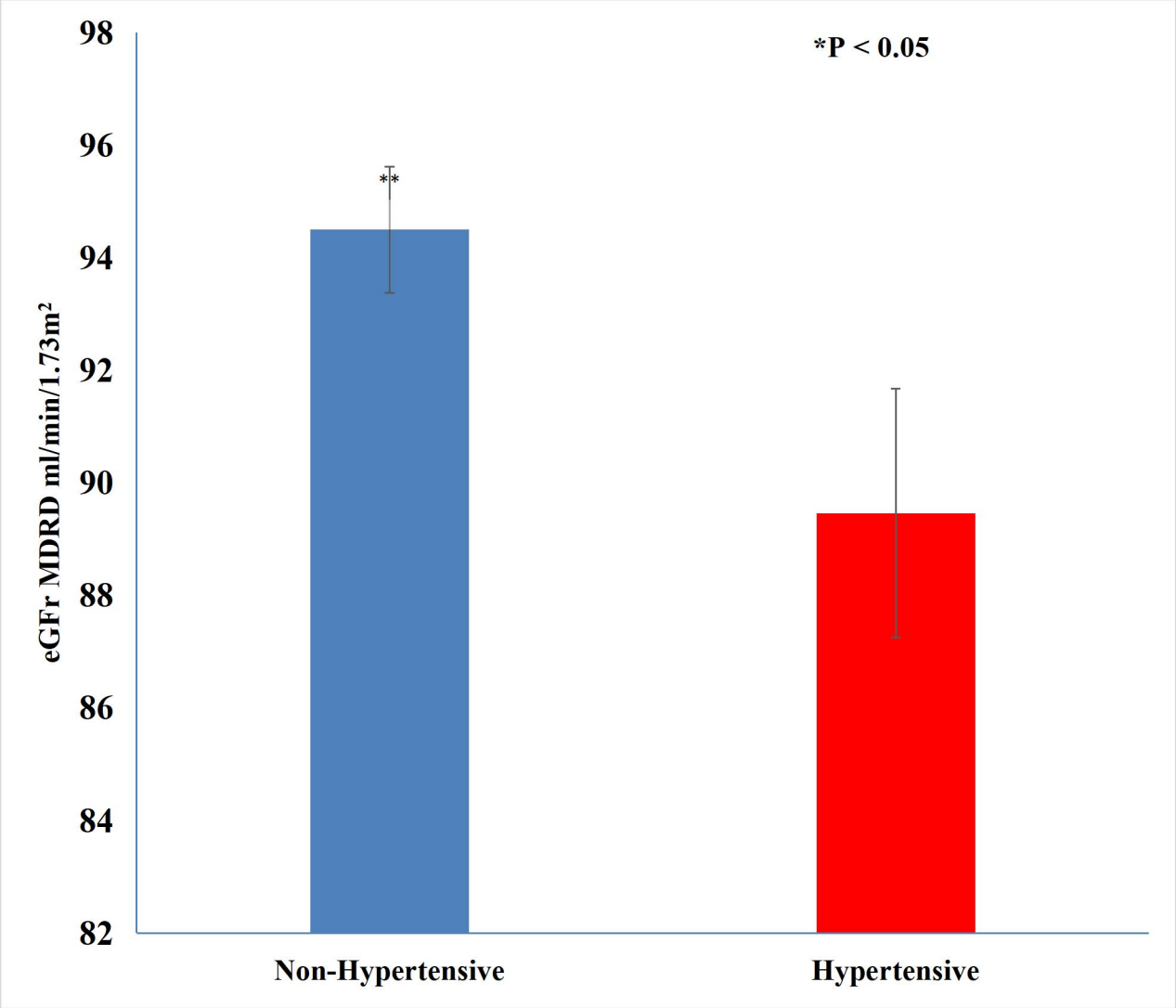

Fig. 4.49: Bar chart graph of estimated Glomerular Filtration Rate (eGFR) with MDRD equation for all Male and Female hypertensive and non-hypertensive subjects. The eGFR was significantly lower in Hypertensive subjects ($P < 0.05$).

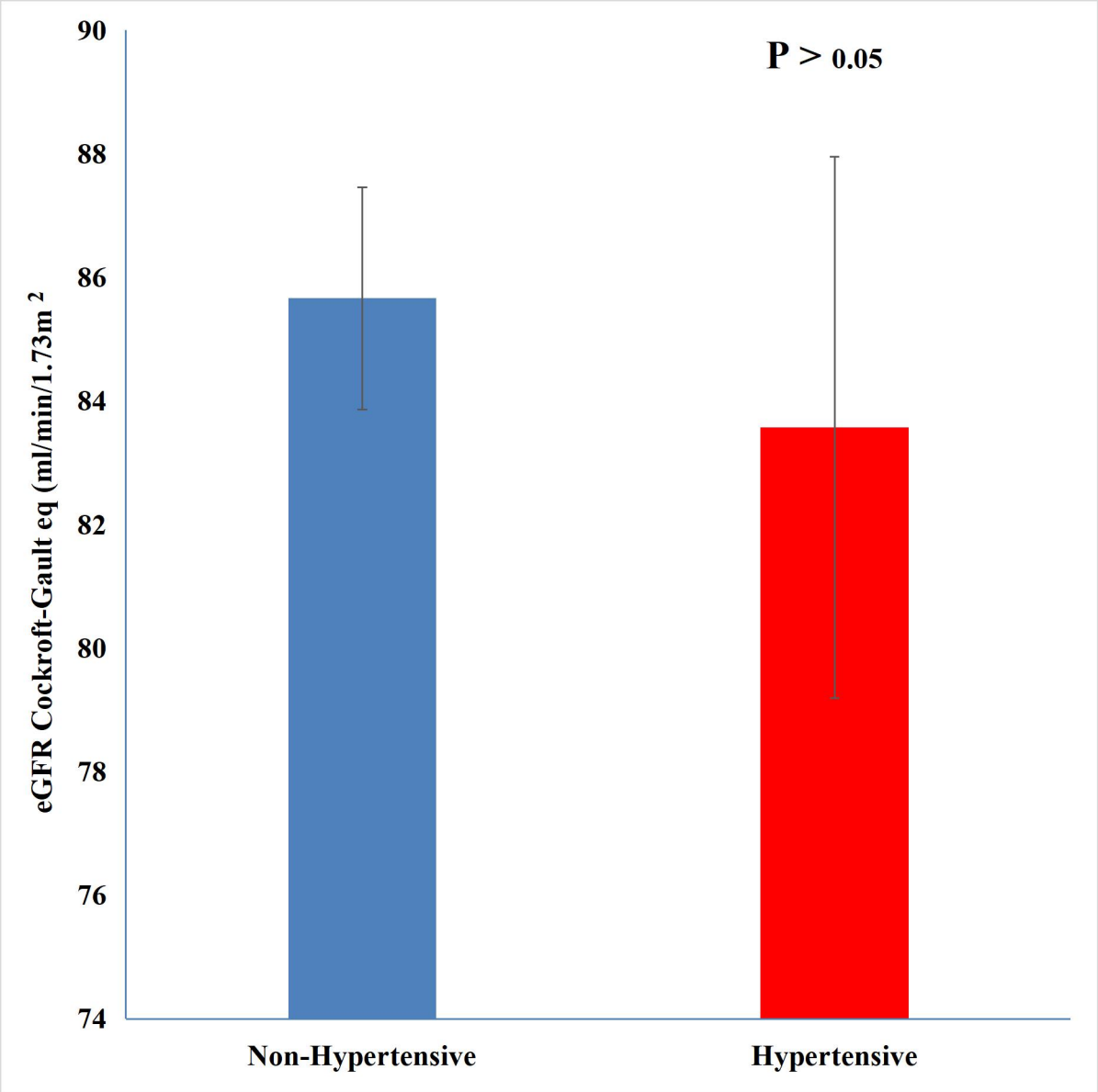

Fig. 4.50: Bar chart graph of estimated Glomerular Filtration Rate (eGFR) with Cockroft-Gault equation for all Male and Female hypertensive and non-hypertensive subjects. The eGFR was not significantly lower in Hypertensive subjects (P > 0.05).

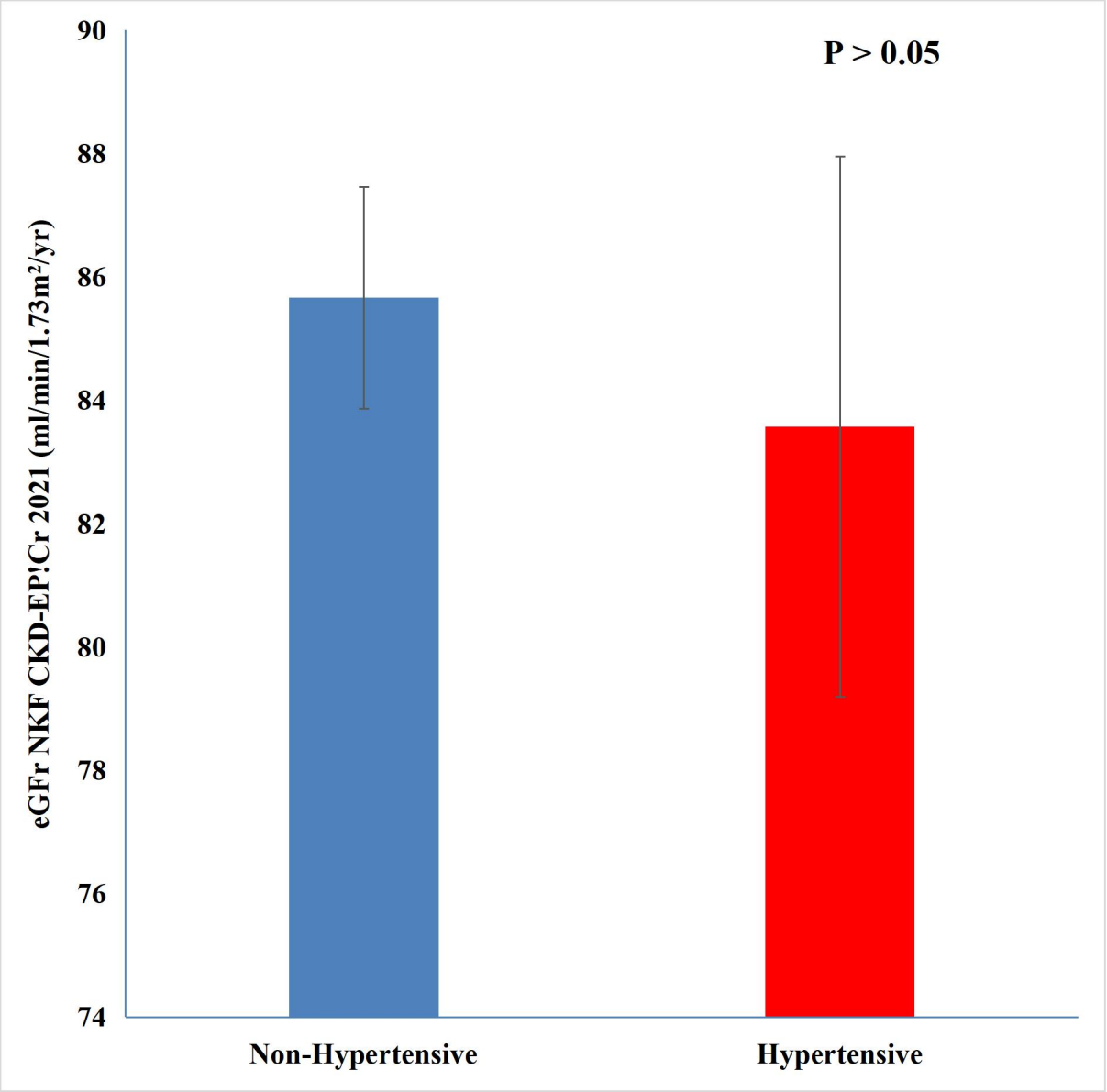

Fig. 4.51: Bar chart graph of estimated Glomerular Filtration Rate (eGFR) with National Kidney Foundation Chronic Kidney Disease Epidemiology creatinine 2021 equation for all Male and Female hypertensive and non-hypertensive subjects. The eGFR was not significantly lower in Hypertensive subjects (P > 0.05).

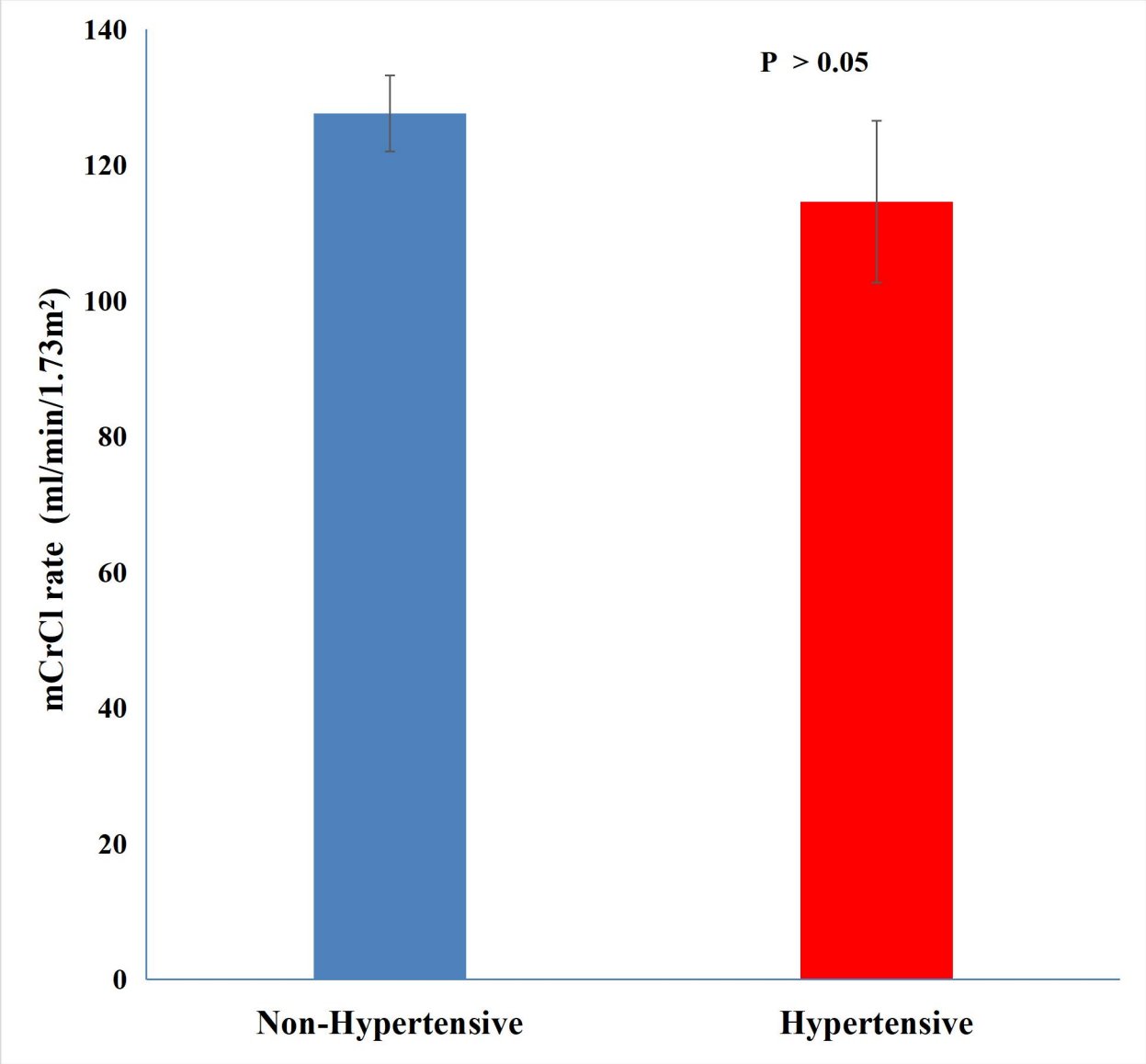

Fig. 4.52: Bar chart graph of measured Glomerular Filtration rate (mGFR) using measured Creatinine Clearance rate (mCrCl) for all Male and Female hypertensive and non-hypertensive subjects. The mCrCl was not significantly lower in Hypertensive subjects ($P > 0.05$).

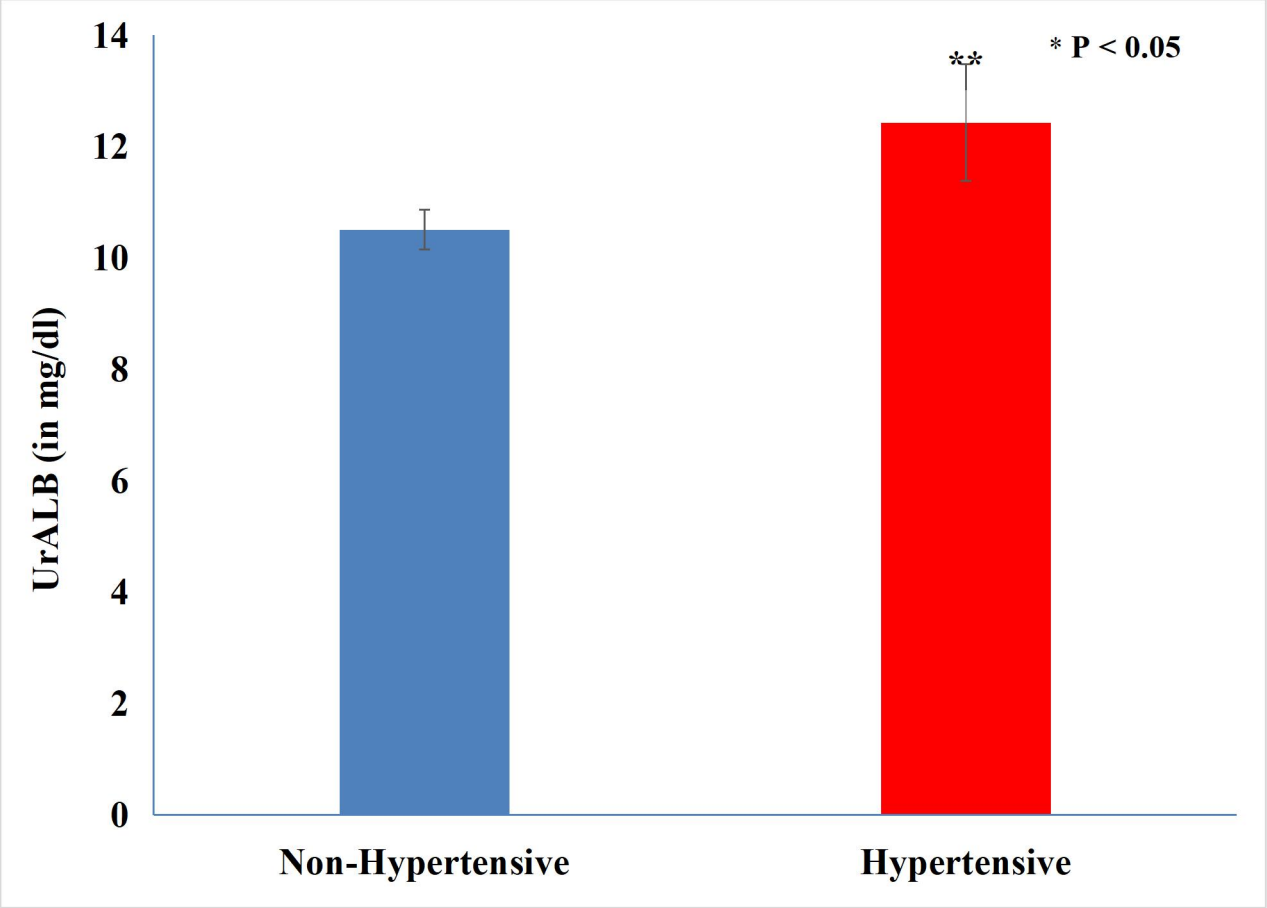

Fig. 4.53: Comparison of Urine Albumin excretion (in mg/dl) of the Hypertensive and Non-Hypertensive Subjects. This was more significant in hypertensive subjects ($P < 0.05$).

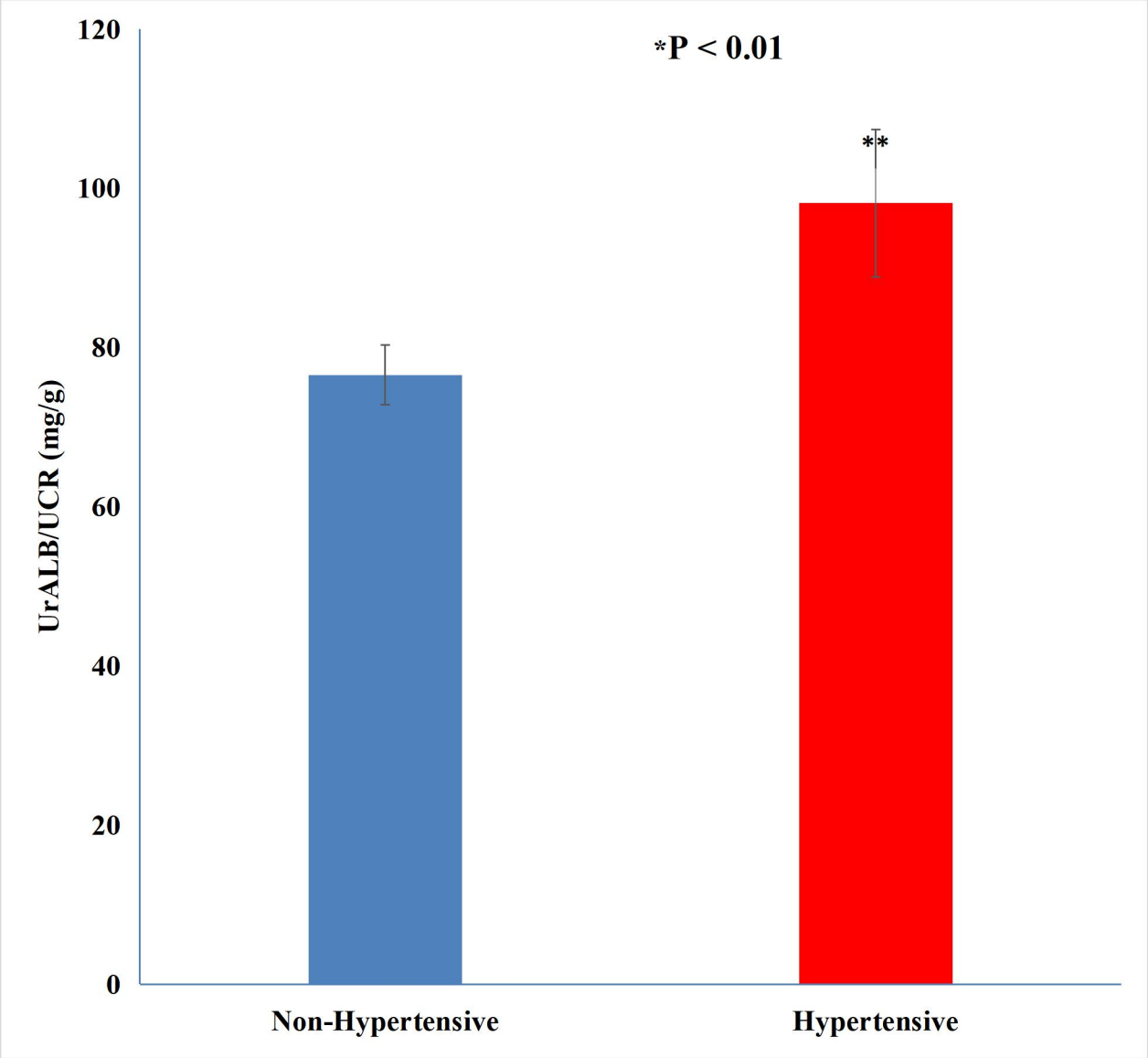

Fig. 4.54: Bar chart graph of Urine Albumin: Urine Creatinine ratio for all Male and Female Hypertensive and non-Hypertensive subjects. There was significantly higher UrALB/UCr in Hypertensive subjects ($P < 0.01$).

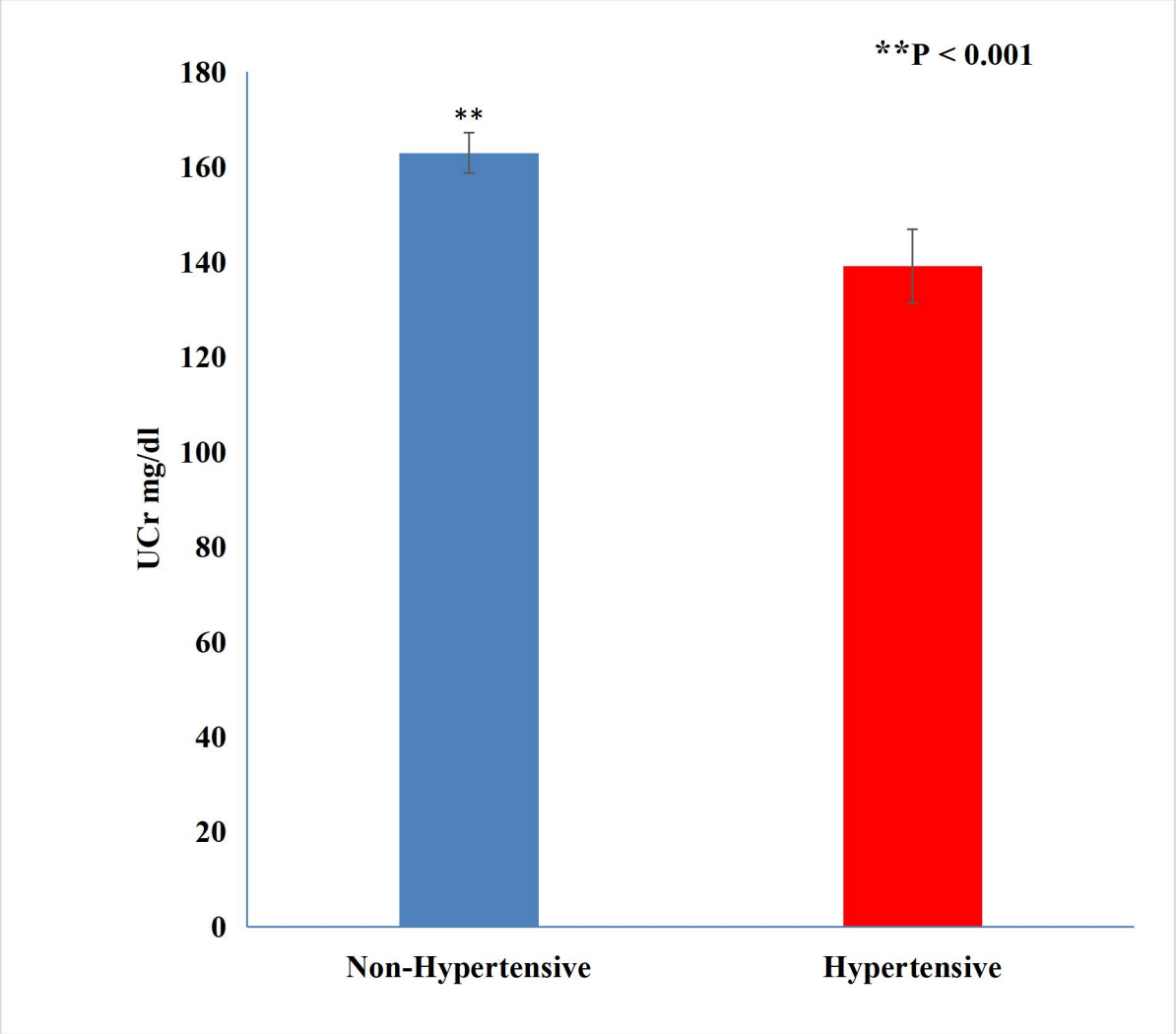

Fig. 4.55: Bar chart graph of Urine creatinine concentration (in mg/dl) for all Male and Female Hypertensive and non-Hypertensive subjects. There was significantly lower UCR in Hypertensive than non-Hypertensive subjects ($P < 0.001$).

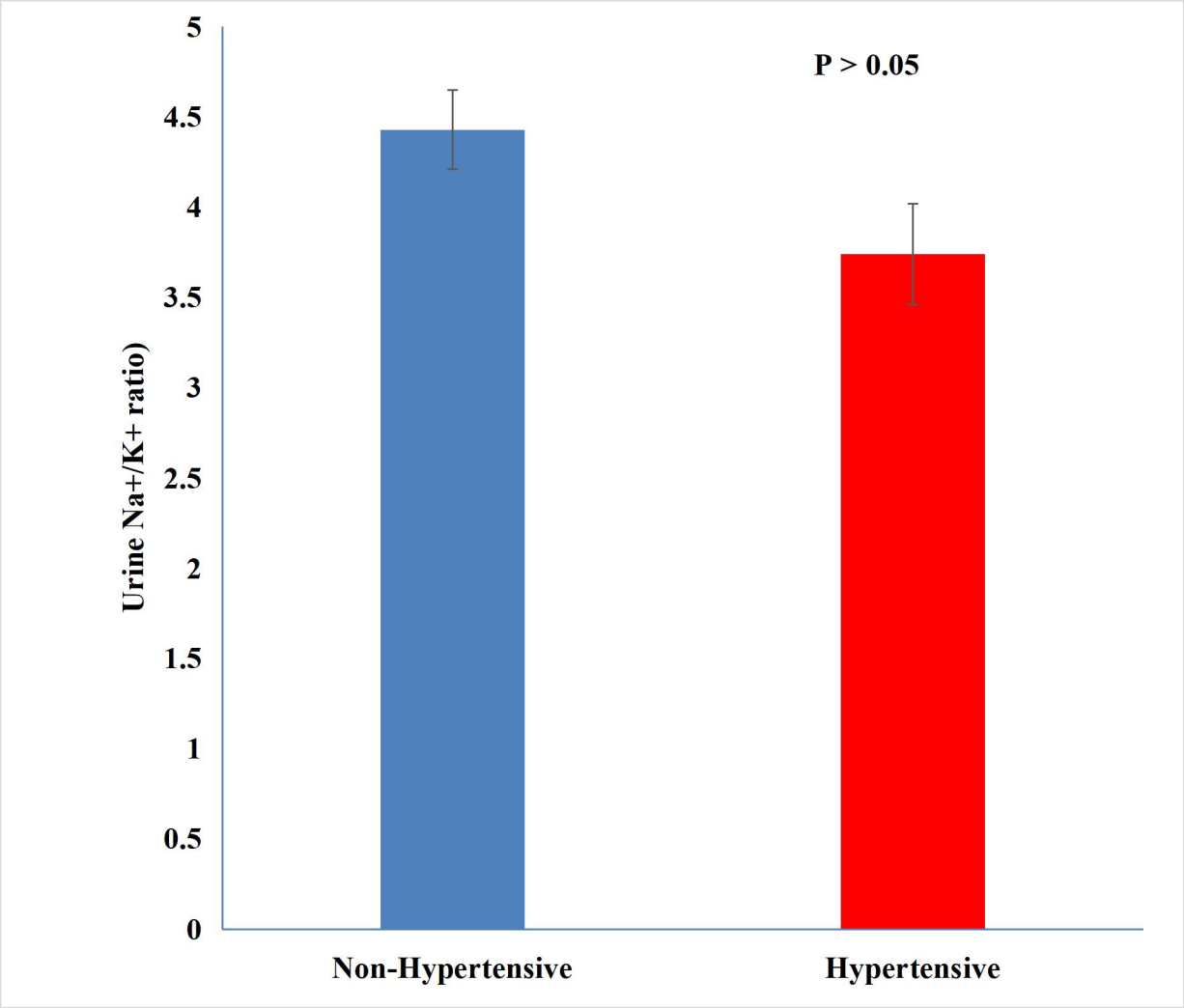

Fig. 4.56: Bar chart graph comparison of Urine Sodium: Potassium ratio for all Male and Female Hypertensive and non-Hypertensive subjects. The mean value of Urine Na+/K+ ratio was not significantly lower in Hypertensive subjects (P > 0.05).

Table 4.11: T-test Summary for Non-Hypertensive and Hypertensive **Males only**

Parameters	Non-Hypertensive and Hypertensive Male subjects	N	Mean ± Sem	T-value	P-value
MAP	Non-Hypertensive	83	87.46 ± 0.78	-12.12	0.00**
	Hypertensive	24	109.85 ± 2.12	0.1	
UCr (mg/dl)	Non-Hypertensive	83	161.3 ± 6.38	0.55	0.59 NS
	Hypertensive	24	153.82±12.51	0.49	
UrCR (g/dl)	Non-Hypertensive	83	0.16 ± 0.01	0.49	0.62 NS
	Hypertensive	24	0.15 ± 0.01	-0.77	
Ur ALB (mg/dl)	Non-Hypertensive	83	10.66 ± 0.6	-0.77	0.44 NS
	Hypertensive	24	11.6 ± 0.94	-0.52	
Ur ALB/UCR	Non-Hypertensive	83	79.59 ± 6.26	-0.52	0.60 NS
	Hypertensive	24	86.42 ± 10.88	0.82	
SCr (mg/dl)	Non-Hypertensive	83	1.14 ± 0.03	0.82	0.41 NS
	Hypertensive	24	1.09 ± 0.04	-0.36	
CrCl (ml/min)	Non-Hypertensive	83	123 ± 9.15	-0.36	0.72 NS
	Hypertensive	24	130.33±20.16	0.24	
CG (ml/min)	Non-Hypertensive	83	91.25 ± 2.86	0.24	0.82 NS
	Hypertensive	24	89.75 ± 6.6	0.23	
EP!Cr (ml/min)	Non-Hypertensive	83	88.88 ± 2.34	0.23	0.82 NS
	Hypertensive	24	87.79 ± 3.29	1.97	
MDRD (ml/min)	Non-Hypertensive	83	109.41 ± 1.06	1.97	0.05*
	Hypertensive	24	105.15 ± 1.63	0.87	
UrNa+/K+	Non-Hypertensive	83	4.68 ± 0.37	0.87	0.39 NS
	Hypertensive	24	4.06 ± 0.37	0.44	
Ur flow (ml/min)	Non-Hypertensive	83	0.86 ± 0.05	-1.09	0.28 NS
	Hypertensive	24	0.99 ± 0.12	0.82	
CrCl (ml/min)	Non-Hypertensive	83	123 ± 9.15	-0.36	0.72 NS
	Hypertensive	24	130.3 ± 20.2	0.00	

Note *P < 0.05 = Significant, **P < 0.01 = Highly Significant, P > 0.05 = Not Significant

Graphical presentation of the T-test comparison for Non-Hypertensive and Hypertensive Male Subjects shown in table 19 above (from fig. 4.57 to 4.66)

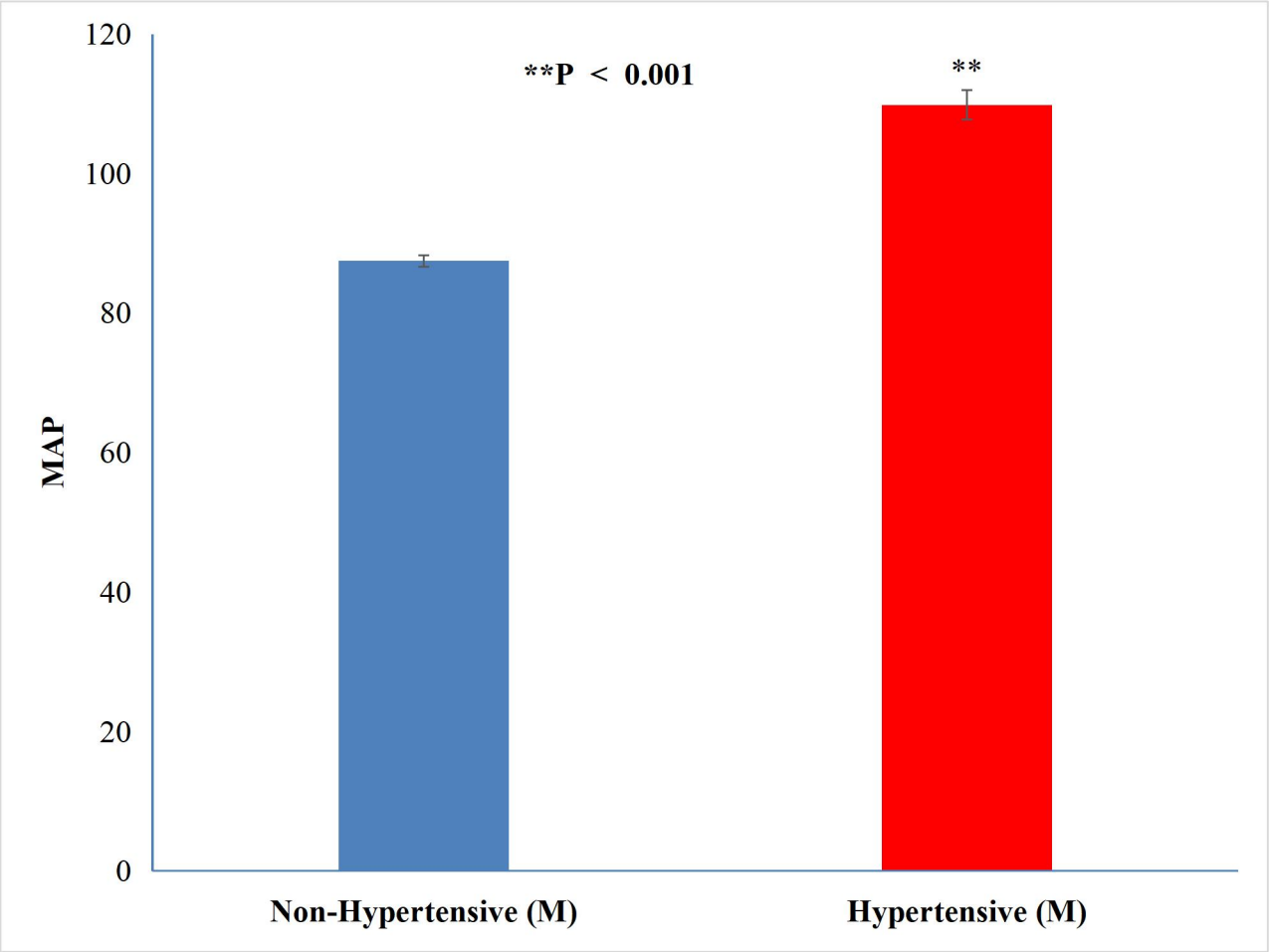

Fig. 4.57: Bar chart graph comparison of MAP of Hypertensive and Non-Hypertensive Male subjects. The mean value of MAP was significantly higher in Hypertensive subjects ($P < 0.05$).

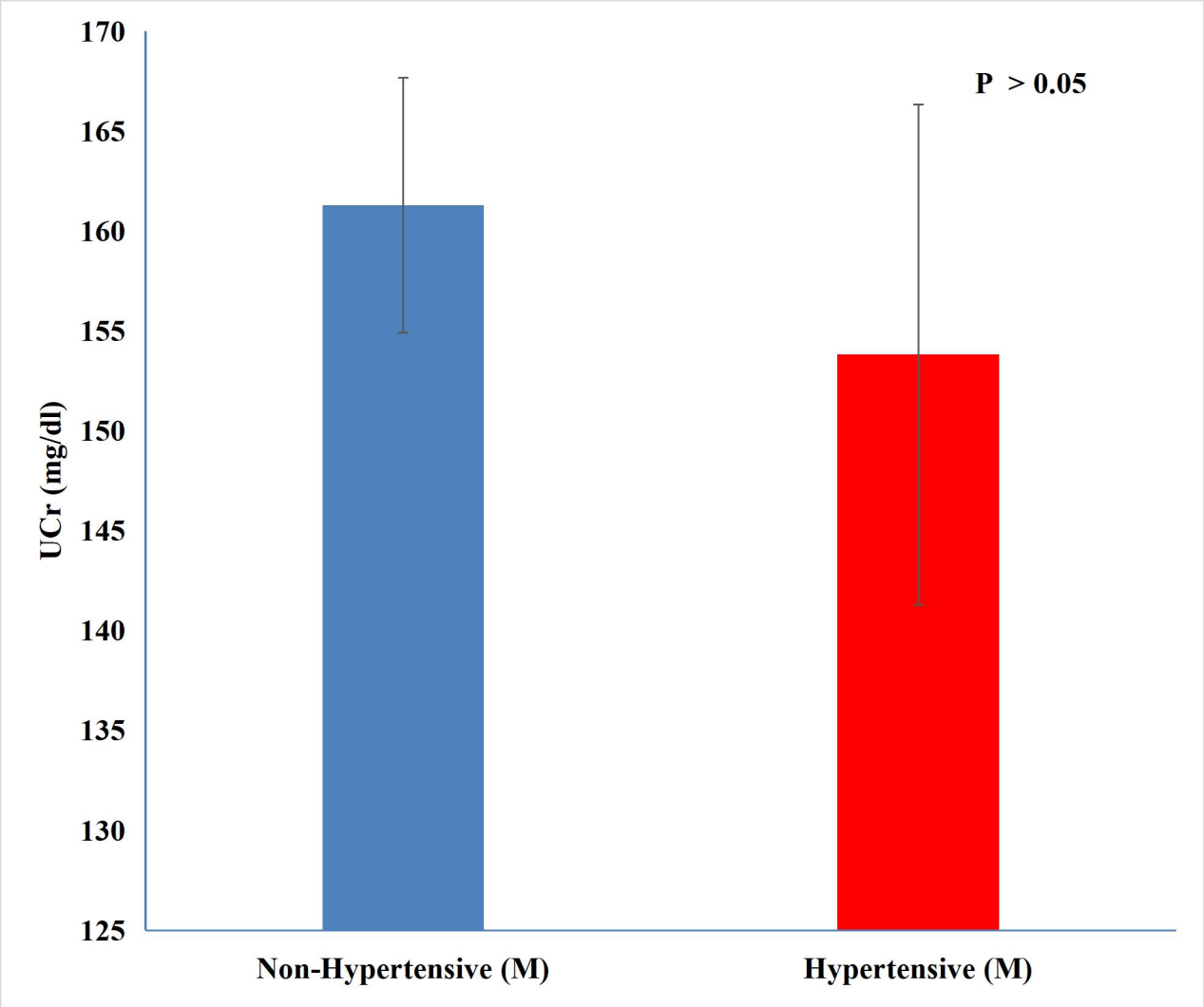

Fig. 4.58 : Bar chart graph Comparison of Urine creatinine (UCr in mg/dl) of the Hypertensive and Non-Hypertensive Male subjects. The mean value of UCr was not significantly higher in non-Hypertensive subjects ($P > 0.05$).

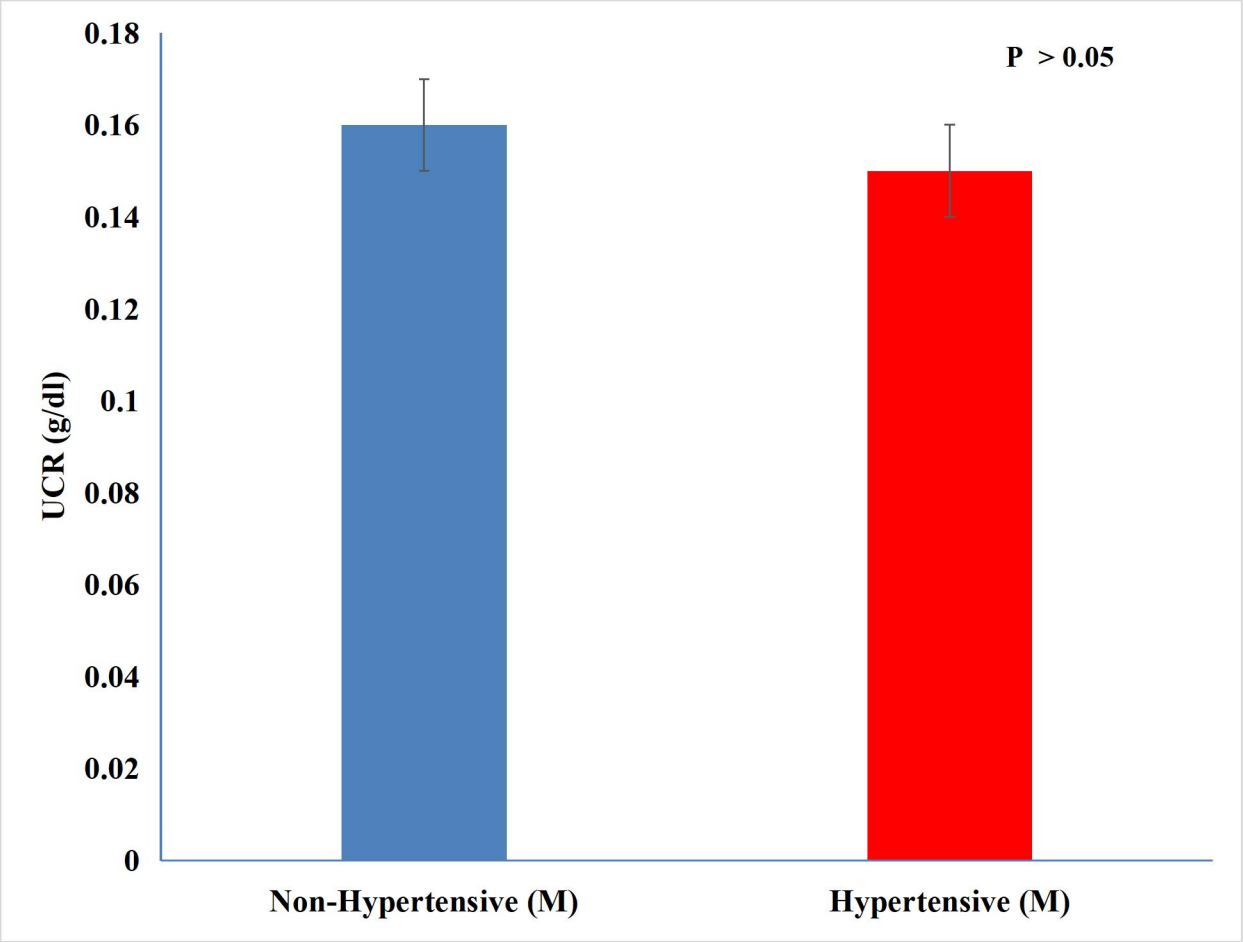

Fig. 4.59: Bar chart graph Comparison of Urine creatinine (Ur CR, g/dl) of the Hypertensive and non-Hypertensive Male subjects. The UCR was not significantly higher in non-hypertensive subjects ($P > 0.05$).

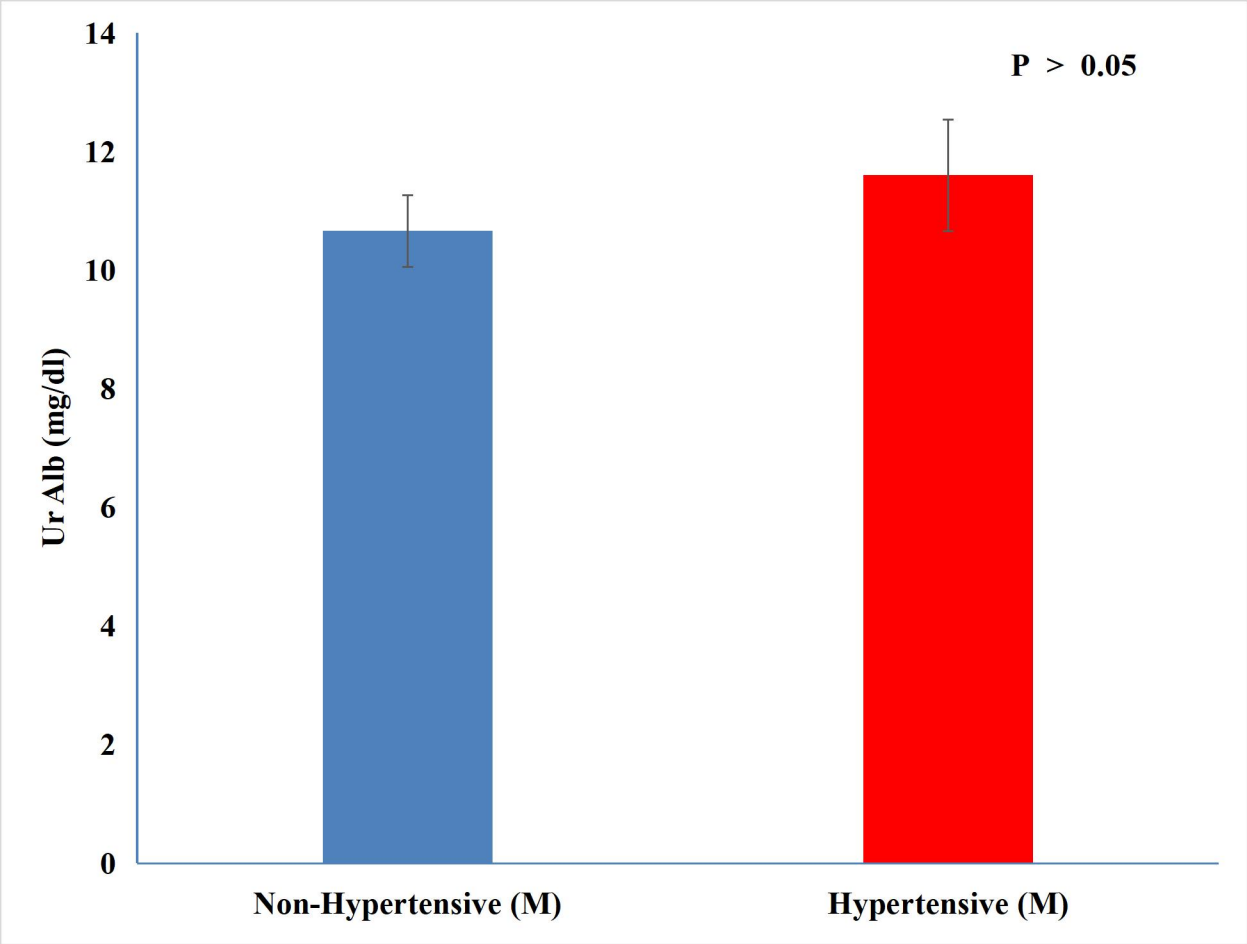

Fig. 4.60: Bar chart graph Comparison of Urine Albumin excretion (Ur Alb, mg/dl) of the Hypertensive and Non-Hypertensive male Subjects. The Ur Alb was not significantly higher in hypertensive subjects (P > 0.05).

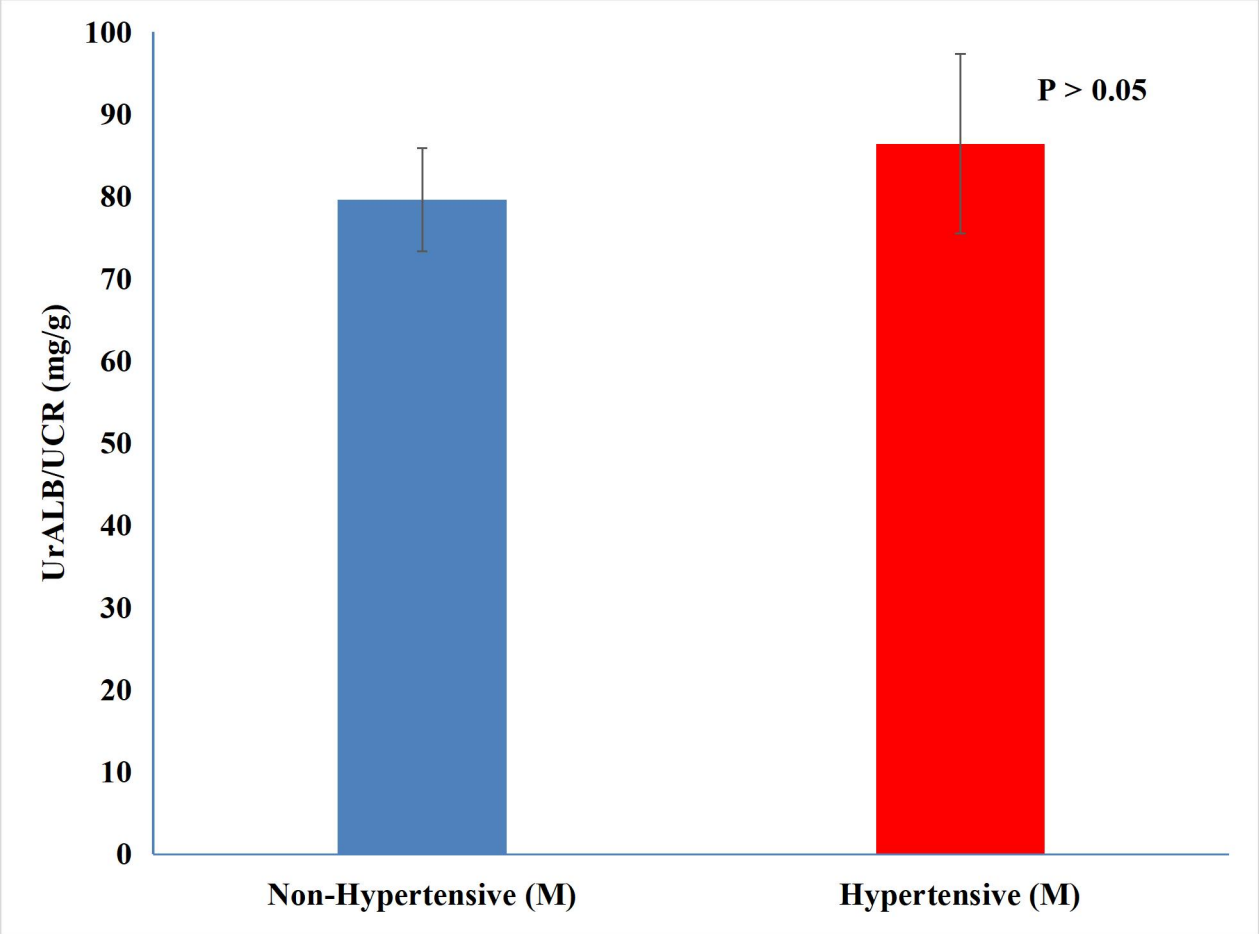

Fig. 4.**61**: Bar chart graph comparison of Urine Albumin Creatinine ratio (UrALB/UCR, mg/g) of the Hypertensive and Non-Hypertensive Male subjects. The UrALB/UCR was not significantly higher in hypertensive subjects ($P > 0.05$).

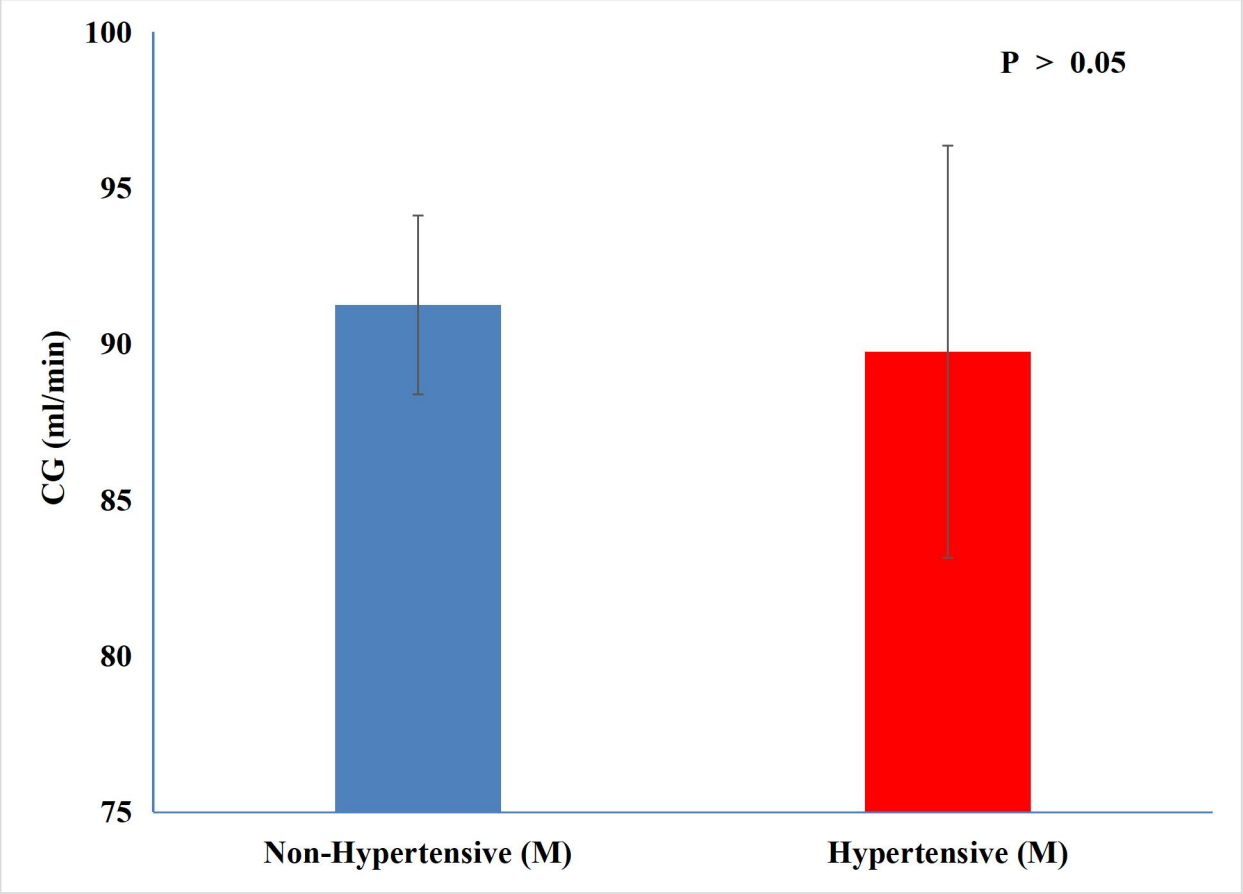

Fig. 4.62: Bar chart graph comparison of Cockroft-Gault eGFR (CG, ml/min) of the Hypertensive and Non-Hypertensive Male Subjects. The CG of hypertensive Subjects was not significantly lower than non-hypertensive ($P > 0.05$).

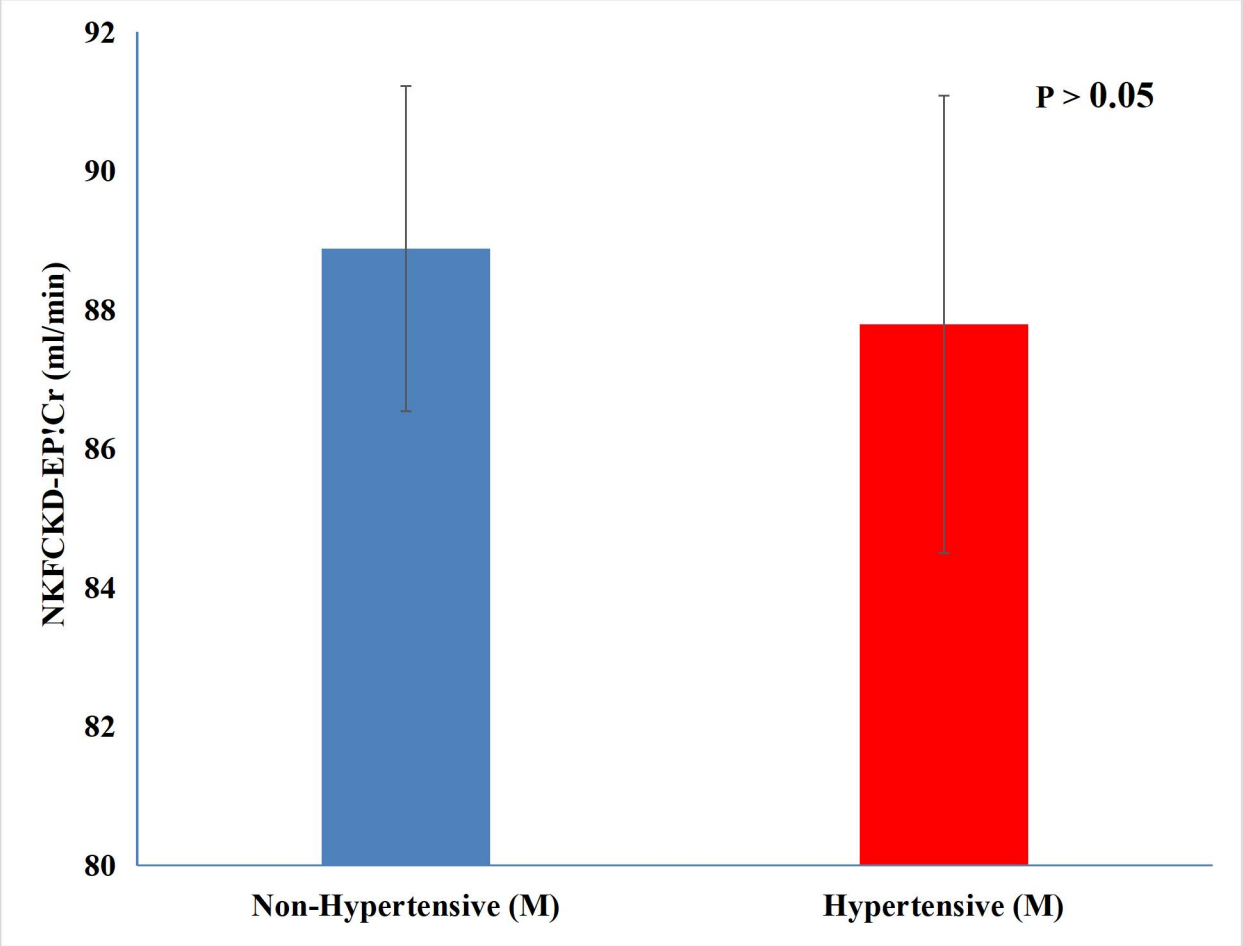

Fig. 4.63: Bar chart graph comparison of NKFCKD-EP!Cr (ml/min/1.73m ^{2}) of the Hypertensive and Non-Hypertensive Male Subjects. The CKD-EP!Cr was not significantly lower in hypertensive Subjects (P > 0.05).

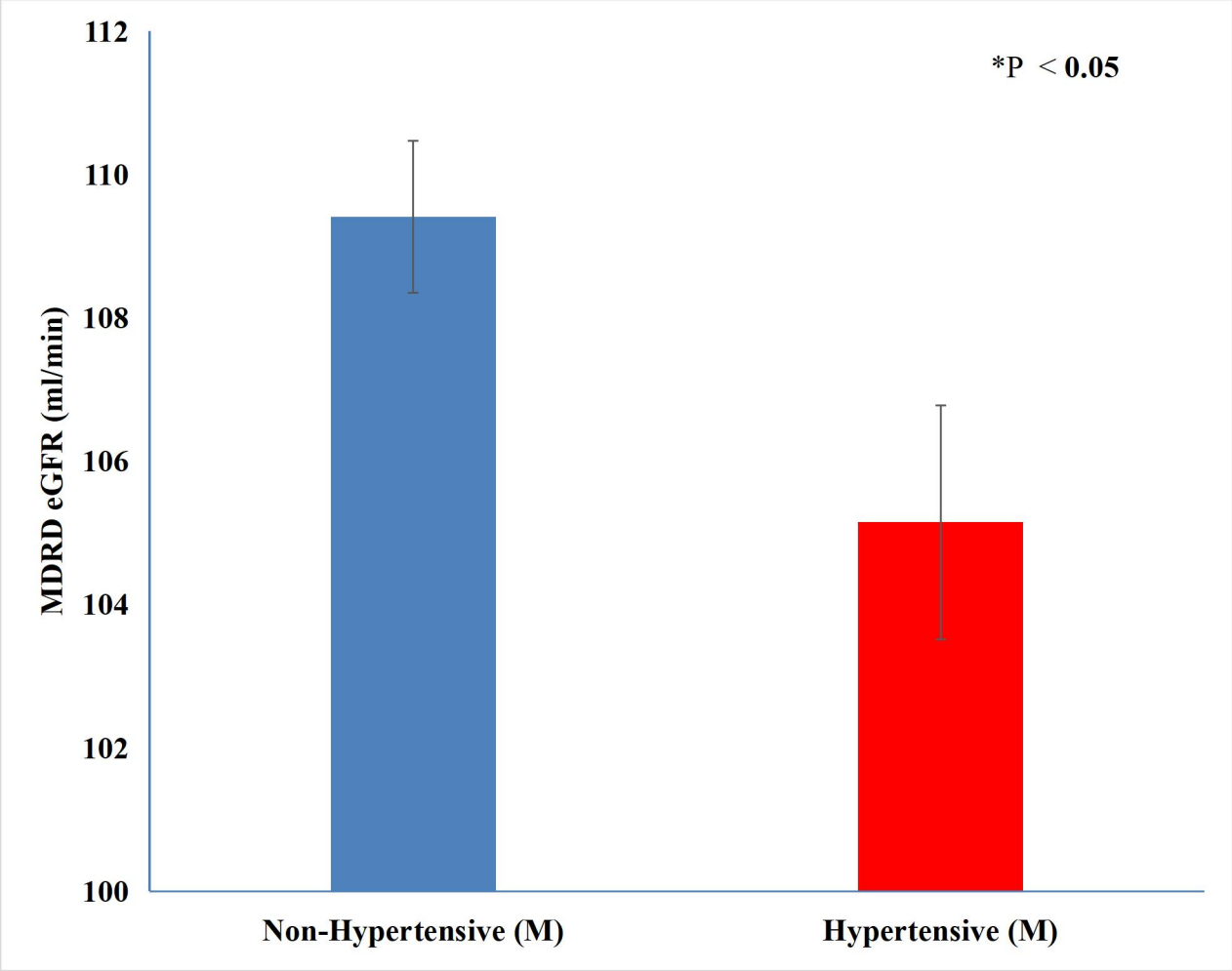

Fig. 4.64: Bar chart graph comparison of Modification of Diet in Renal Disease eGFR (MDRD, ml/min/1.73 m ^{2}) of the Hypertensive and Non-Hypertensive male Subjects. The MDRD eGFR was significantly lower in hypertensive than in non-hypertensive Subjects ($P < 0.05$).

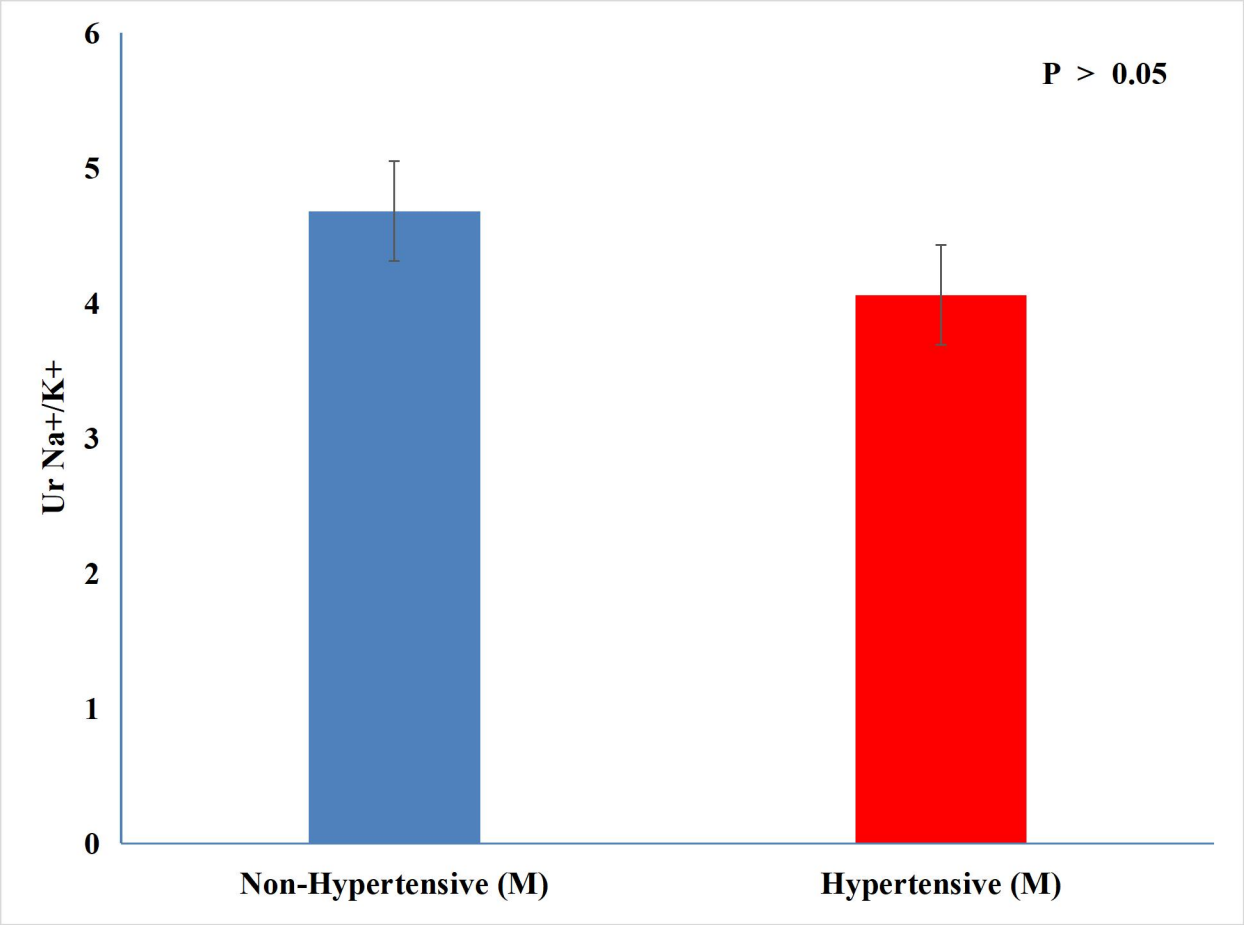

Fig. 4.65: Bar chart graph comparison of Urine Sodium Potassium ratio (Ur Na+/K+) of the Hypertensive and Non-Hypertensive Male Subjects. The Ur Na+/K+ was not significantly higher in non-hypertensive Subjects (P > 0.05).

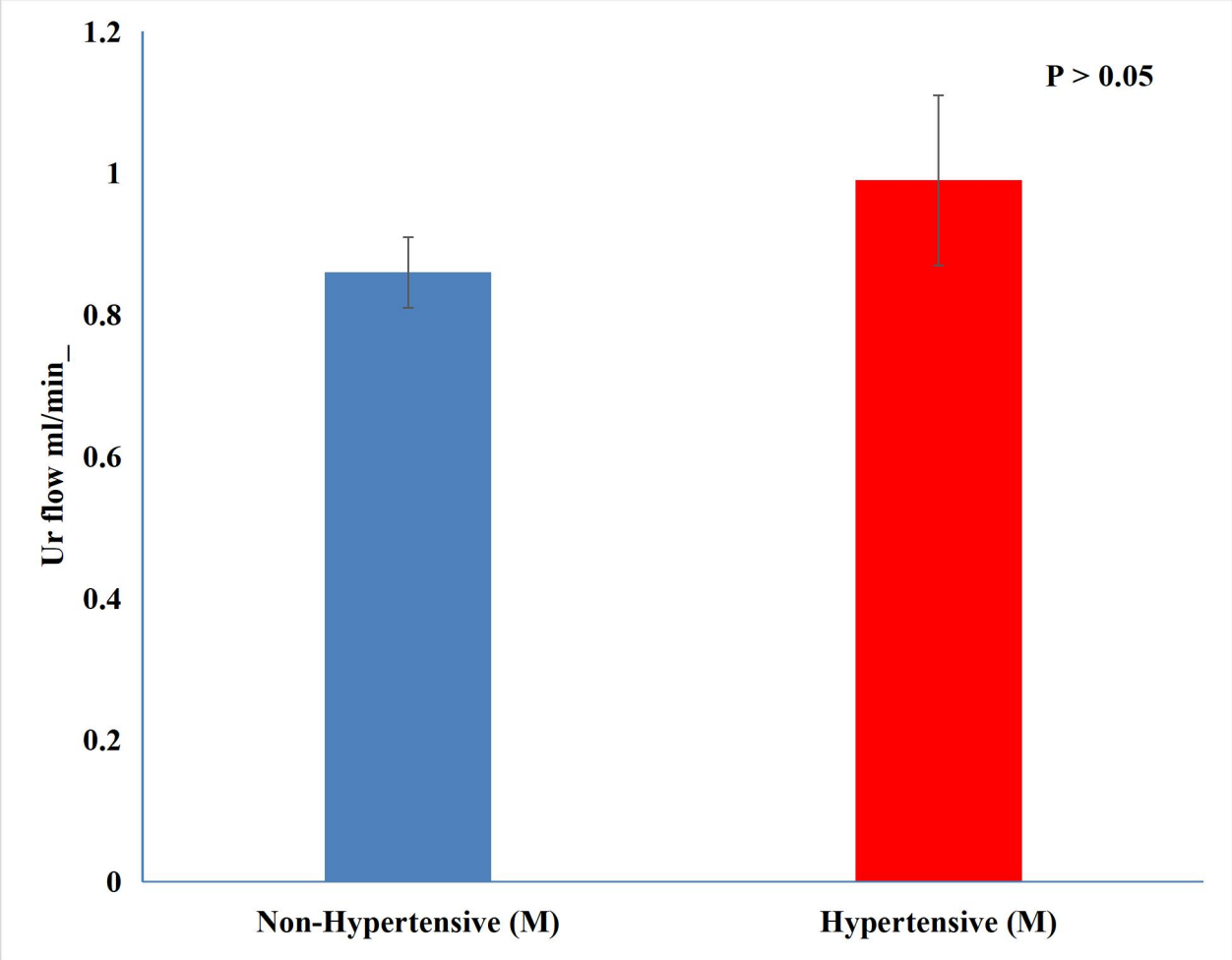

Fig. 4.66: Bar chart graph comparison of Urine flow rate (ml/min) of Hypertensive and Non-Hypertensive Male Subjects. The Ur flow was not significantly higher in Hypertensive Subjects ($P > 0.05$).

Table 4.12: T - test Summary for the Hypertensive and Non-Hypertensive Subjects (**Females**)

Parameters	**Non-hypertensive and Hypertensive Females**	**N**	**Mean ± Sem**	**T-value**	**P-value**
MAP	Non-Hypertensive	106	84.79±0.81	-11.76	**0.001
	Hypertensive	29	109.31±2.68		
Ur Cr (mg/dl)	Non-Hypertensive	106	164.54±5.78	3.10	**0.001
	Hypertensive	29	127.47±8.73		
Ur CR (g/dl)	Non-Hypertensive	106	0.16±0.01	2.38	*0.02
	Hypertensive	29	0.14±0.01		
Ur ALB (mg/dl)	Non-Hypertensive	106	10.38±0.45	-2.15	*0.03
	Hypertensive	29	13.04±1.71		
Ur ALB/UCR	Non-Hypertensive	106	74.23±4.64	-2.82	**0.01
	Hypertensive	29	106.27±13.63		
SCr (mg/dl)	Non-Hypertensive	106	0.98±0.02	-0.67	0.51 NS
	Hypertensive	29	1.01±0.05		
CG (ml/min)	Non-Hypertensive	106	81.55±2.26	0.75	0.45 NS
	Hypertensive	29	77.64±5.45		
EP!Cr (ml/min)	Non-Hypertensive	106	80±1.68	1.91	0.06 NS
	Hypertensive	29	72.76±3.89		
MDRD (ml/min)	Non-Hypertensive	106	82.92±0.66	4.72	*0.001
	Hypertensive	29	76.48±0.97		
UrNa+/K+	Non-Hypertensive	106	4.27±0.28	1.48	0.14 NS
	Hypertensive	29	3.41±0.39		
Ur flow (ml/min)	Non-Hypertensive	106	0.77±0.03	-0.38	0.70 NS
	Hypertensive	29	0.8±0.06		
CrCl (ml/min)	Non-Hypertensive	106	131.38±7.16	1.95	*0.05
	Hypertensive	29	101.83±12.57		

Note * P < 0.05 = Significant, **P < 0.01= Highly significant, P > 0.05= Not significant(NS)

Graphical presentation of the T-test comparison for Non-Hypertensive and Hypertensive Female Subjects shown in table 15 above (from fig. 4.67 to 4.78)

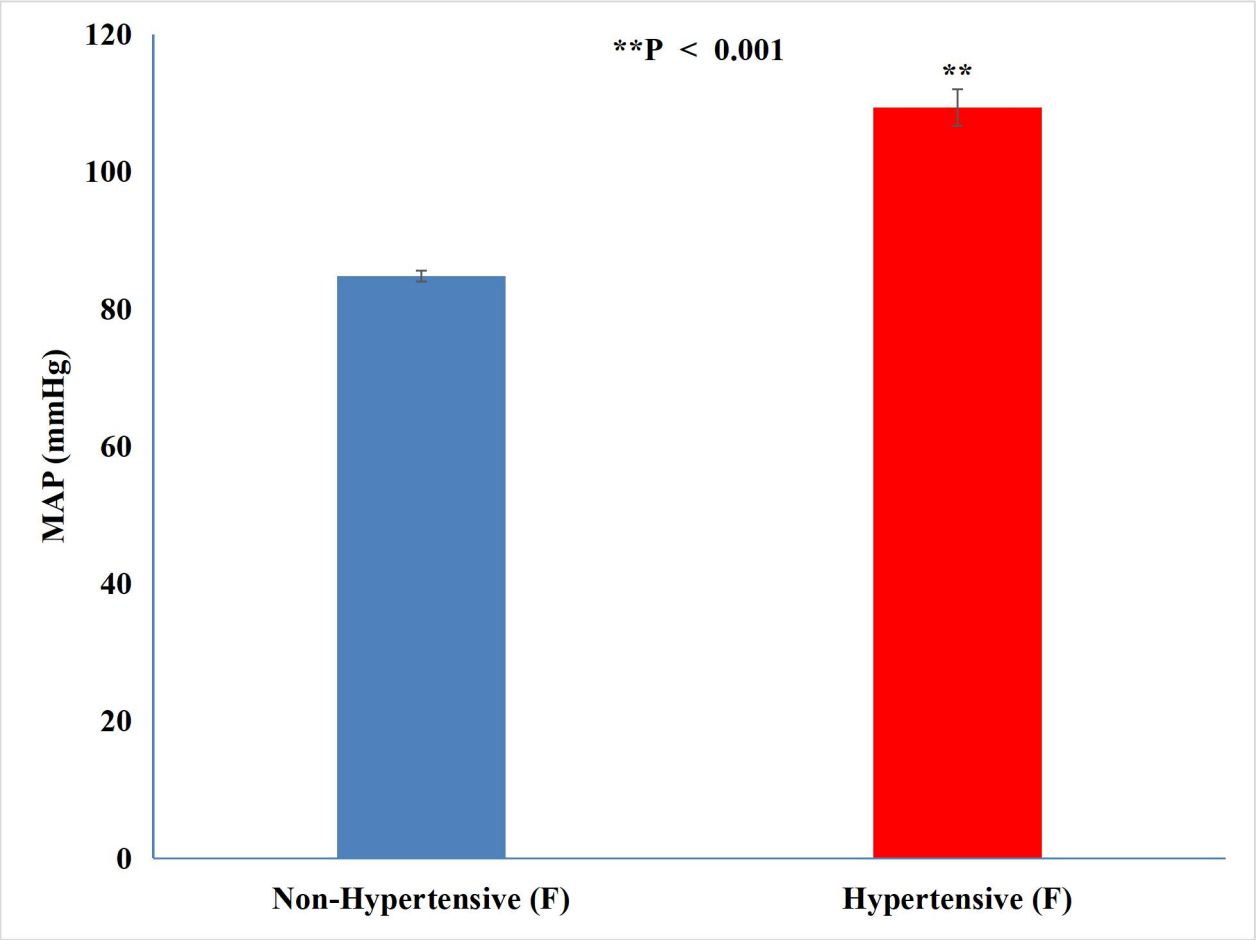

Fig. 4.67: Bar chart graph comparison of Mean Arterial Pressure (MAP, mmHg) of the Hypertensive and Non-Hypertensive Female Subjects. There was significantly higher MAP in Hypertensive than non-Hypertensive Female Subjects ($P < 0.001$).

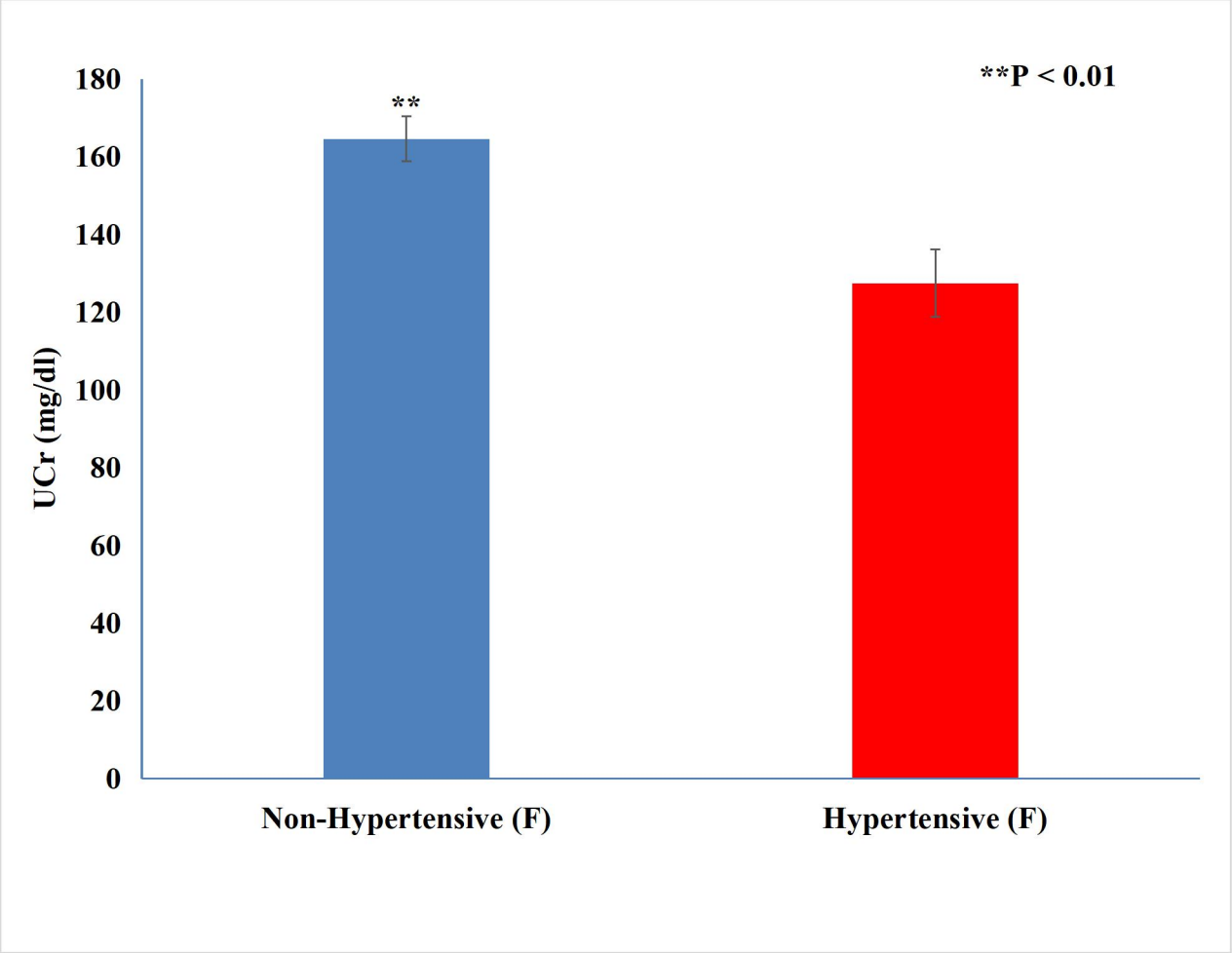

Fig. 4.68: Bar chart graph Comparison of Urine Creatinine (UCr in mg/dl) of the Hypertensive and Non-Hypertensive Female Subjects. The UCr was significantly greater in non-Hypertensive ($P < 0.01$).

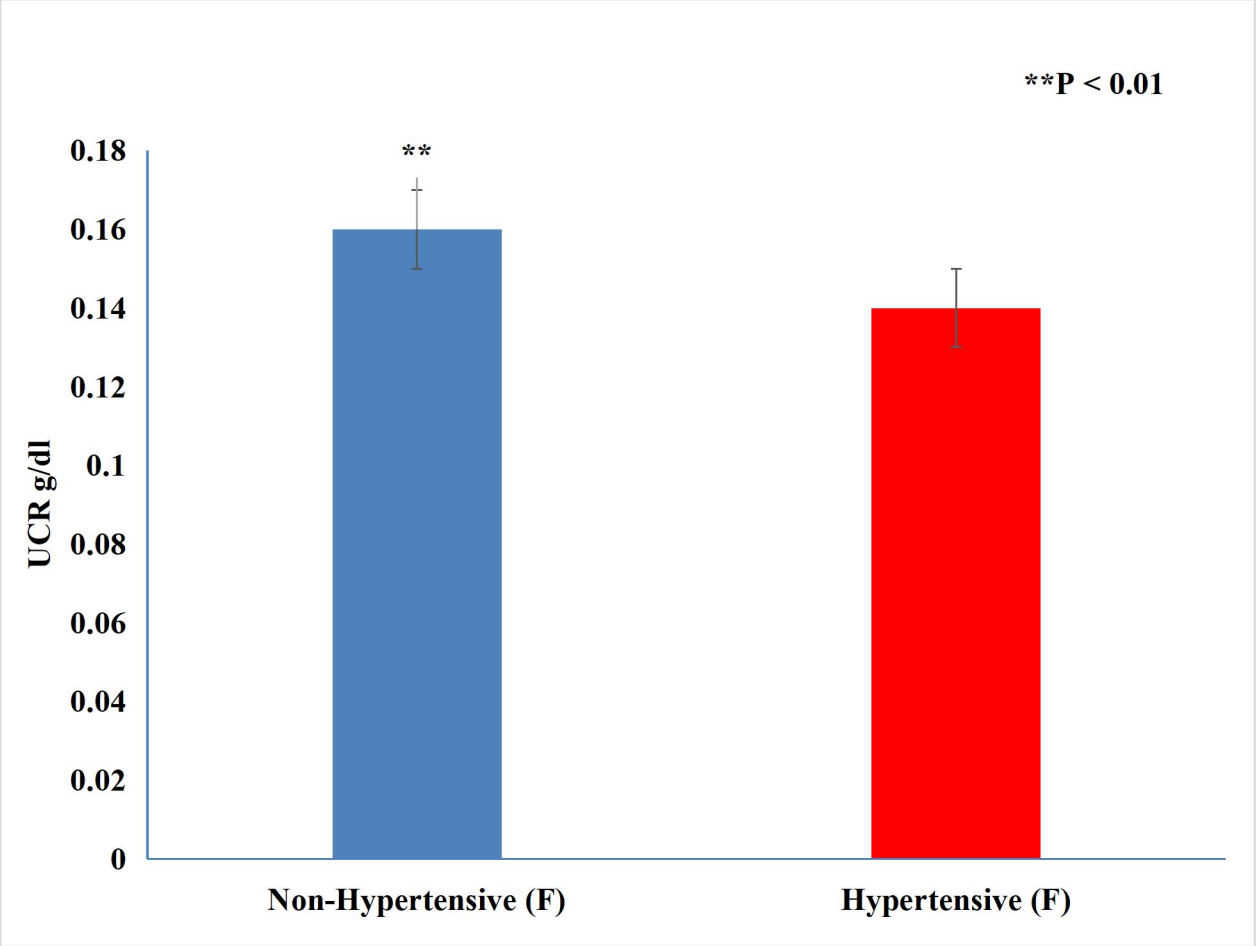

Fig. 4.69: Bar chart graph Comparison of Urine Creatinine (UCR in g/dl) of the Hypertensive and Non-Hypertensive Female Subjects. The UCR was significantly greater in non-Hypertensive Female Subjects ($P < 0.01$).

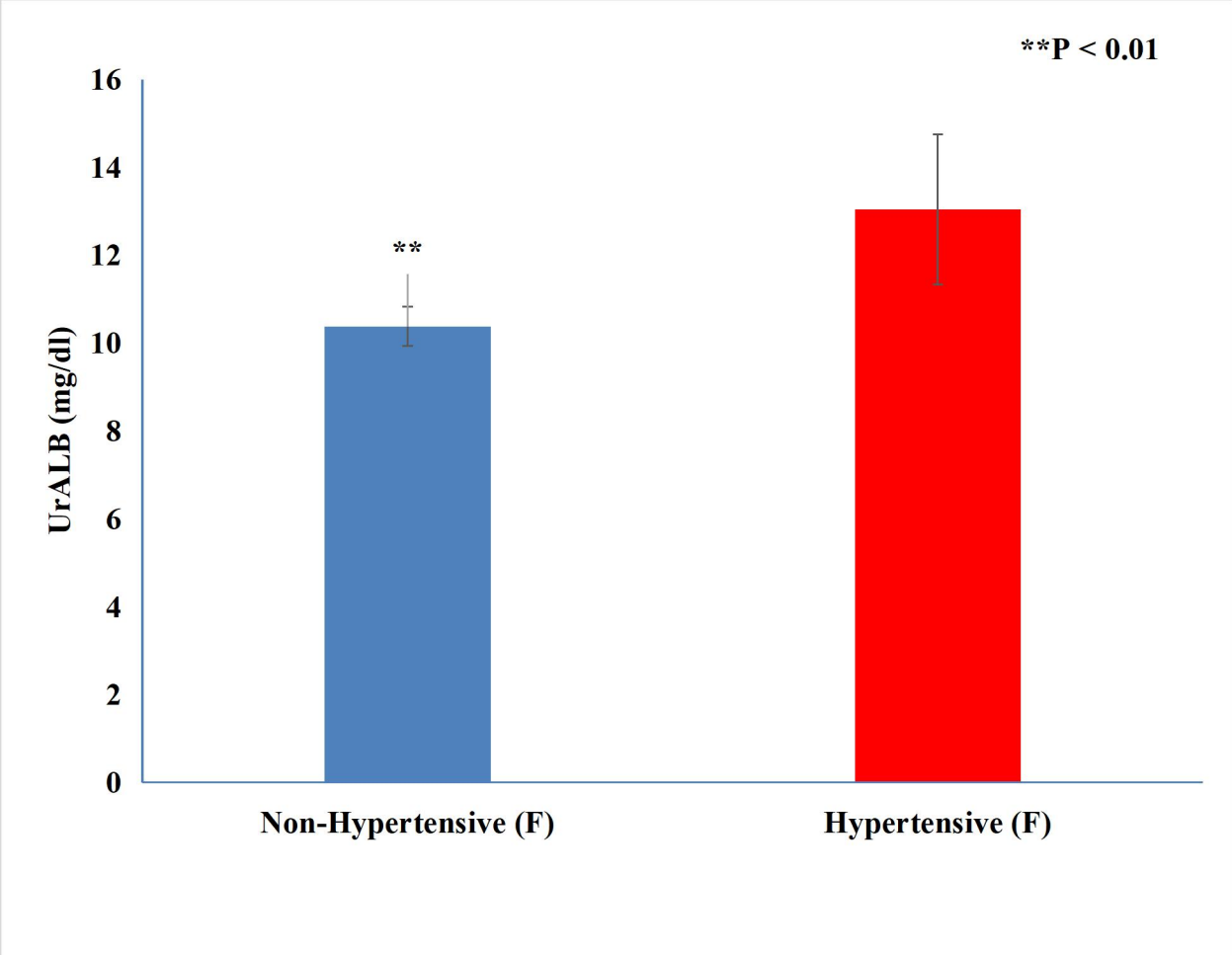

Fig. 4.70: Bar chart graph comparison of Urine Albumin excretion (UrALB in mg/dl) of the Hypertensive and Non-Hypertensive Female Subjects. The mean UrALB was significantly greater in Hypertensive Females.

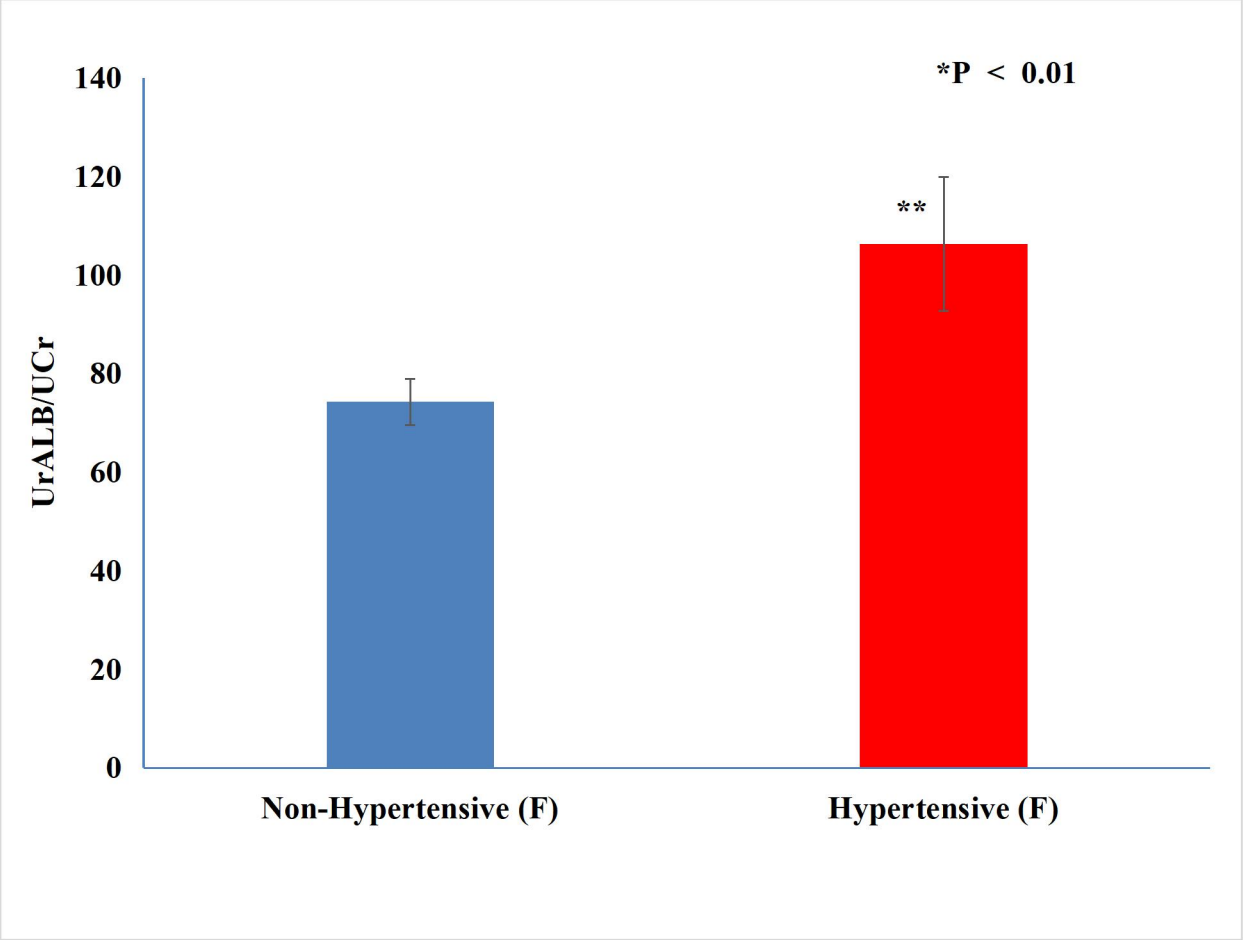

Fig. 4.71: Bar chart graph comparison of Urine Albumin Creatinine ratio (UrALB/UCR mg/g) of the Hypertensive and Non-Hypertensive Female Subjects. The UrALB/UCR ratio was significantly greater in Hypertensive Females ($P < 0.01$).

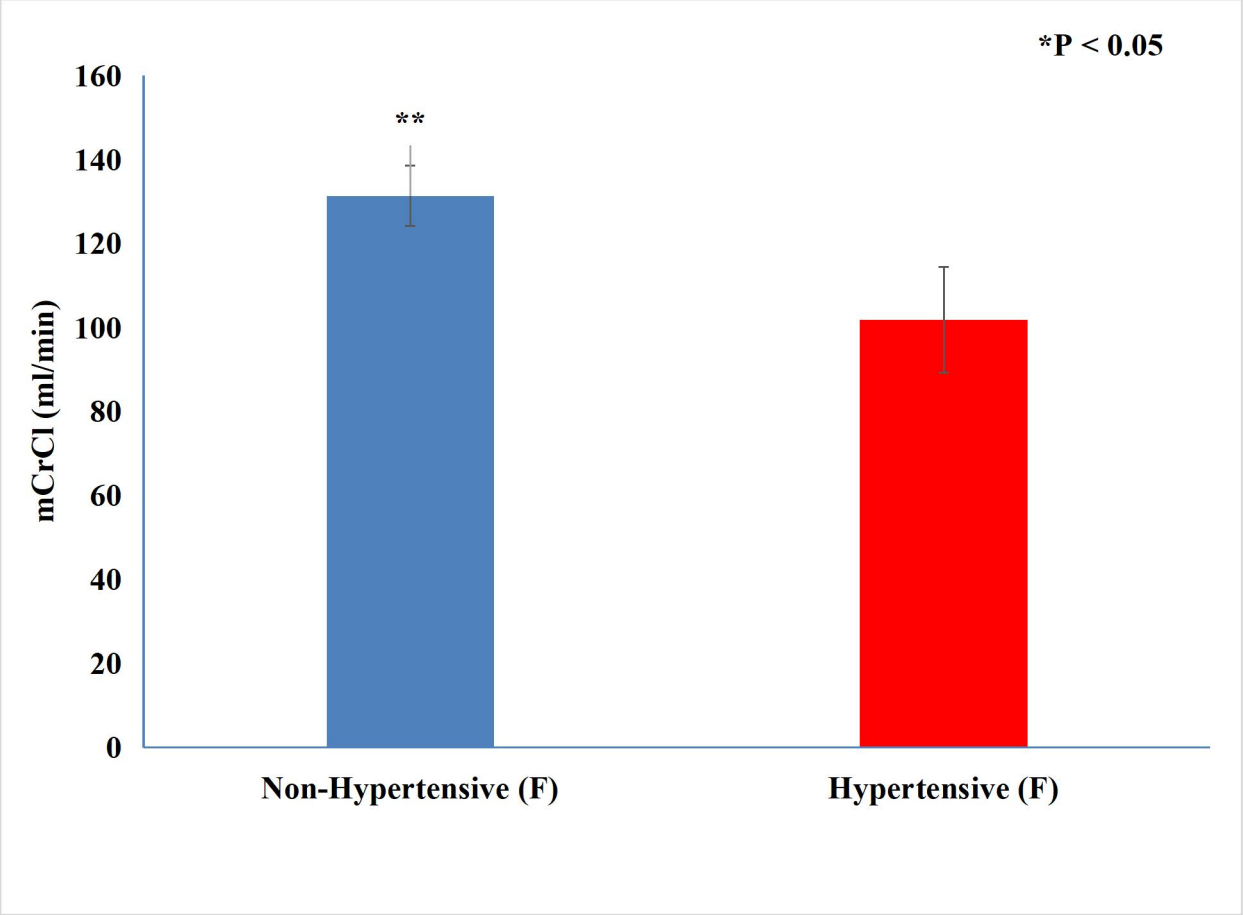

Fig. 4.72: Bar chart graph comparison of measured Creatinine clearance (mCrCl in ml/min/1.73m^2) of the Hypertensive and Non-Hypertensive Female Subjects. The mean mCrCl was significantly lower in Female Hypertensive Subjects ($P < 0.05$).

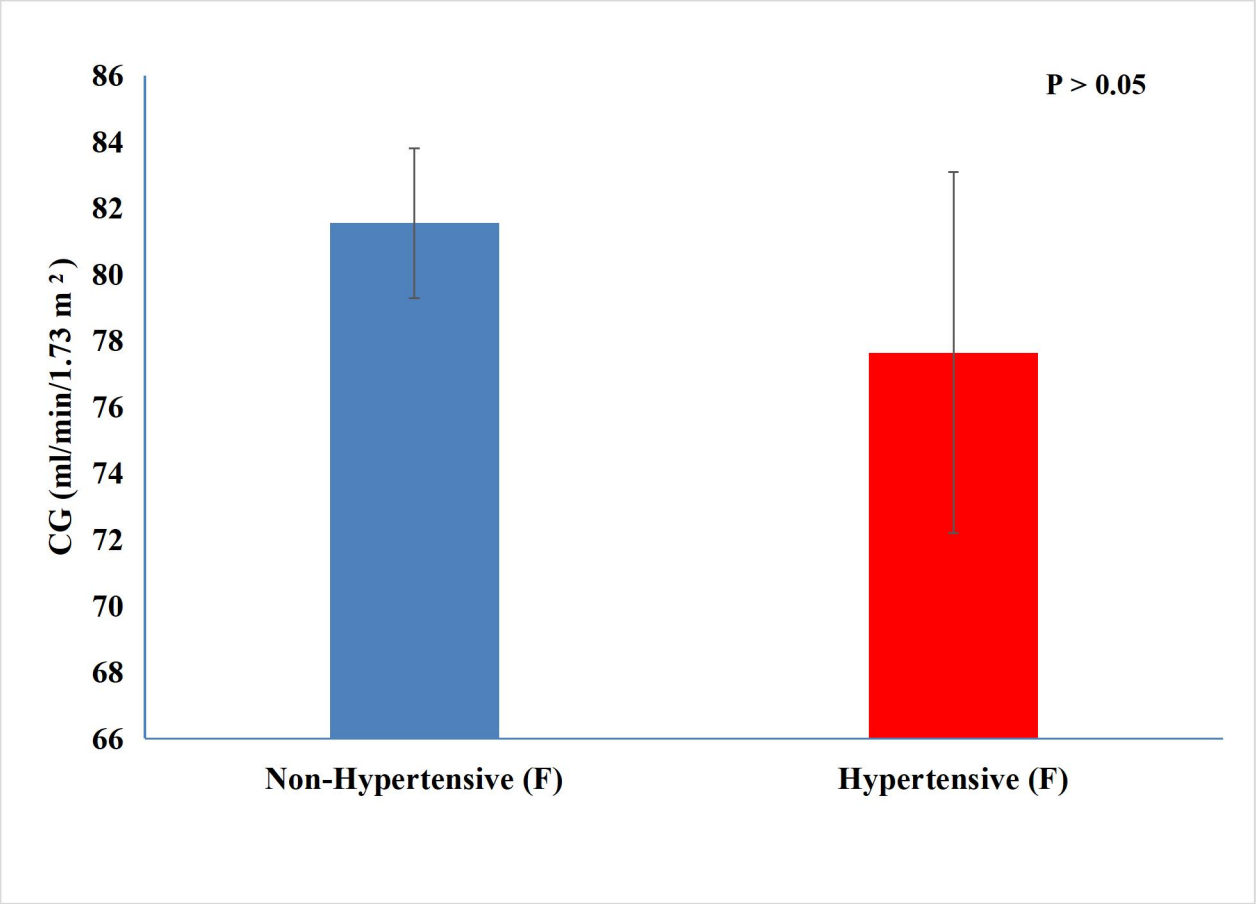

Fig. 4.73: Bar chart graph comparison of Cockroft-Gault eGFR (CG in ml/min/1.73 m ^{2}) of the Hypertensive and Non-Hypertensive Female Subjects. The CG was not significantly lower in Hypertensive Female Subjects ($P > 0.05$).

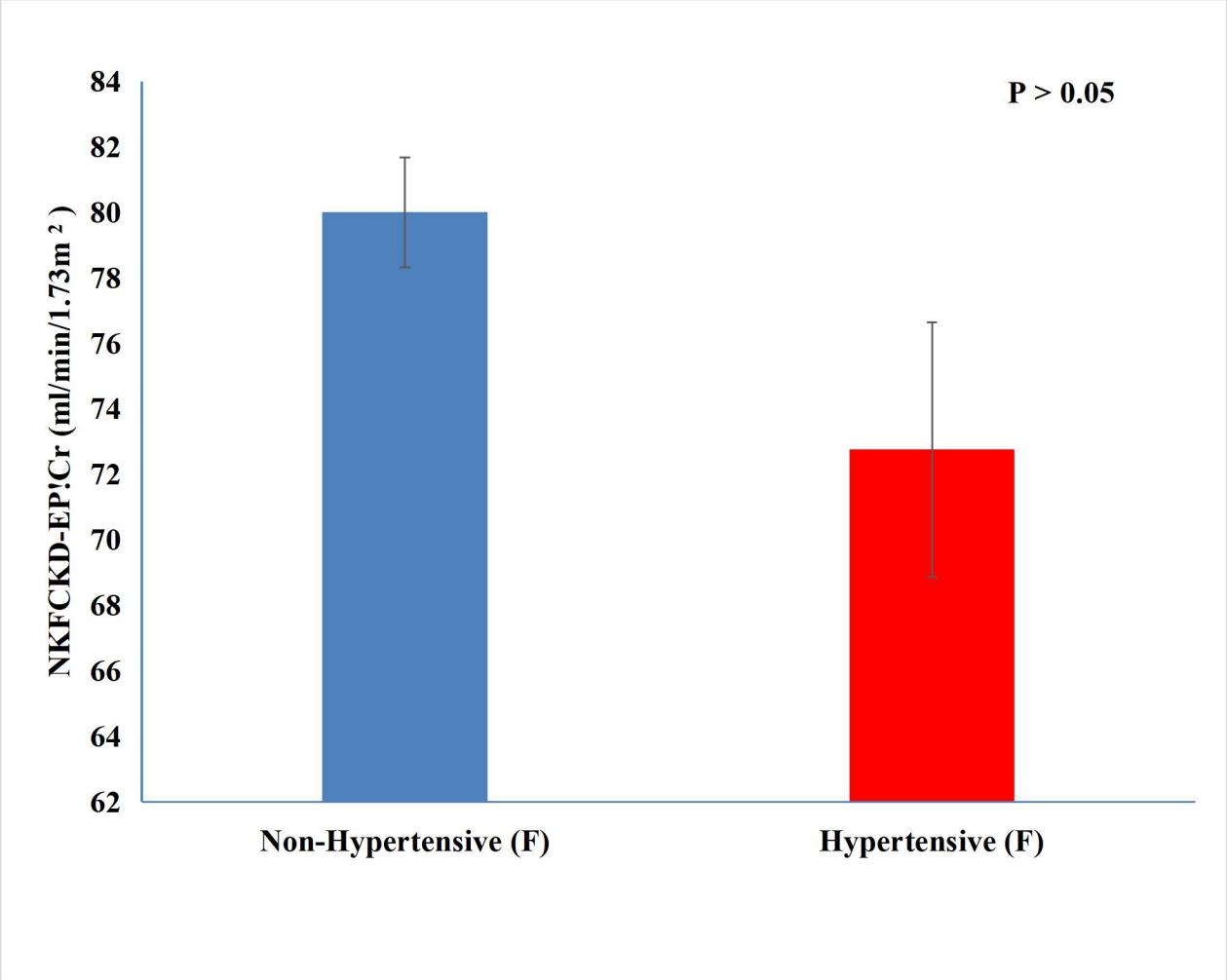

Fig. 4.74: Bar chart graph comparison of CKD-EP!Cr eGFR (in ml/min/1.73 m ^{2}) of the Hypertensive and Non-Hypertensive Female Subjects. The CKD-EP!Cr eGFR was not significantly lower in Hypertensive Female Subjects ($P > 0.05$).

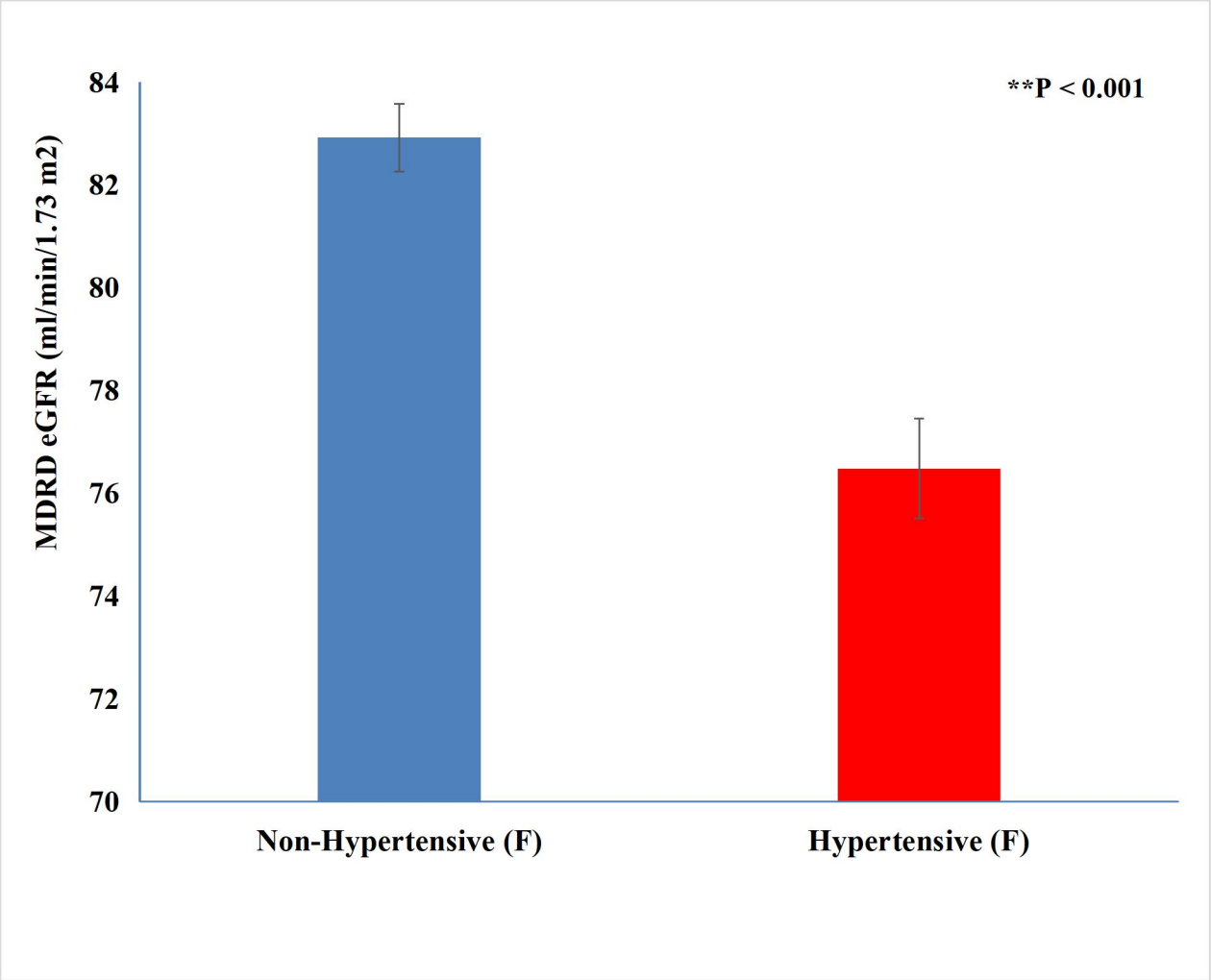

Fig. 4.75: Bar chart graph comparison of Modification of Diet in Renal Disease eGFR (MDRD in ml/min/1.73m ^{2}) of Hypertensive and Non-Hypertensive Female Subjects. The MDRD eGFR was significantly lower in Hypertensive Subjects ($P < 0.001$).

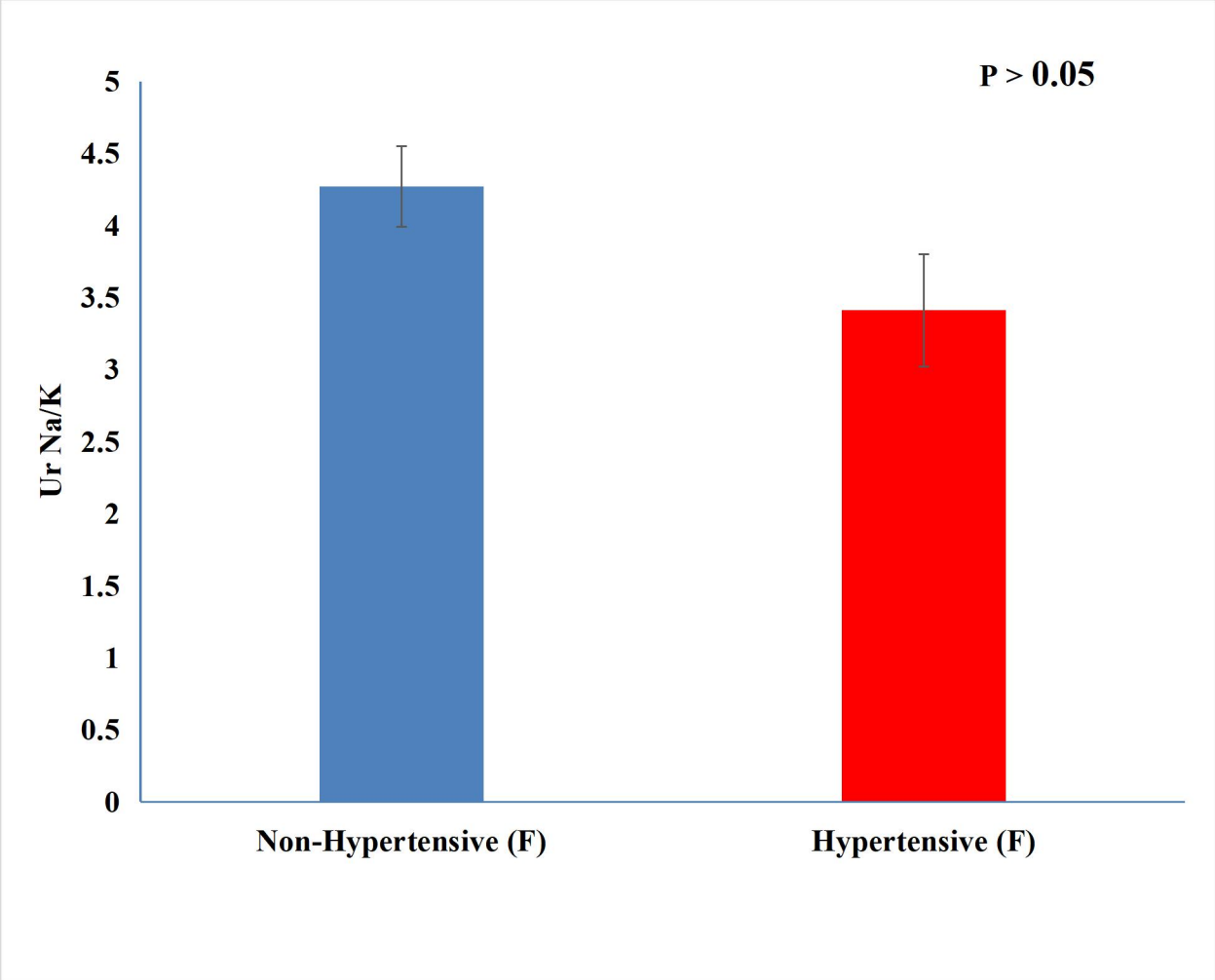

Fig. 4.76: Bar chart graph Comparison of Urine Sodium Potassium ratio (UrNa+/K+) of the Hypertensive and Non-Hypertensive Female Subjects. The UrNa+/K+ was not significantly lower in Hypertensive Female Subjects ($P > 0.05$).

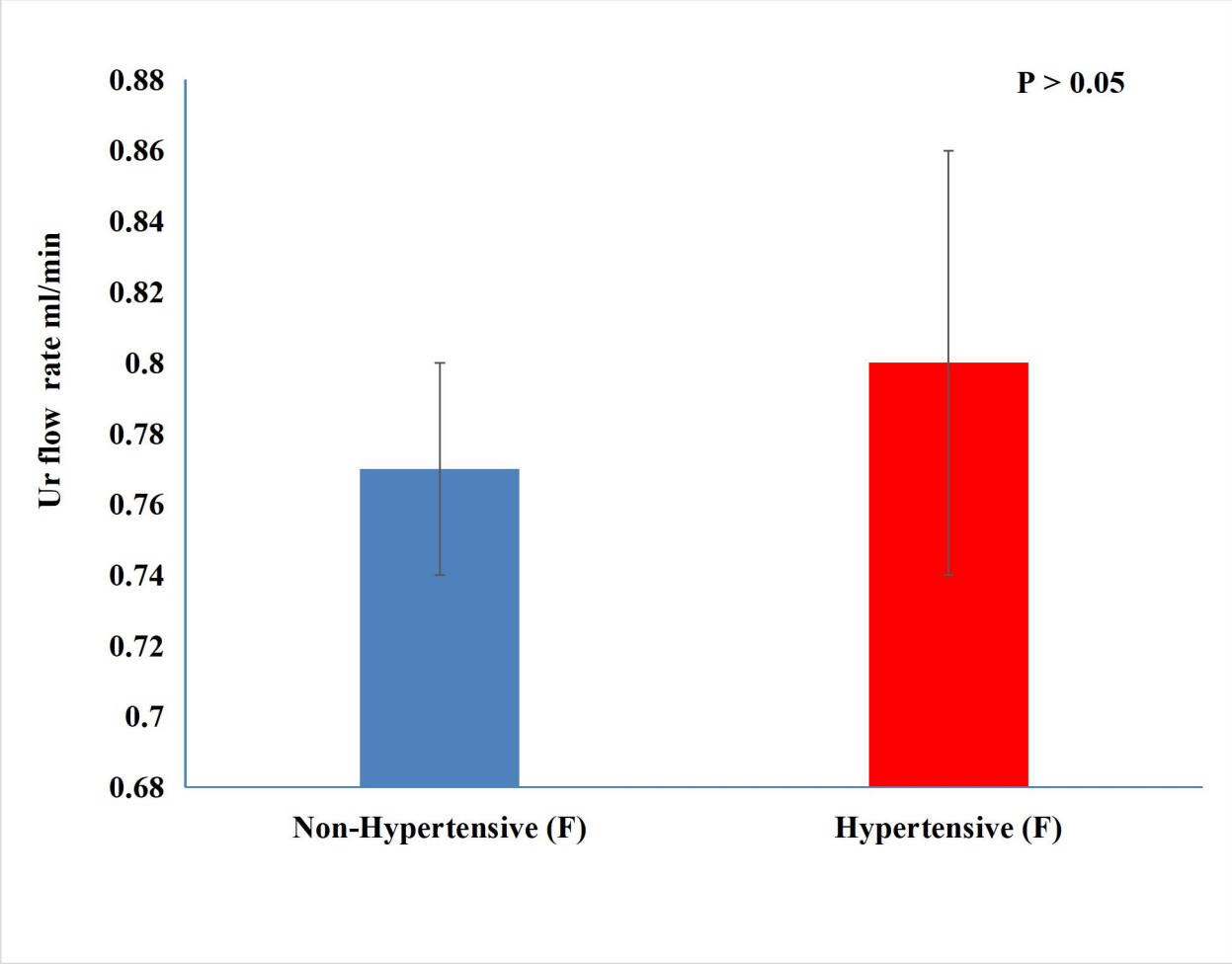

Fig. 4.77: Bar chart graph Comparison of Urine flow rate in ml/min of Hypertensive and Non-Hypertensive Female Subjects. The Ur flow was not significantly higher in Hypertensive Female Subjects ($P > 0.05$).

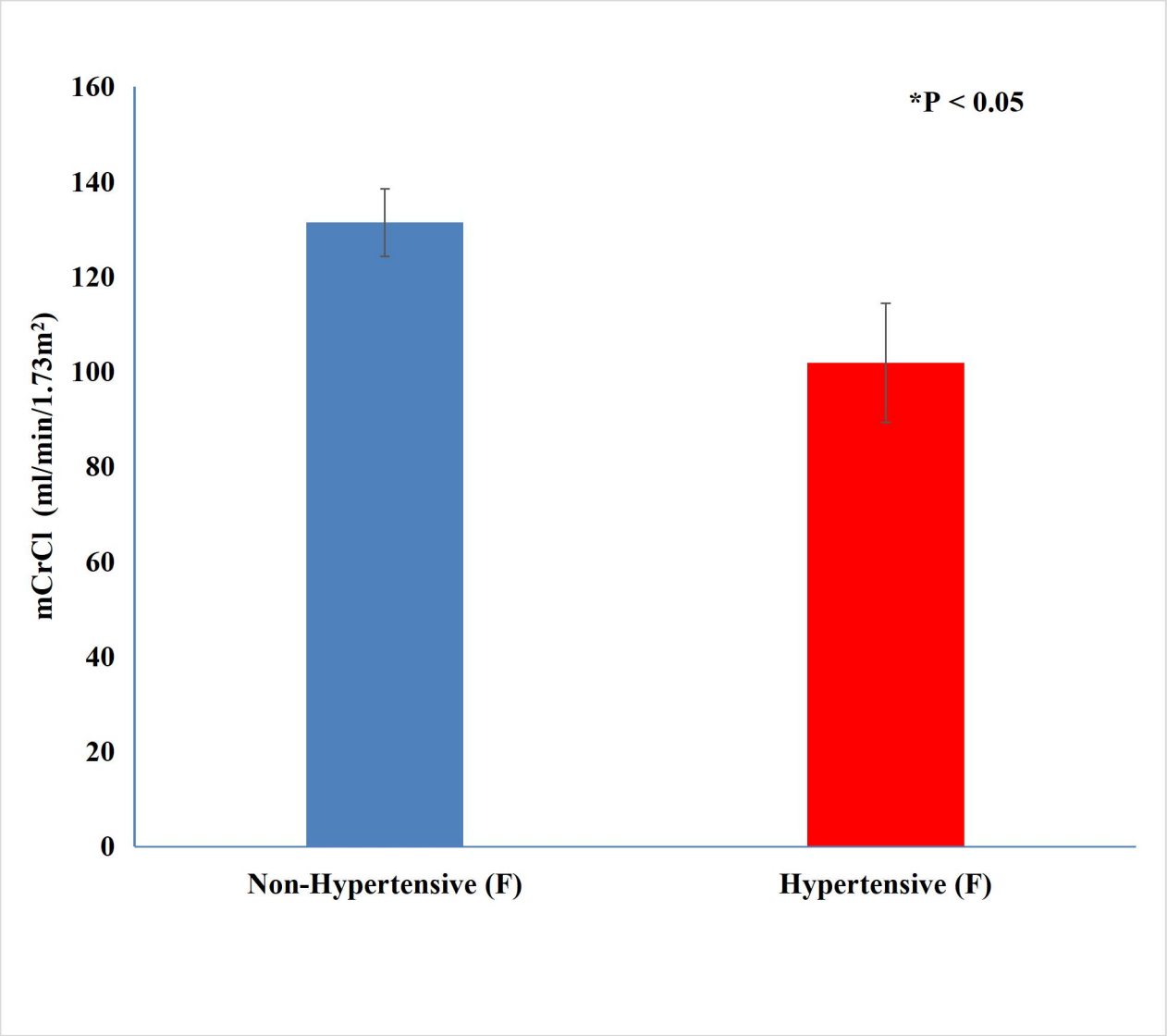

Fig. 4.78: Comparison of measured Creatinine clearance (mCrCl in ml/min/1.73 m ^{2}) of the Hypertensive and Non-Hypertensive Female Subjects. The mCrCl was significantly lower in the Hypertensive Female Subjects ($P < 0.05$).

Table 4.13: Percentage of CKD detected by GFR methods and UALB/CR (or UACR) from 243 Apparently Healthy Subjects

GFR method	Mean GFR ± Sem (ml/min/1.73m^2)	CKD numbers (GFR < 60ml/min/1.73m ^{2})	RATE (%)
MDRD eq.	93.44 ±1.01	None	0.00
Cyst C alone eq.	72.90 ±3.88	72	29.9
Comb NKF CKD-EP! Cr-Cyst C 2021 eq.	79.62 ±2.64	47	19.54
CG eq.	85.22 ±1.69	38	15.7
Simple Cyst C eq.	104.47 ± 6.08	33	13.8
mCrCl	124.86 ±5.09	24	9.91
NKF CKD-EP!Cr 2021	82.95 ±1.27	21	8.7
UALB/CR (UACR) ratio	Mean 81.12 ±3.58	219 (UACR > 30mg/g)	90.5

MDRD eq. = Modification of Diet in Renal Disease eGFR equation

Cyst C alone eq. = NKF Cystatin C alone eGFR equation

Comb NKF CKD-EP! Cr-Cyst C 2021 eq. = Combined National Kidney Foundation Chronic Kidney Disease Epidemiology Creatinine Cystatin C 2021 equation

CG eq. = Cockroft-Gault eGFR equation

Simple Cyst C eq. = Simple Cystatin C equation.

mCrCl = measured Creatinine Clearance method

NKF CKD-EP!Cr 2021 eq. = National Kidney Foundation Chronic Kidney Disease Epidemiology Creatinine 2021 equation

UALB/CR (UACR) ratio = Urine Albumin Creatinine ratio

Table 4.14: Summary of Descriptive statistics of Age, UCR, SCr, CG eGFR, CKD-EP!Cr, MDRD eGFR and mCrCl for Subjects of 30 years and above

	AGE (in years)	UCr (mg/dl)	SCr (mg/dl)	CG (ml/min)	CKD-EP!Cr (ml/min)	MDRD (ml/min)	mCrCL (ml/min)
Mean	48.69	128.00	1.01	84.30	81.04	87.32	91.99
± Sem	0.82	3.38	0.02	2.08	1.59	1.07	3.33

UCr mg/dl = Urine Creatinine in mg/dl

SCr mg/dl = Serum Creatinine in mg/dl

CG ml/min = Cockroft-Gault eGFR in ml/min/1.73 m^2

CKD-EP!Cr ml/min = National Kidney Foundation Chronic Kidney Disease-Epidemiology Creatinine 2021 equation in ml/min/1.73 m^2

MDRD ml/min = Modification of Diet in Renal Disease equation in ml/min/1.73 m^2

mCrCL ml/min = measured Creatinine Clearance in ml/min/1.73 m^2

REGRESSION GRAPHS FOR SUBJECTS OF AGE 30 YEARS AND ABOVE (fig. 4.79 to 4.84)

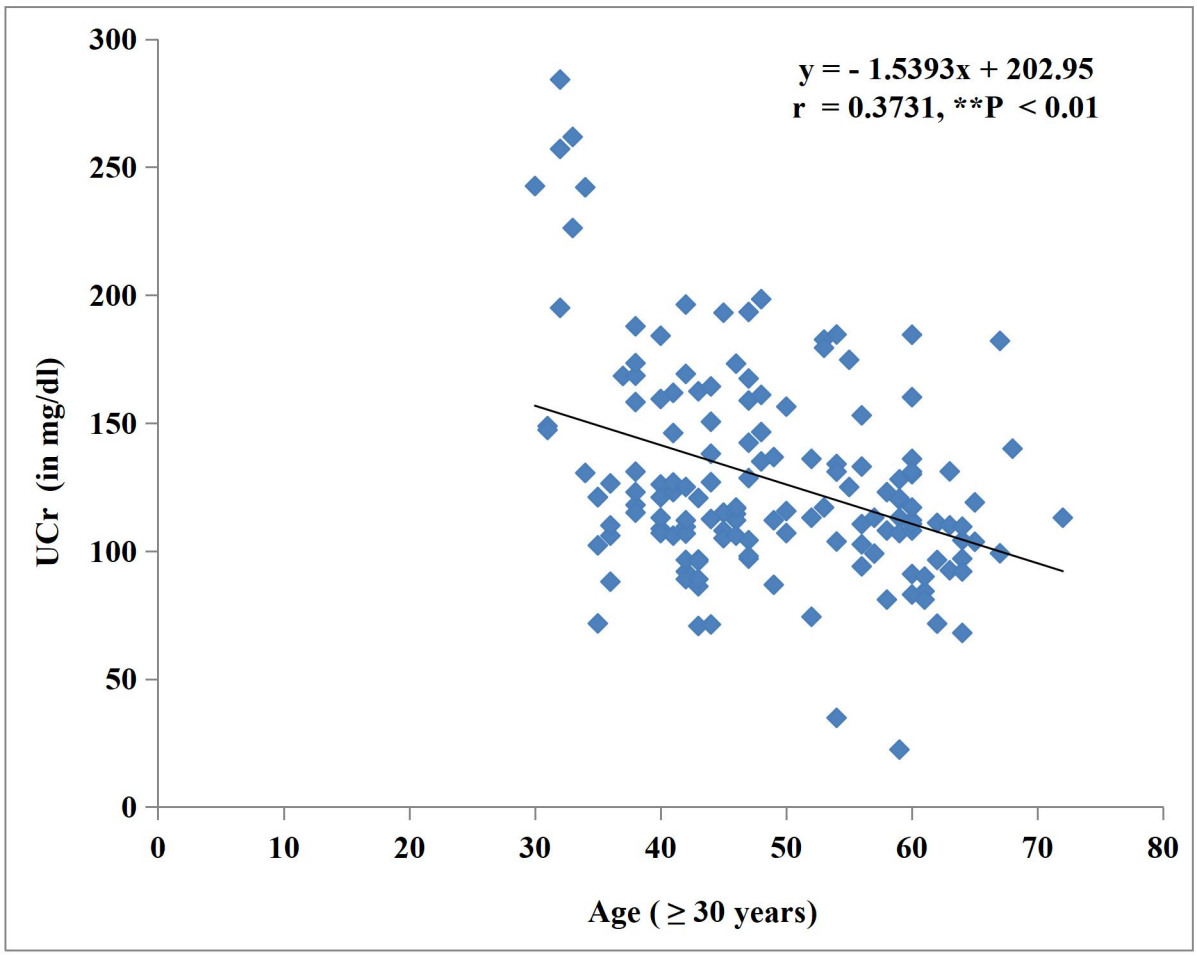

Fig. 4.79: Linear regression of Urine Creatinine vs. Age (≥ 30 years). There was significant decrease in urine creatinine excretion ($P < 0.01$). It declined at the rate of 1.54 mg/dl per year.

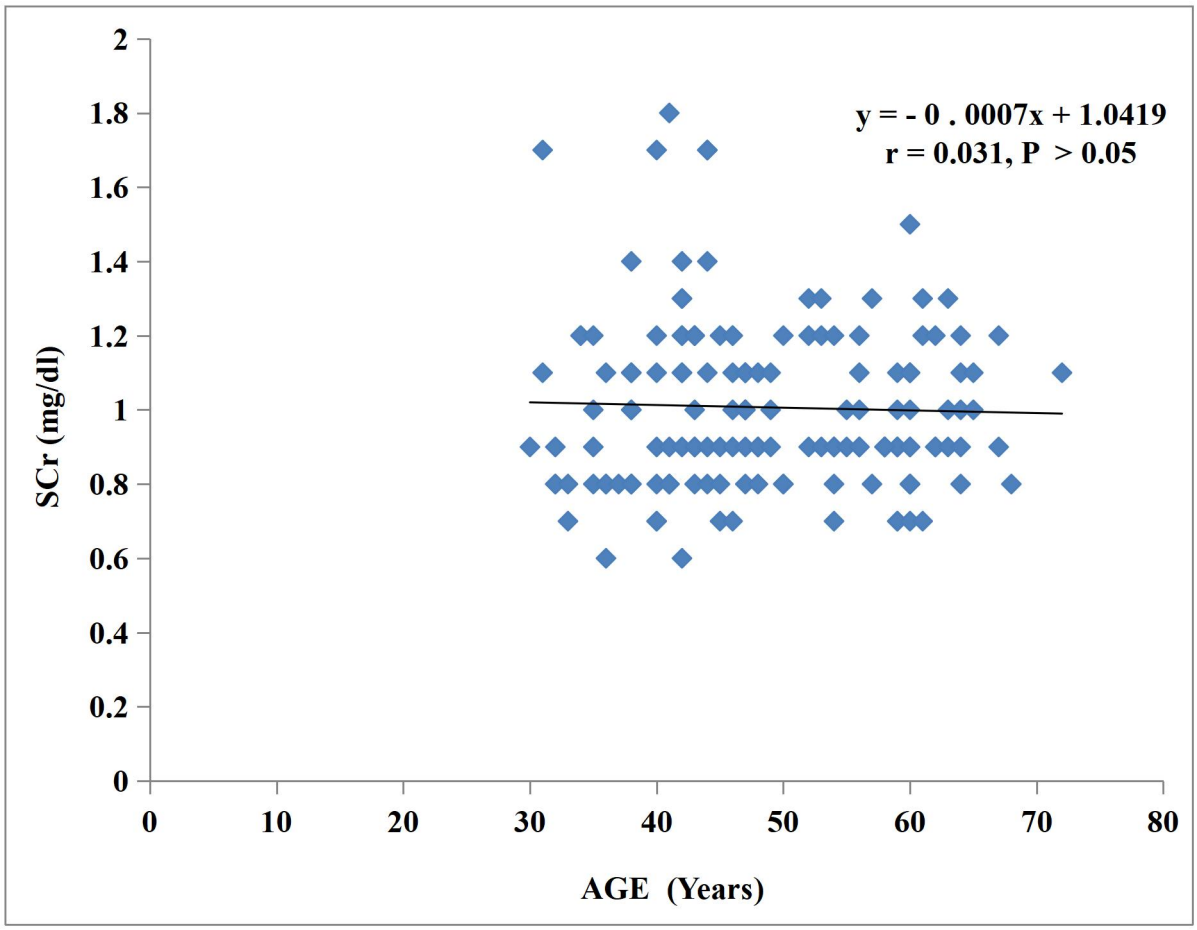

Fig. 4.80: Linear regression of SCr vs. Age (≥ 30 years & above). The annual decrease in serum creatinine was not significant (P > 0.05).

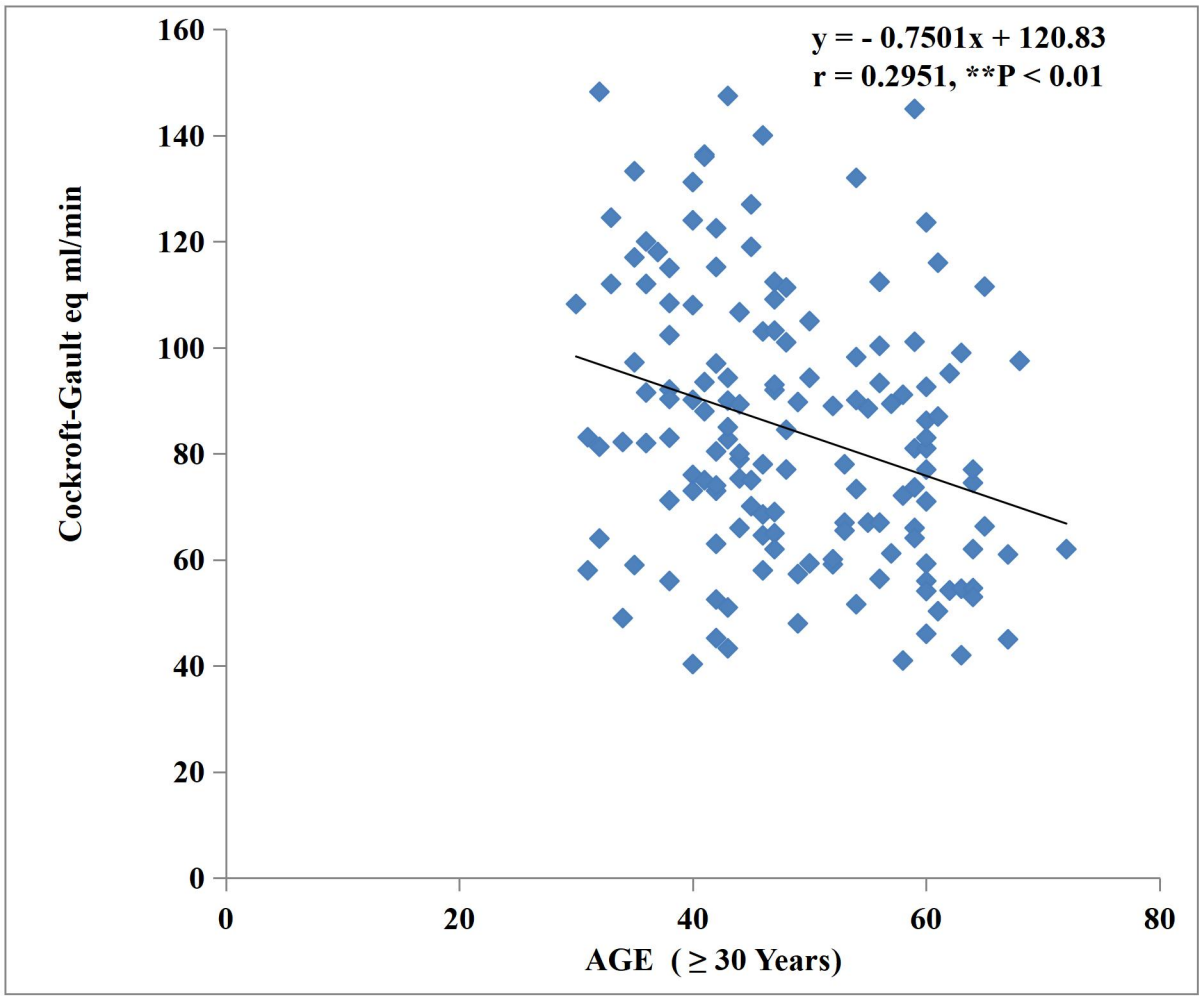

Fig. 4.81: Regression of mean Cockroft-Gault eGFR (in ml/min/1.73 m ^{2}) vs. Age (in years) for Male and Female Subjects ≥ 30 years. There was significant decline in eGFR ($P < 0.01$) which occurred at the rate of 0.7501 ml/min/yr.

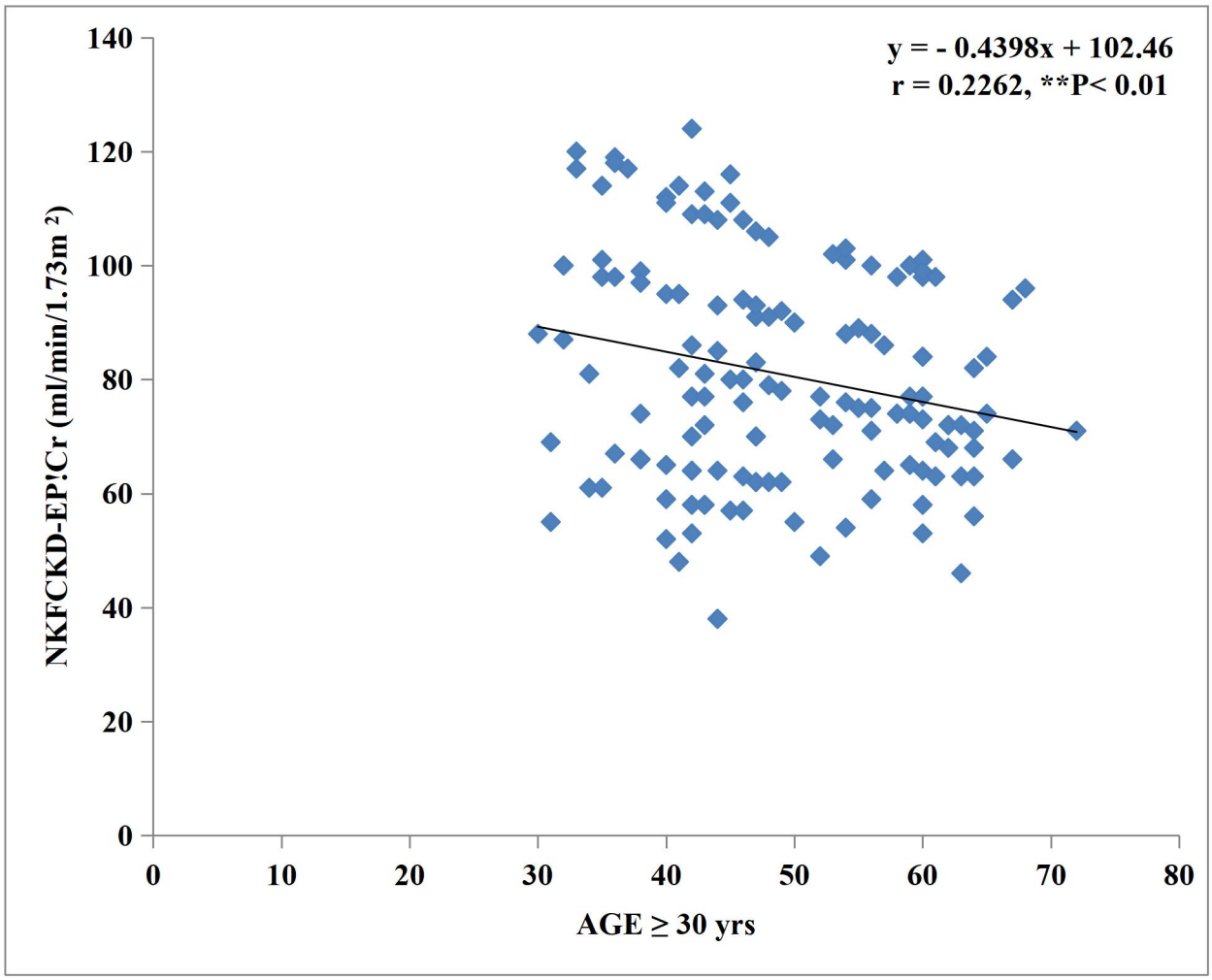

Fig. 4.82: Regression of mean NKF CKD-EP!Cr 2021 eGFR (in ml/min/1.73 m ^{2}) vs. Age (in years) for Male and Female Subjects ≥ 30 years. There was significant decline in eGFR ($P < 0.01$) which occurred at the rate of 0. 44 ml / min /yr.

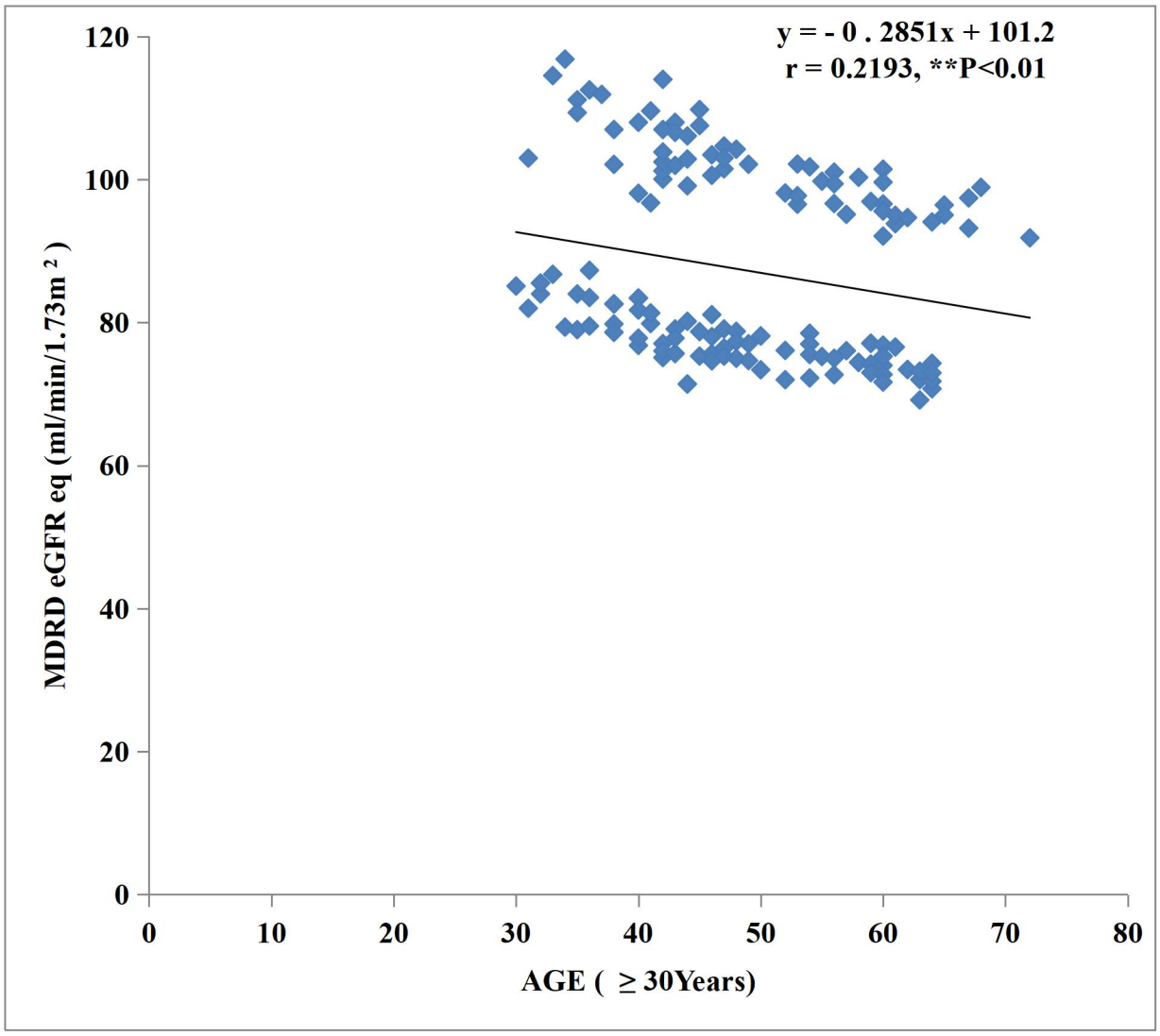

Fig. 4.83: Regression of mean MDRD eGFR (in ml/min/1.73 m ^{2}) vs. Age (in years) for Male and Female Subjects ≥ 30 years. There was significant decline in eGFR (P < 0.01) which occurred at the rate of 0.29 ml/min/yr.

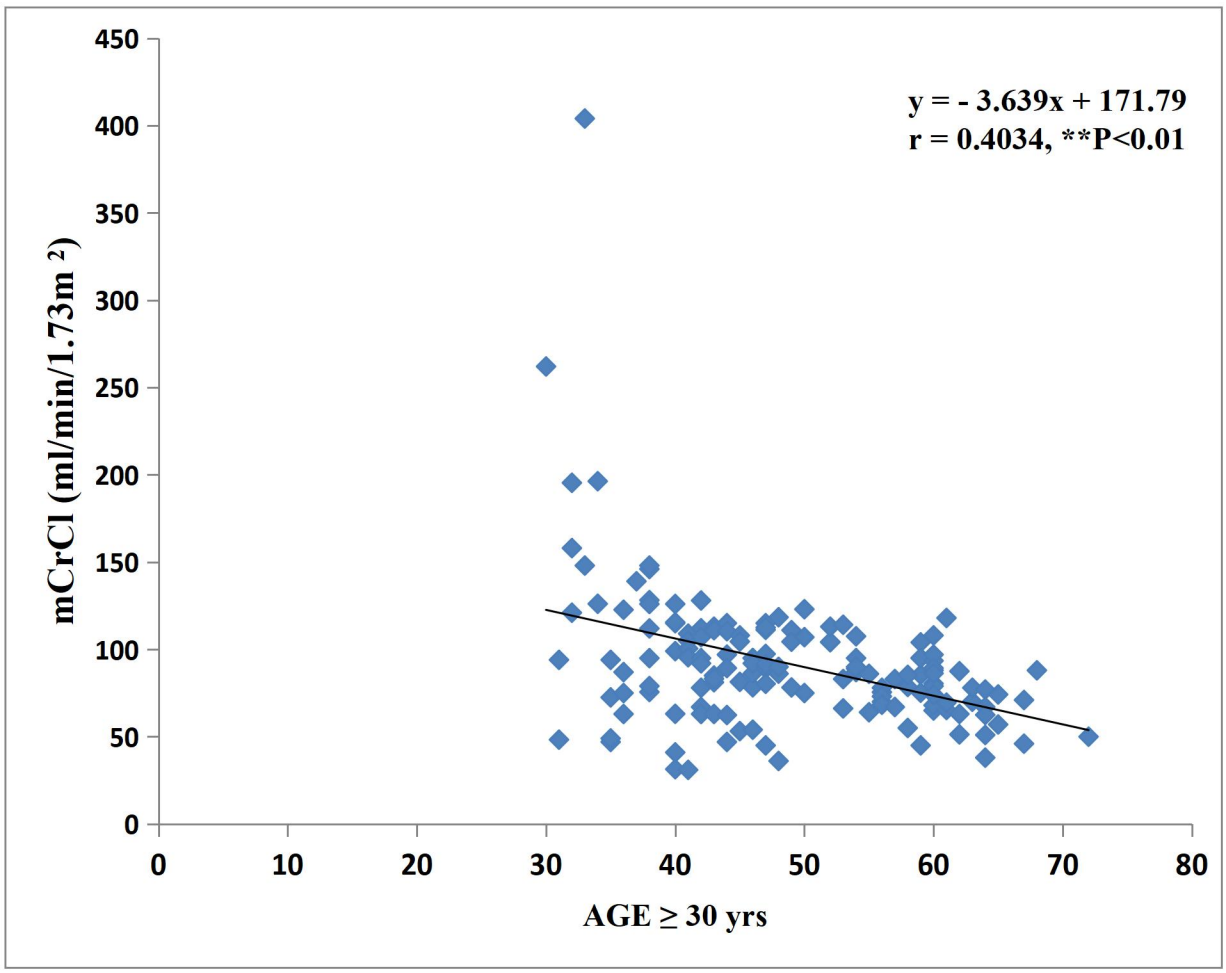

Fig. 4.84: Regression of mean measured Creatinine Clearance (in ml/min/1.73 m ^{2}) vs. Age (in years) for Male and Female Subjects ≥ 30 years. There was significant decline in eGFR ($P < 0.01$) which occurred at the rate of 3.64 ml/min/yr.

Table 4.15: Descriptive statistics of Age, UCr, SCr, CG eq., CKD-EP! Cr eGFR equation., MDRD equation, and mCrCl for Subjects less than 30 years

	Age (in years)	UCr (mg/dl)	SCr (mg/dl)	CG eq. (ml/min)	CKD-EP!Cr eGFR (ml/min)	MDRD eq. (ml/min)	mCrCl (ml/min)
Mean	21.78	205.86	1.13	86.69	86.01	103.23	177.53
± Sem	0.27	5.20	0.03	2.89	2.08	1.51	9.96

UCr mg/dl = Urine Creatinine in mg/dl

SCr mg/dl = Serum Creatinine in mg/dl

CG ml/min = Cockroft-Gault eGFR in ml/min/1.73 m^2

CKD-EP!Cr ml/min = National Kidney Foundation Chronic Kidney Disease-Epidemiology Creatinine 2021 equation in ml/min/1.73 m^2

MDRD ml/min = Modification of Diet in Renal Disease equation in ml/min/1.73 m^2

mCrCl ml/min = measured Creatinine Clearance in ml/min/1.73 m^2

REGRESSION GRAPHS OF GFR FOR SUBJECTS LESS THAN 30 YEARS (figures 4.85 to 4.90)

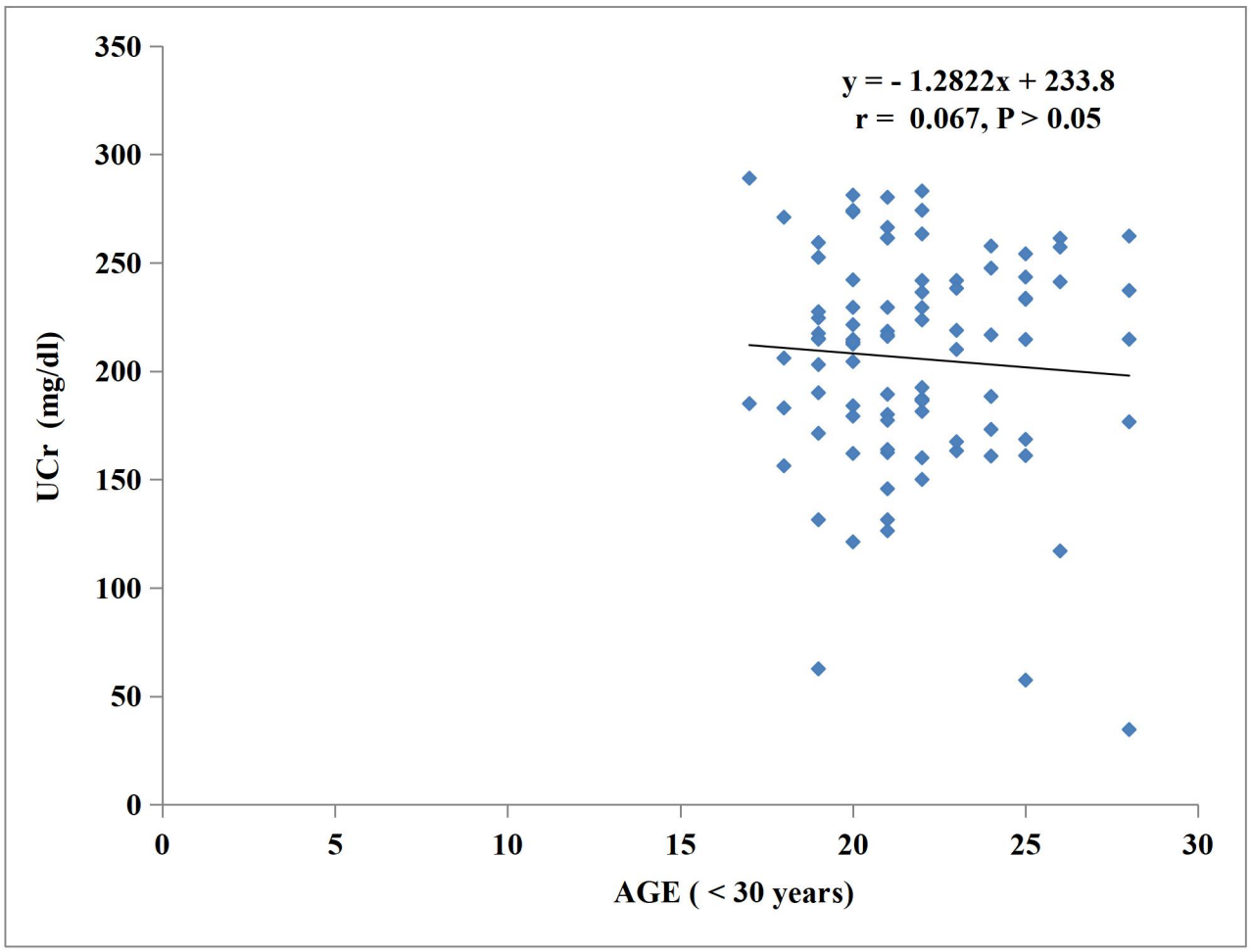

Fig. 4.85: Linear regression of Urine Creatinine (in mg/dl) vs. Age for subjects < 30 years. The decline in Urine Creatinine excretion was not significant (P > 0.05) which showed that the younger age group excreted more Creatinine in urine.

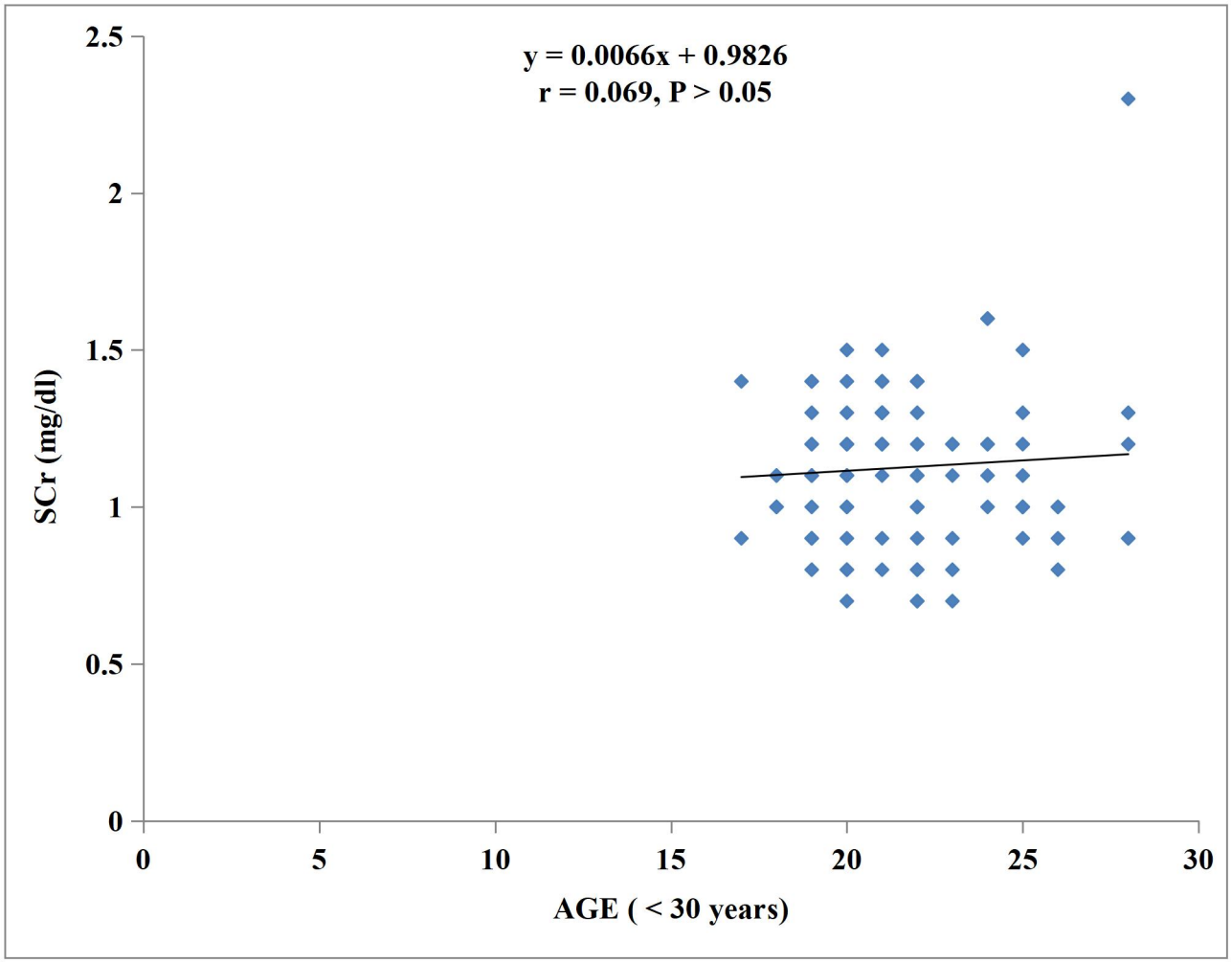

Fig. 4.86: Linear regression of mean Serum Creatinine (in mg/dl) vs. Age (<30 years). There annual increase in serum creatinine was not significant (P > 0.05).

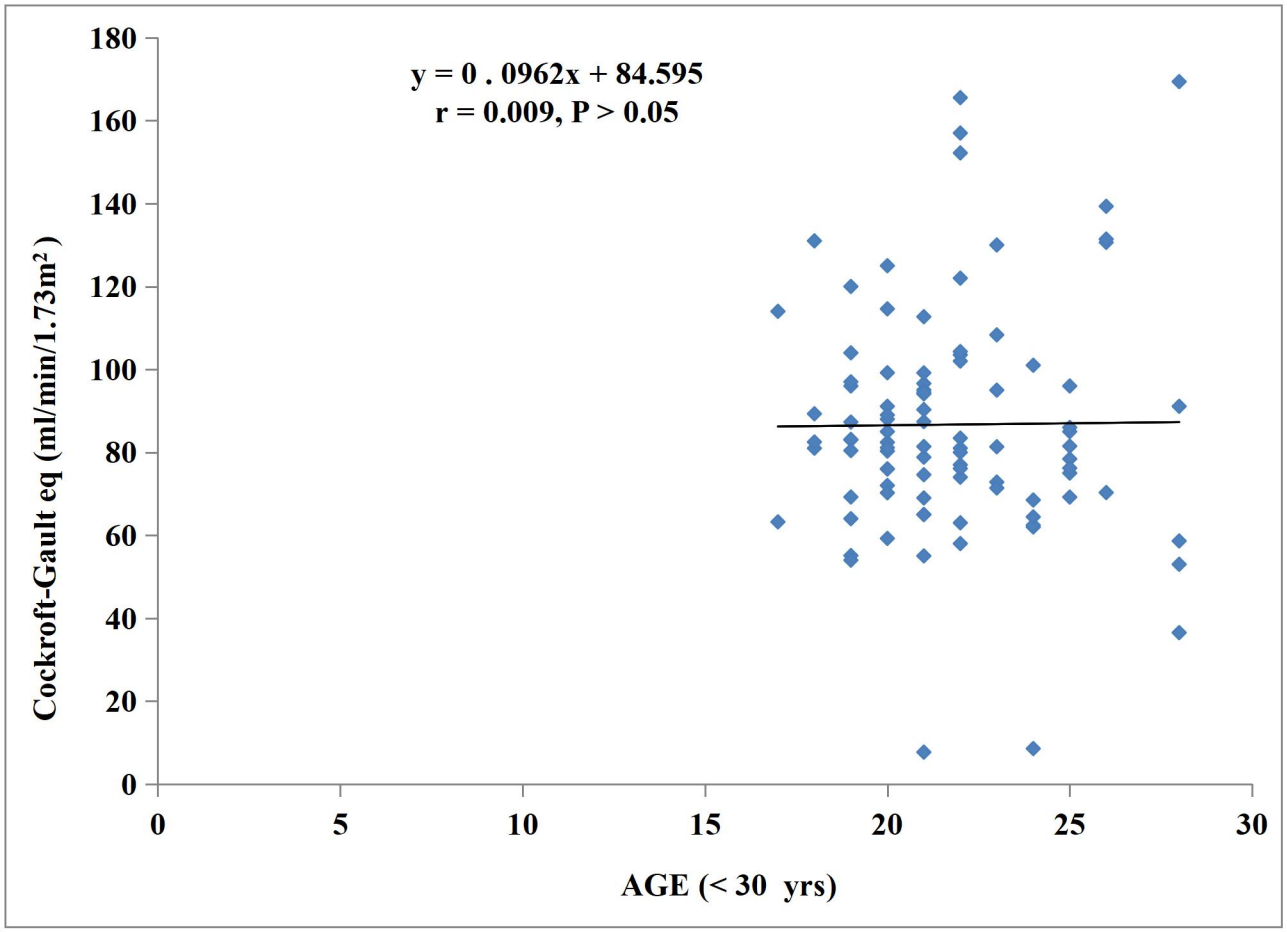

Fig 4.87: Linear regression of Cockroft-Gault eGFR eq. (ml/min) vs. Age (< 30 years). The annual increase in eGFR was not significant (P > 0.05). It occurred at the rate of 0.0962 ml/min.

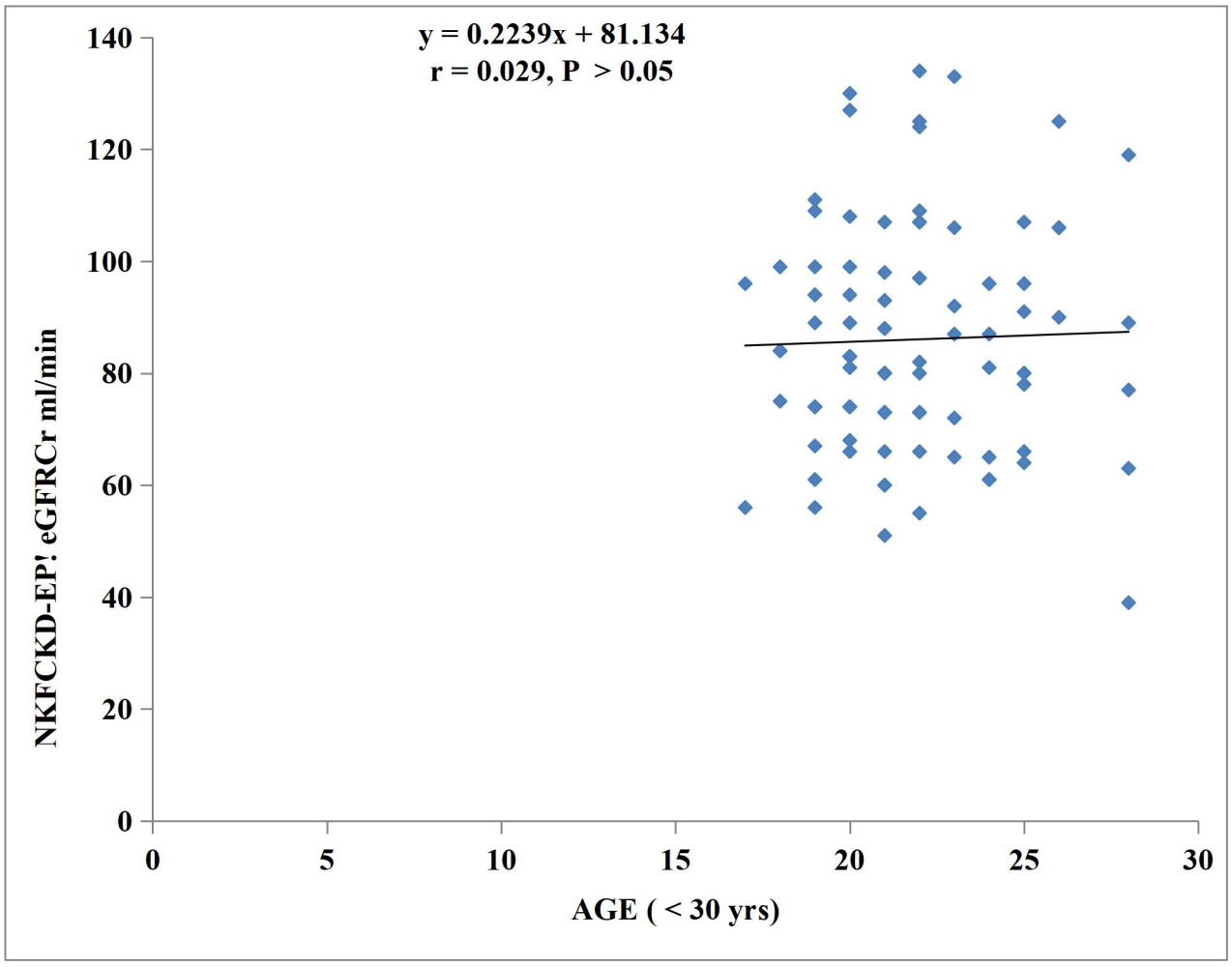

Fig. 4.88: Linear regression of National Kidney Foundation Chronic Kidney Disease Epidemiology 2021 eGFR Cr eq. (in ml/min) vs. Age (< 30 years). The annual increase in eGFR was not significant (P > 0.05).

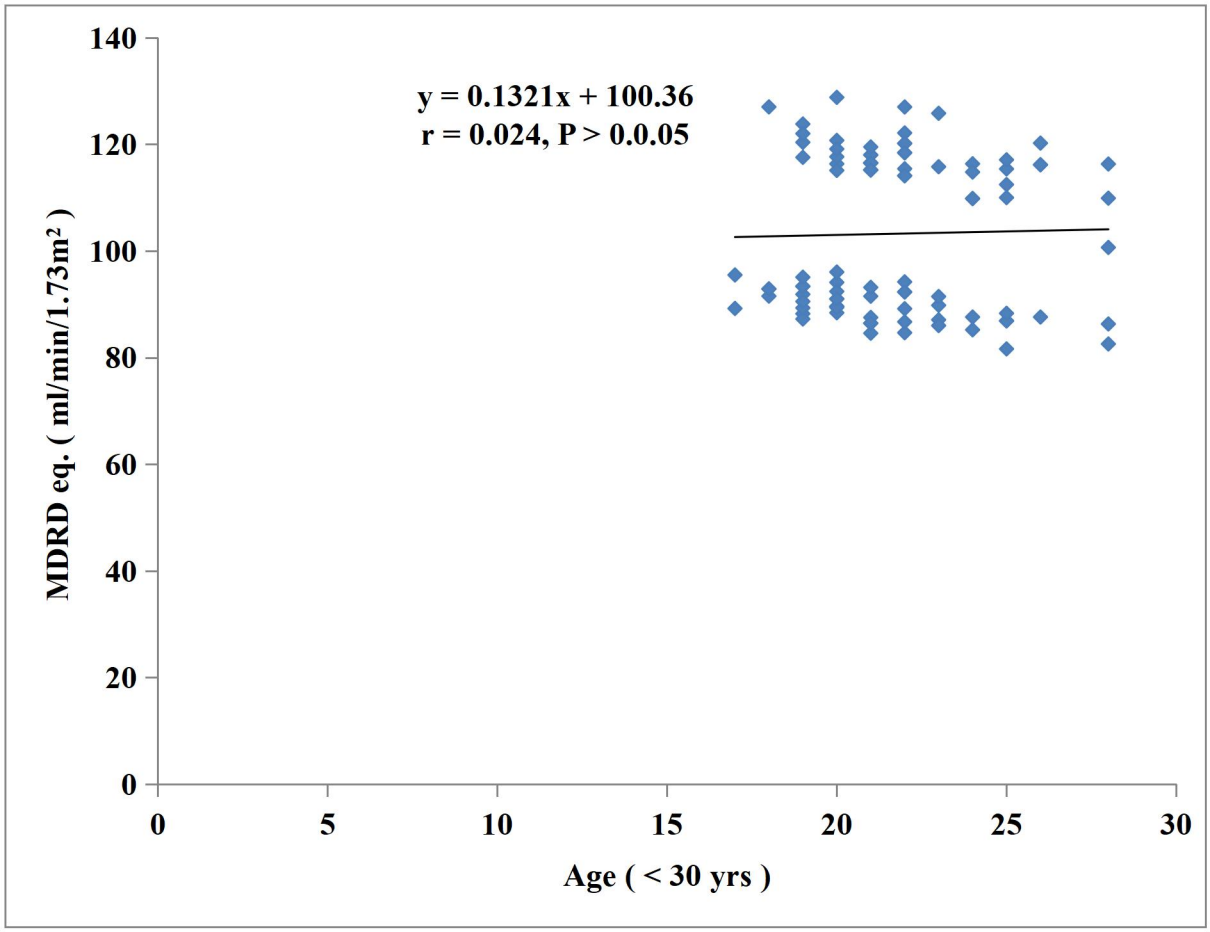

Fig. 4.89: Linear regression of Modification of Diet in Renal Disease eGFR eq. (in ml/min) vs. Age (< 30 years). The annual rate of increase in eGFR was not significant (P > 0.05).

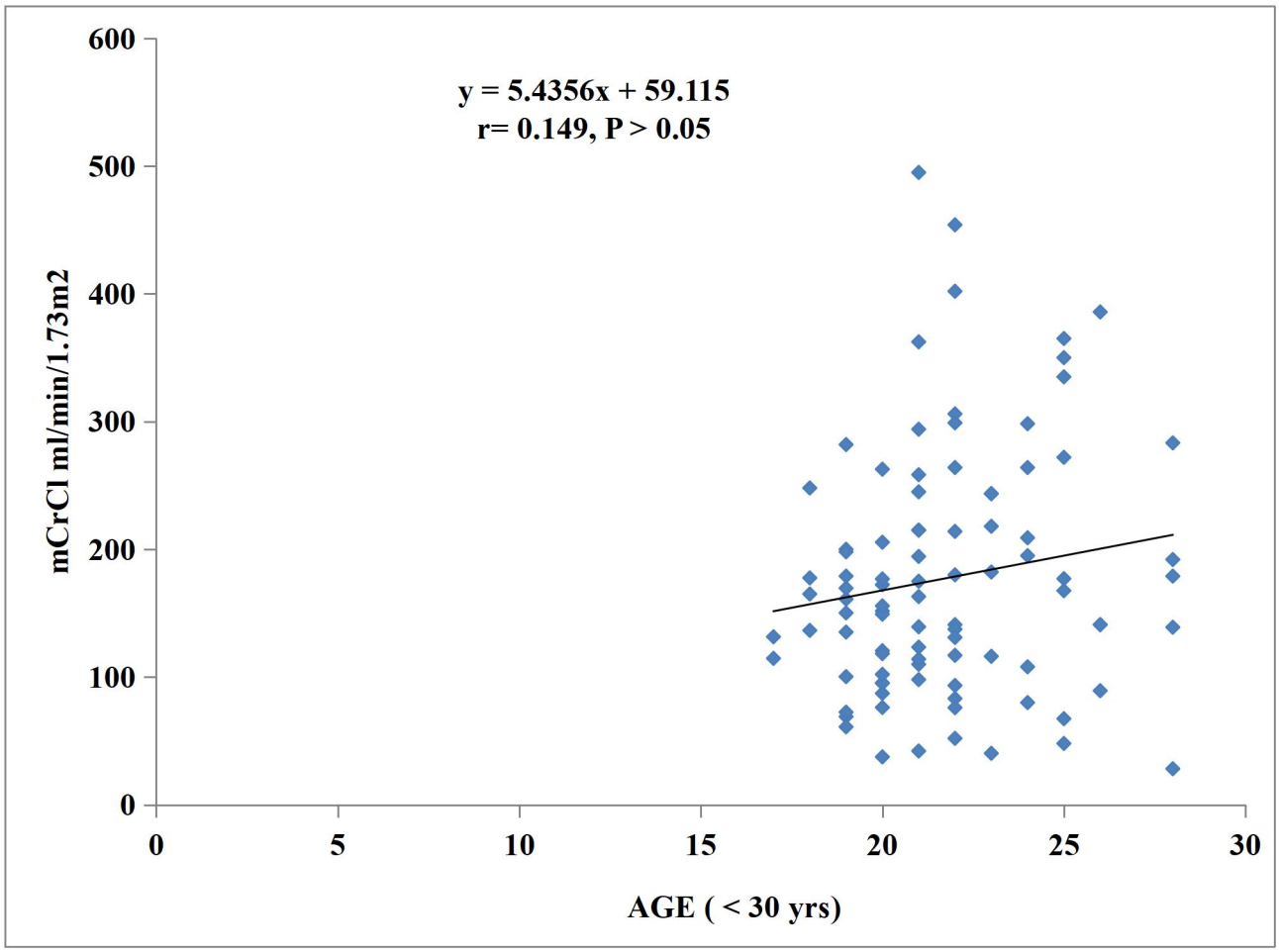

Fig. 4.90: Linear regression of measured Creatinine Clearance (in ml/min) vs. Age (<30 years). There was annual increase in GFR at the rate of 5.44ml/min/1.73 m^2/yr which was not significant (P > 0.05).

Table 4.16: Rates of increase and decline in annual GFR values using four GFR methods in three Age categories

GFR method ($ml/min/1.73m^2$)	Age < 30 (yrs) Increase in GFR (in ml/min/yr)	Age 30 - 70 (yrs.) Decline in GFR (in ml/min/yr)	Age 18 - 70 (yrs.) Decline in GFR in (in ml/min/yr)
Cockroft-Gault eq.	0. 0962 (P > 0.05)	- 0.7501**(P < 0.05)	- 0.258* (P < 0.05)
NKF CKD-EP! Cr 2021eq.	0. 2259 (P > 0.05)	- 0.4398**(P < 0.05)	-0.246** (P <0.01)
MDRD eq.	0. 1321(P > 0.05)	- 0.2851**(P < 0.05)	-0.503**(P < 0.01)
mCrCl	5. 4356 (P > 0.05)	- 3.639**(P < 0.05)	-2.681**(P < 0.01)

The values with negative signs indicated annual rates of decline or decrease in Glomerular Filtration Rates. The values without negative signs indicated annual rates of increase in Glomerular Filtration Rates. The value P > 0.05 indicates not significant. The values P < 0.05 and P <0.01 are significant. There were significant rates of decline GFR in columns 3 and 4.

Table 4.17: T- test comparison for Subjects age groups (less than 30 and ≥ 30 plus years)

Parameters	**Age < 30 | ≥ 30 years**	**N**	**Mean ± Sem**	**T**	**P-Value**
AGE.(Years)	<30 years	93	21.78±0.27	-25.543	**0.001
	≥30 years	150	48.73±0.81		
BMI (kg/m ^{2})	<30 years	93	20.11±0.34	-7.134	**0.001
	≥30 years	150	24.23±0.4		
PR	<30 years	93	76.33±1.23	0.395	0.69^{NS}
	≥30 years	150	75.78±0.8		
SBP	<30 years	93	118.12±1.26	-3.936	**0.001
	≥30 years	150	127.25±1.65		
DBP	<30 years	93	69.05±0.88	-6.68	**0.001
	≥30 years	150	78.72±1		
MAP	<30 years	93	85.22±0.96	-5.813	**0.001
	≥30 years	150	94.84±1.16		
SCr (mg/dl)	<30 years	93	1.19 ± 0.07	3.019	**0.001
	≥30 years	150	1.0 ± 0.02		
CrCL (ml/min)	<30 years	93	177.53±9.96	9.642	**0.001
	≥30 years	150	91.74±3.32		
Cockcroft-Gault (ml/min)	<30 years	93	86.69±2.89	0.702	0.48 ^{NS}
	≥30 years	150	84.26±2.07		
NKFCKD-EPI.Cr.2021 (ml/min)	<30 years	93	86.01±2.08	1.937	*0.05
	≥30 years	150	80.99±1.58		
MDRD (ml/min)	<30 years	93	103.23±1.51	8.878	**0.001
	≥30 years	150	87.23±1.07		
Ur.ALB.(mg/dl)	<30 years	93	9.67±0.47	-2.8	0.01 ^{NS}
	≥30 years	150	11.74±0.51		
Ur.ALB/Ucr (mg/gm)	<30 years	93	52.83±4.33	-1.571	0.12 ^{NS}
	≥30 years	150	63.12±4.4		
Urine.Na+/K+ ratio	<30 years	93	5.06±0.3	3.607	**0.001
	≥30 years	150	3.73±0.22		

Graphs of T- test comparison for Subjects age groups < 30 years and ≥ 30 years (Fig. 4.91 to 4.104)

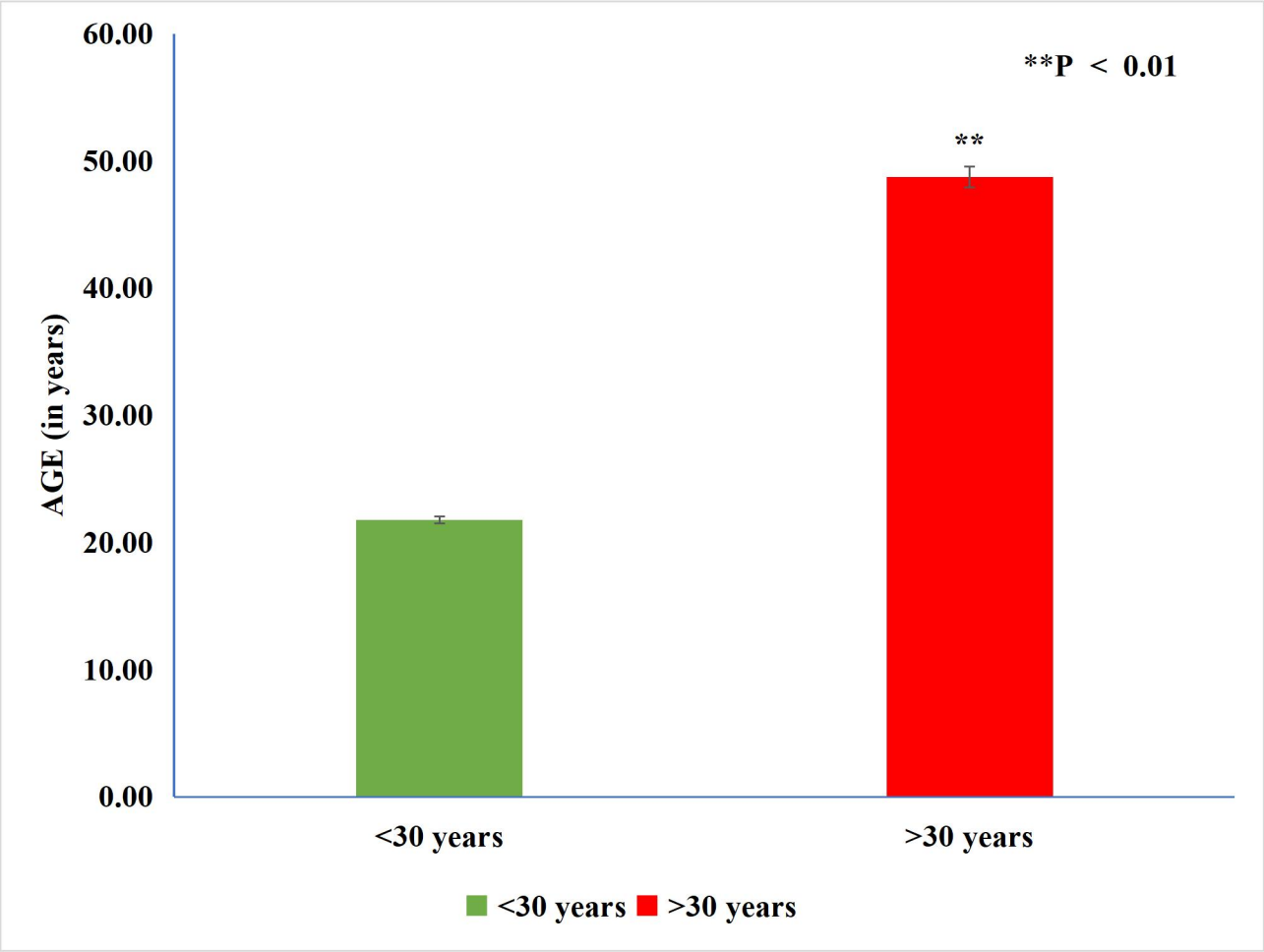

Fig. 4.91: Comparison of Age of the Subjects (< 30 and ≥ 30 years). The graph showed significant mean age difference between the two groups (P < 0.01).

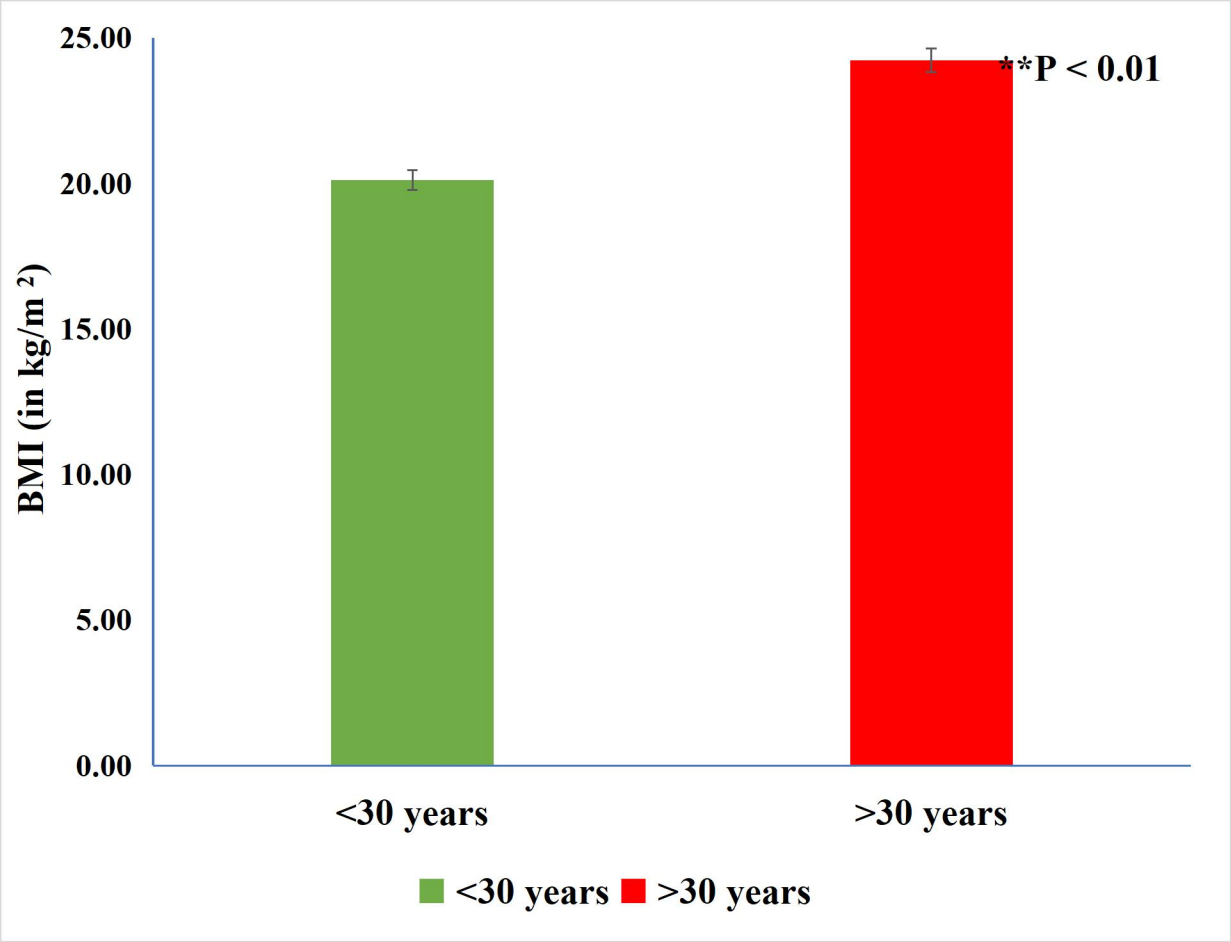

Fig. 4.92: Comparison of Body Mass Index of the Subjects < 30 and ≥30 years. There was significantly higher BMI in subjects ≥30 years.

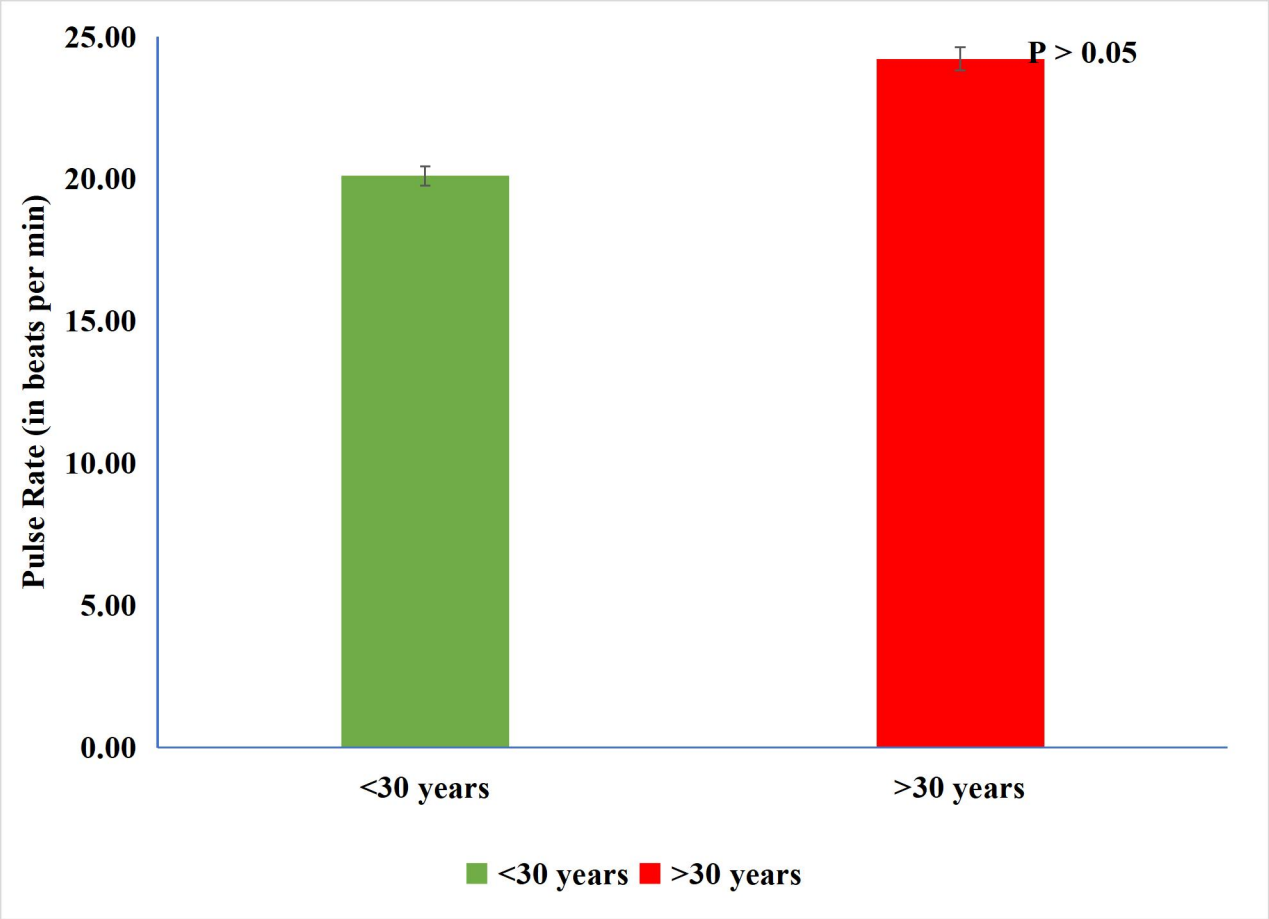

Fig. 4.93: Comparison of Pulse Rate (in beats per min) of the Subjects (< 30 and ≥ 30 years). There was no significant difference between the two groups (P > 0.05).

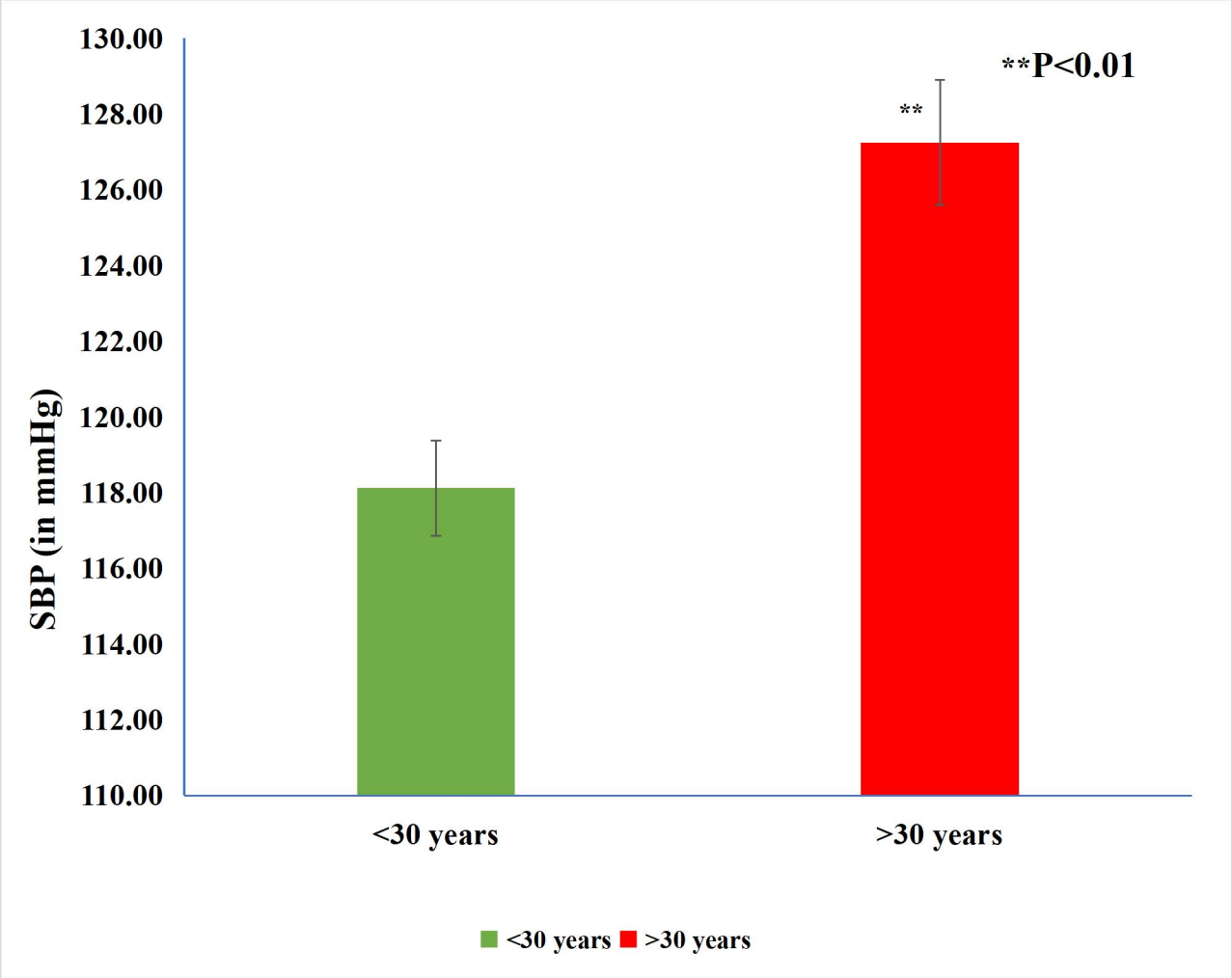

Fig. 4.94: Comparison of Systolic Blood Pressure (in mmHg) of the Subjects in the age groups (< 30 and ≥ 30 years). There was significantly higher SBP in the older age group ($P < 0.01$).

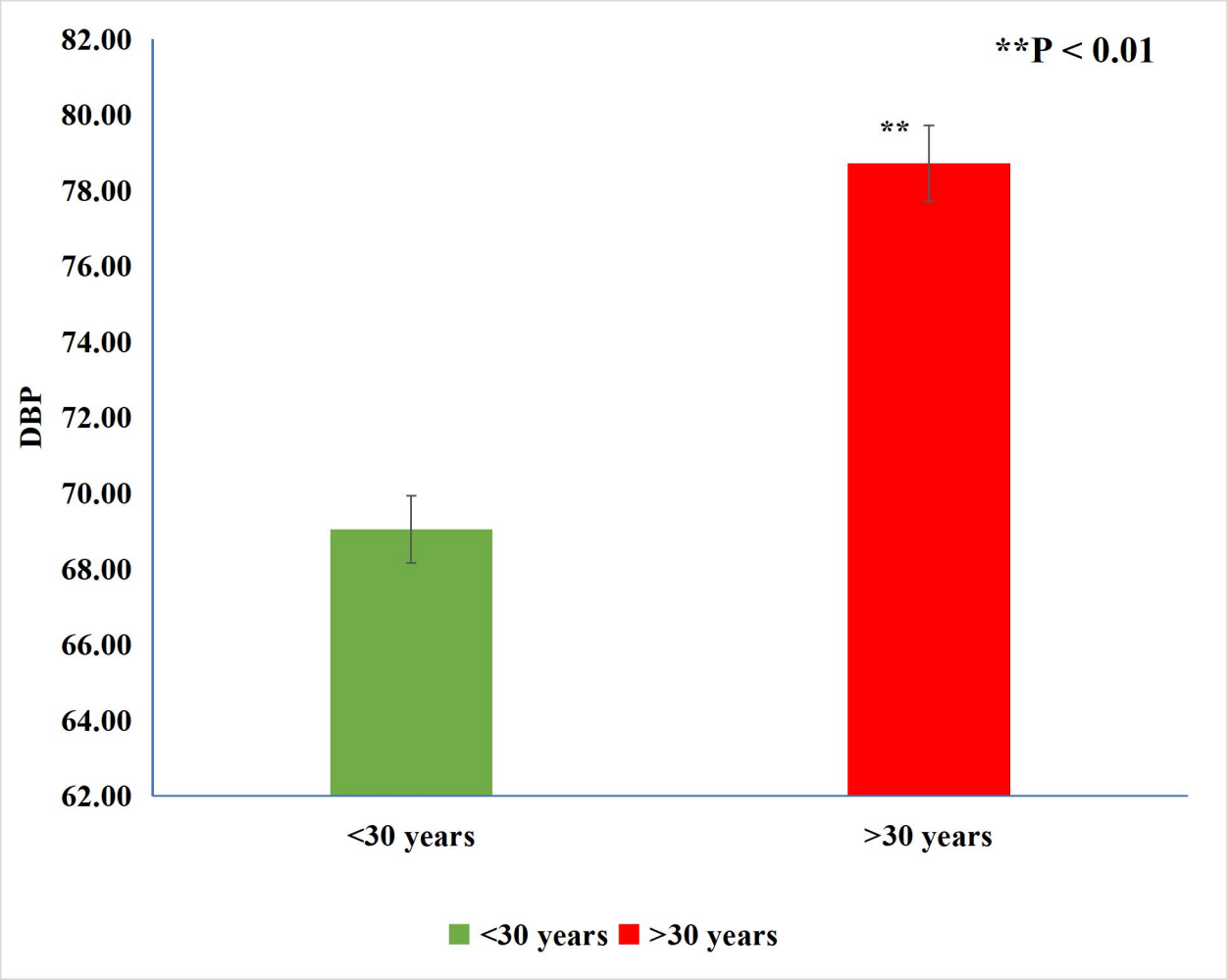

Fig. 4.95: Comparison of Diastolic Blood Pressure of the Subjects in the age groups (< 30 and ≥ 30 years). The older age group (≥ 30 years) had significantly higher DBP (P < 0.01).

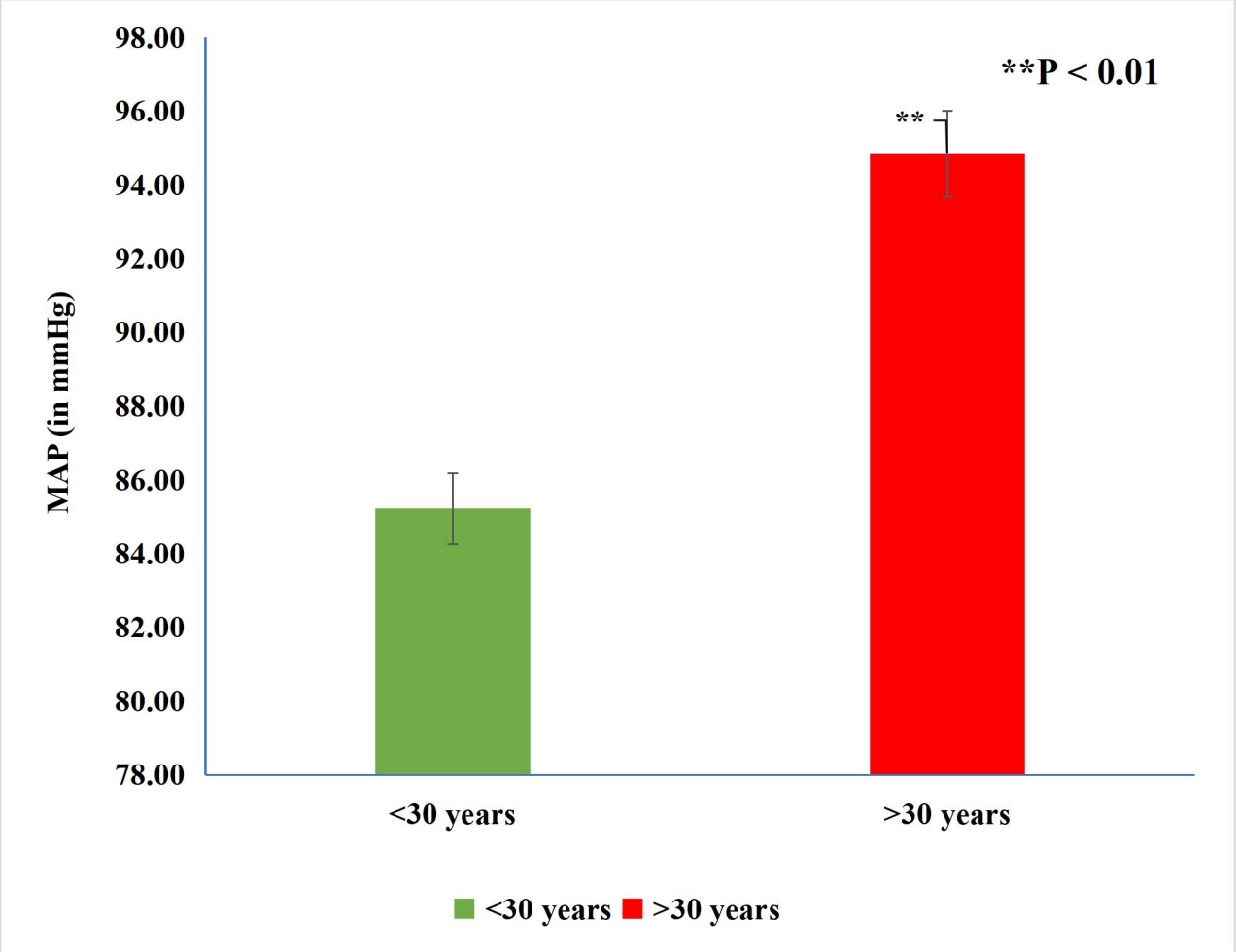

Fig. 4.96: Comparison of Mean Arterial Pressure of the Subjects in the age groups (< 30 and ≥ 30 years). The older age group (≥ 30 years) had significantly higher MAP (P < 0.01).

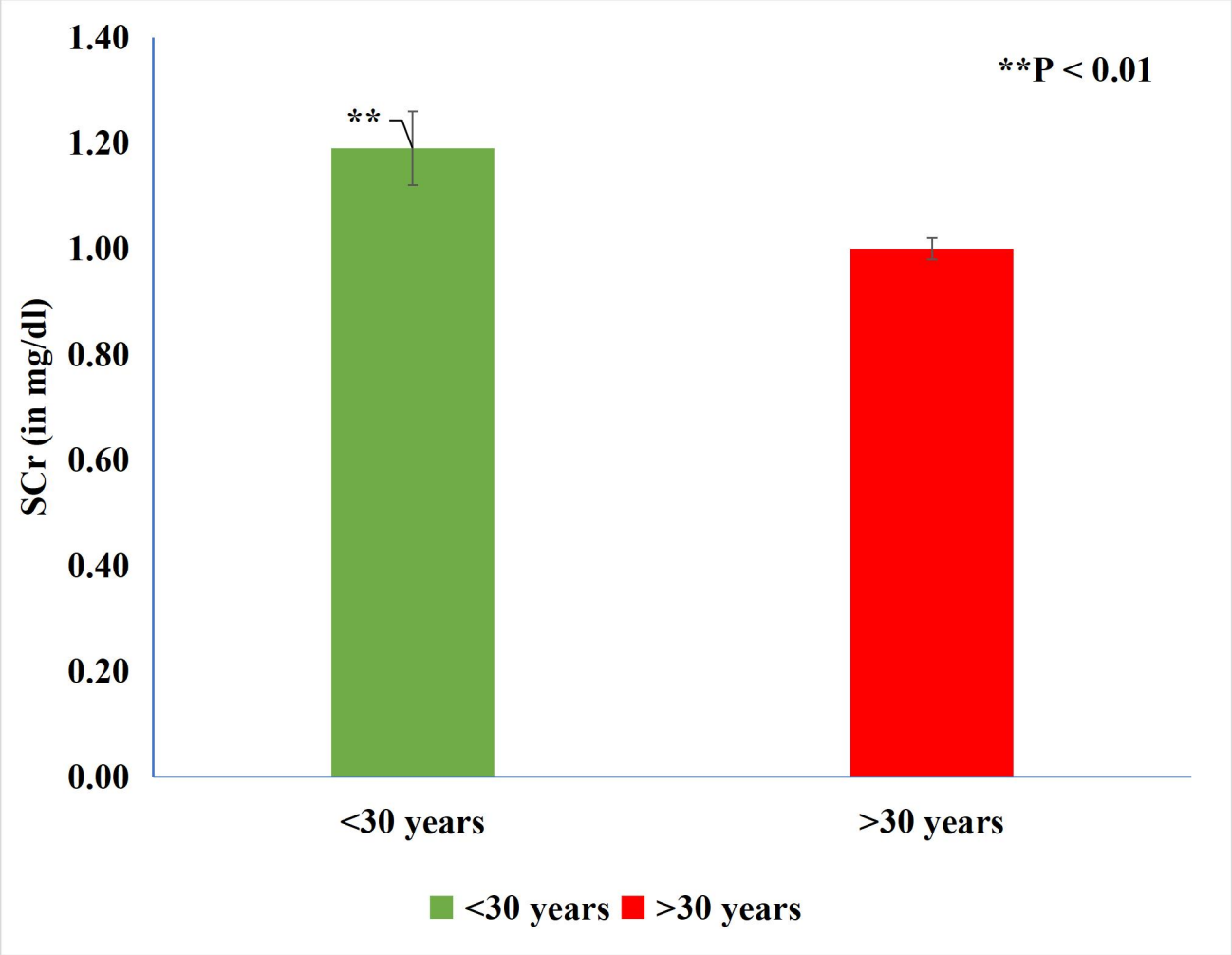

Fig. 4.97: Comparison of Serum Creatinine (in mg/dl) of the Subjects age groups (< 30 and ≥30 years). The younger age group (< 30 years) had significantly higher Serum Creatinine (P < 0.01).

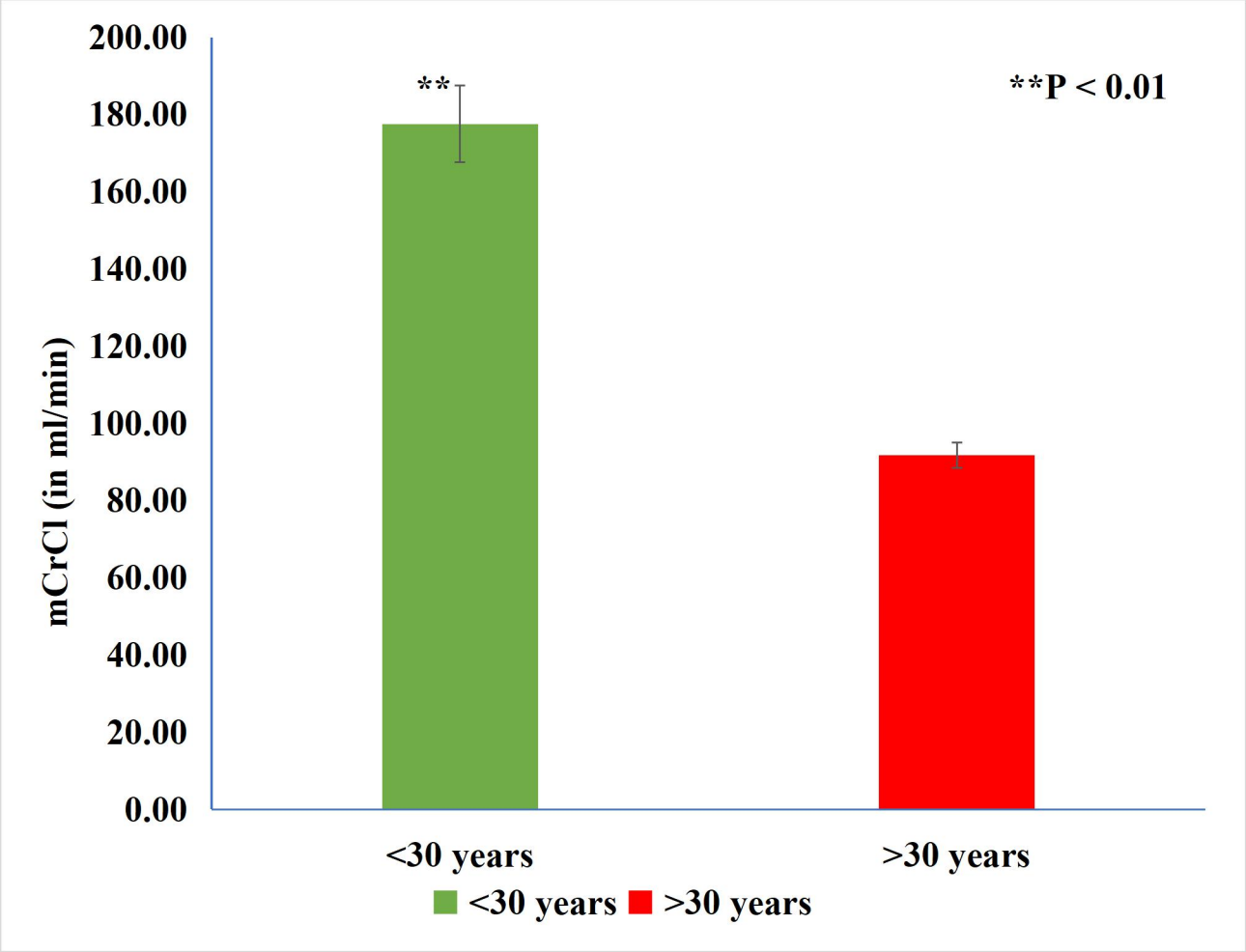

Fig. 4.98: Comparison of measured Creatinine Clearance (in ml/min/1.73 m^2) of the Subjects in the age groups (< 30 and ≥ 30 years). There was significantly higher mean mCrCl in the younger age group (P < 0.01).

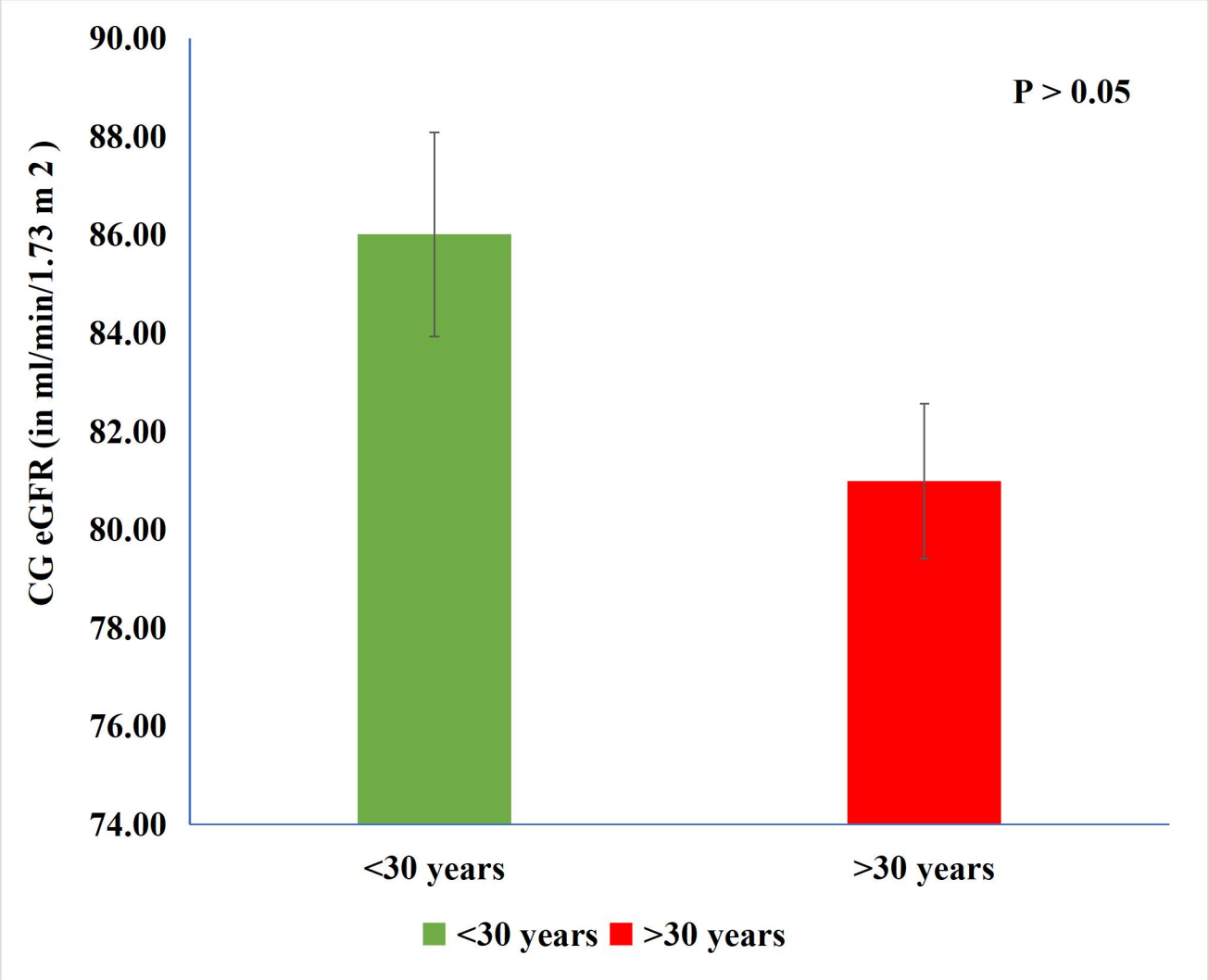

Fig. 4.99: Comparison of Cockroft-Gault eGFR (in ml/min/1.73m ^{2}) of the Subjects age groups (< 30 and ≥ 30 years). The difference in mean CG eGFR between the groups was not significant (P > 0.05).

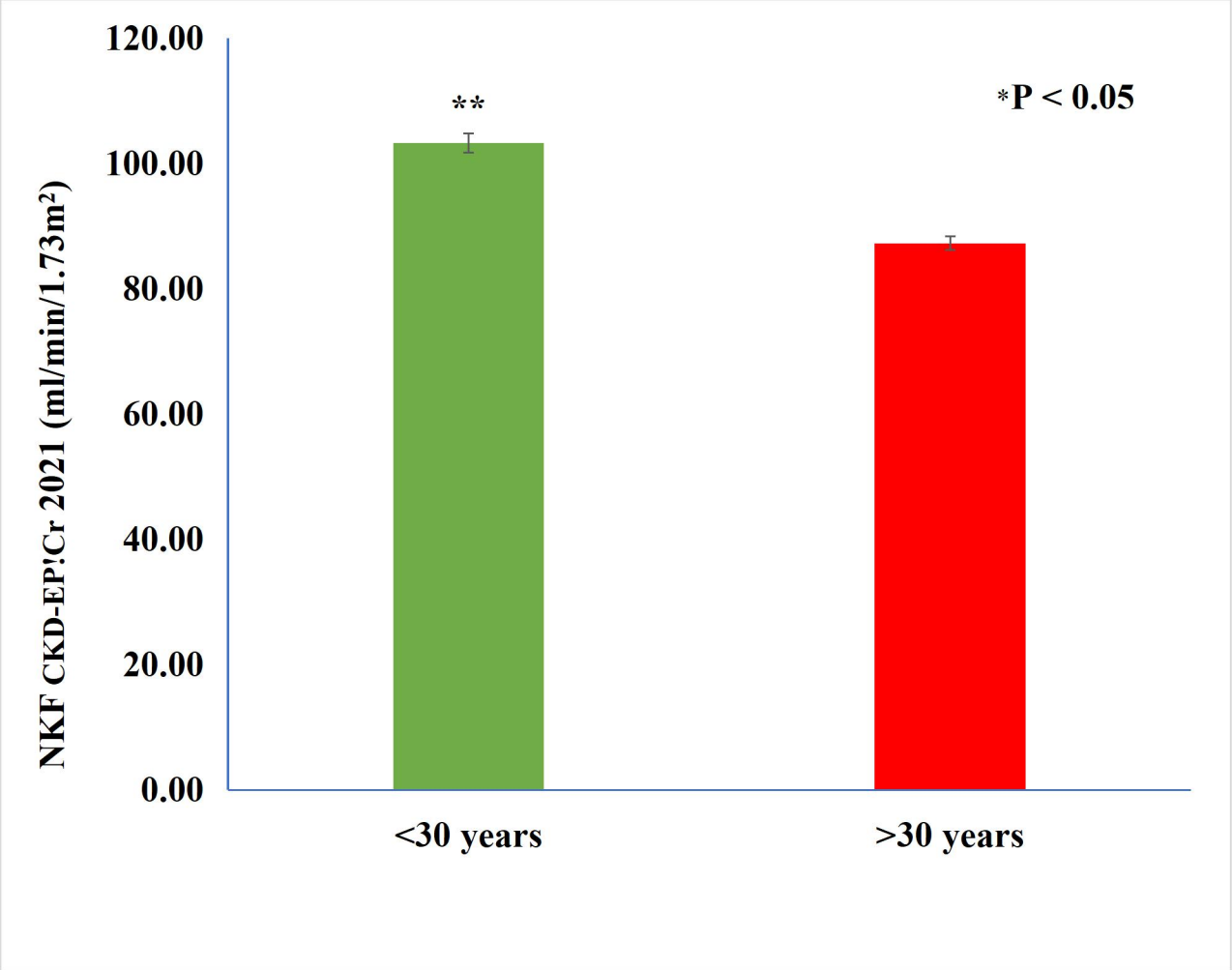

Fig. 4.100: Comparison of mean NKF CKD-EP!Cr.2021 eGFR (ml/min/1.73m ^{2}).of the Subjects (< 30 and ≥ 30 (years). There was significantly greater eGFR in younger age group (< 30 years).

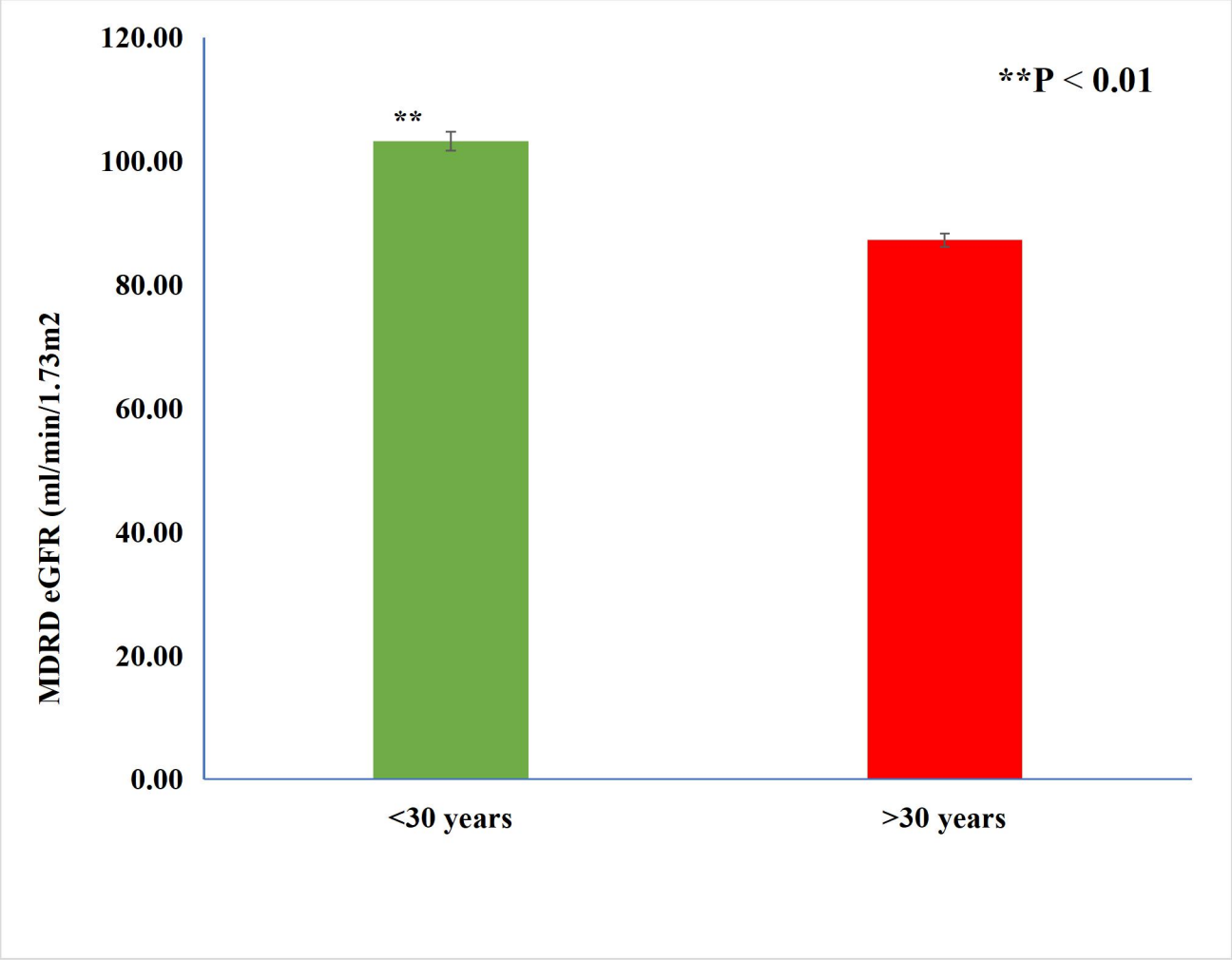

Fig. 4.101: Comparison of mean MDRD eGFR (in ml/min/1.73m ^{2}) of the Subjects (< 30 and ≥30 years). There was significantly greater GFR in the younger age group (P < 0.01).

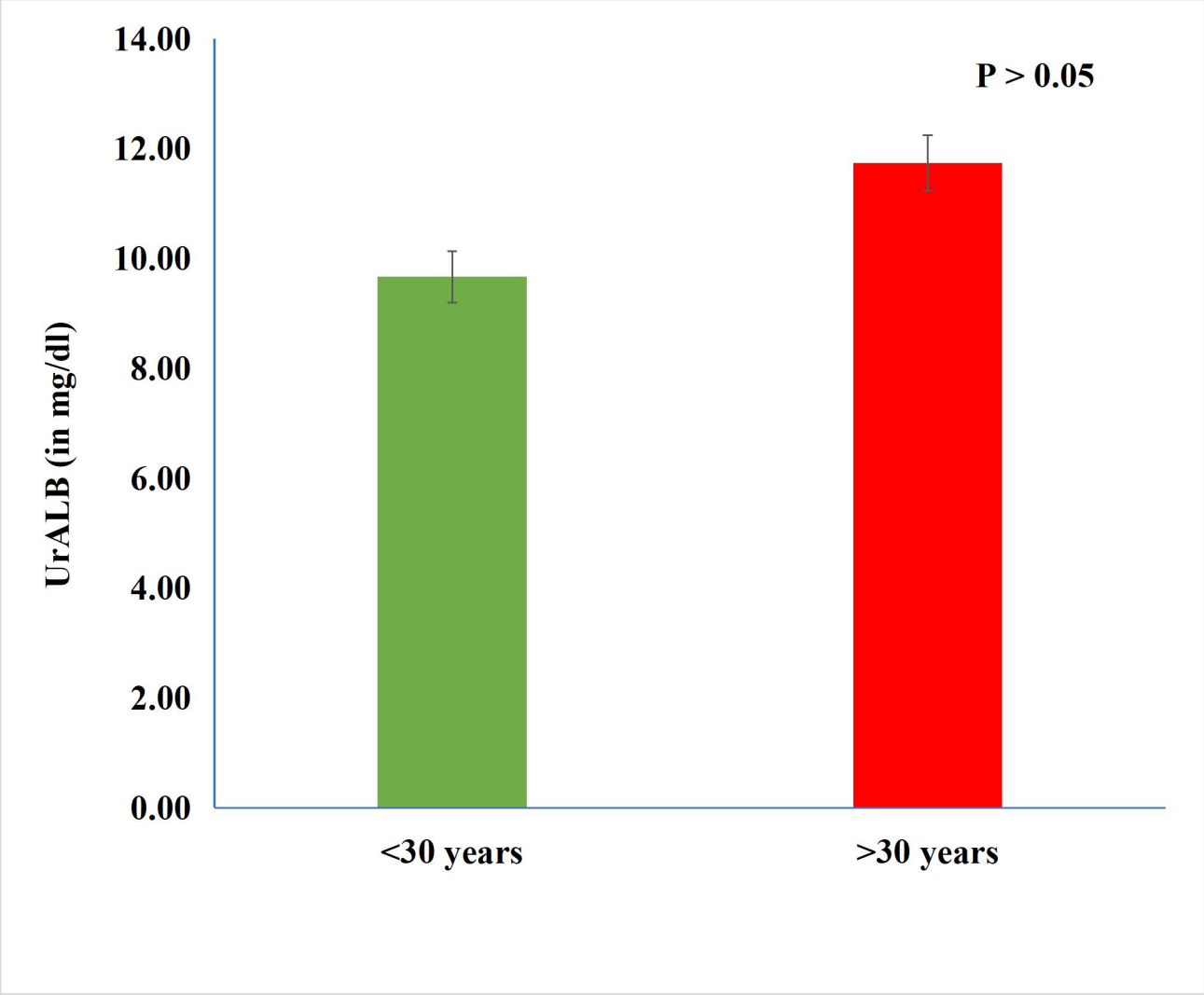

Fig. 4.102: Comparison of Urine Albumin excretion (in mg/dl) of the Subjects age groups (< 30 and ≥ 30 years). Urine Albumin excretion was not significantly greater in the older age group (P > 0.05).

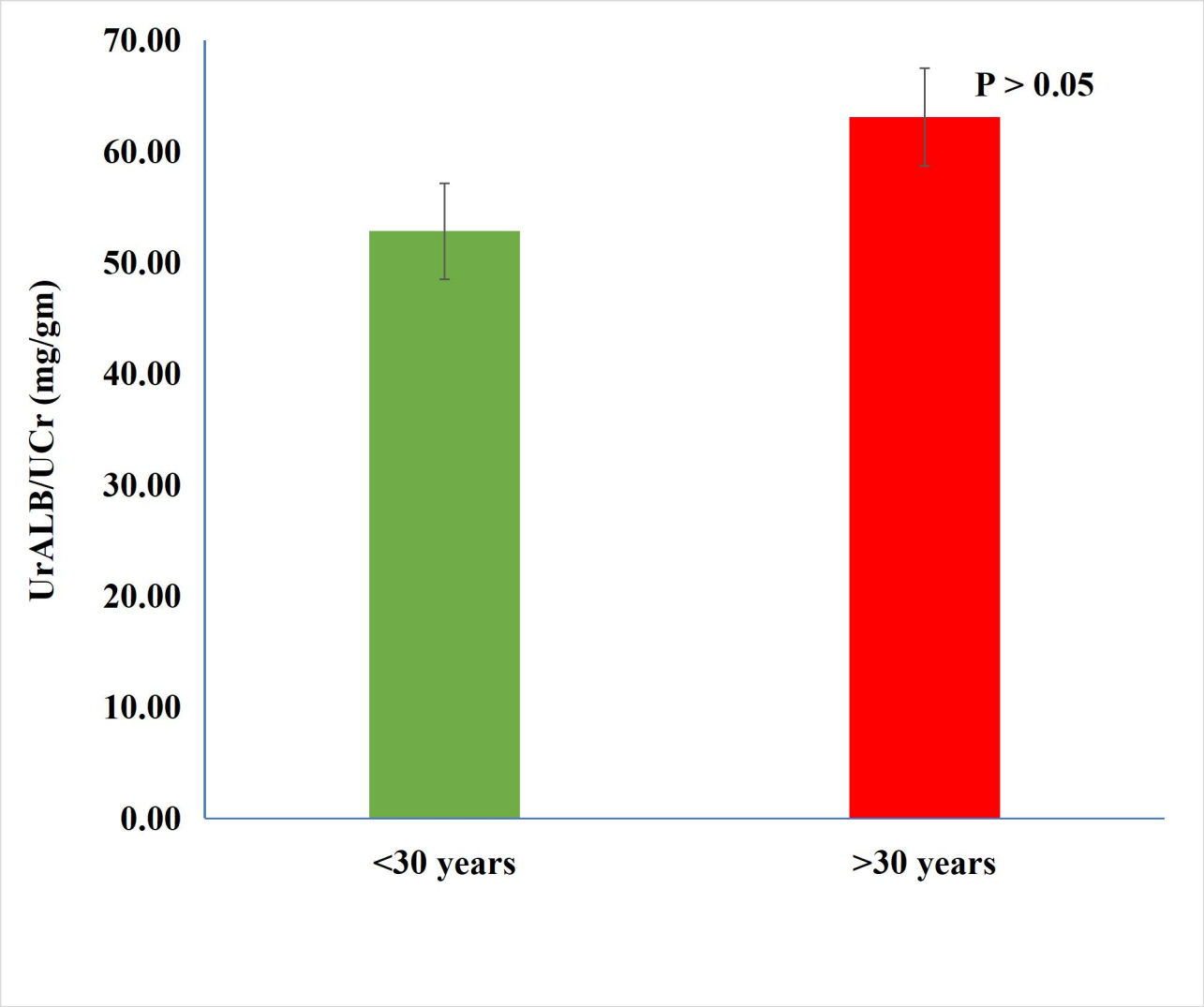

Fig. 4.103: Comparison of Urine Albumin/Urine Creatinine ratio (in mg/gm) of the Subjects age groups (< 30 and ≥30 years). UrALB/CR (or ACR) was not significantly greater in the older age group (P > 0.05).

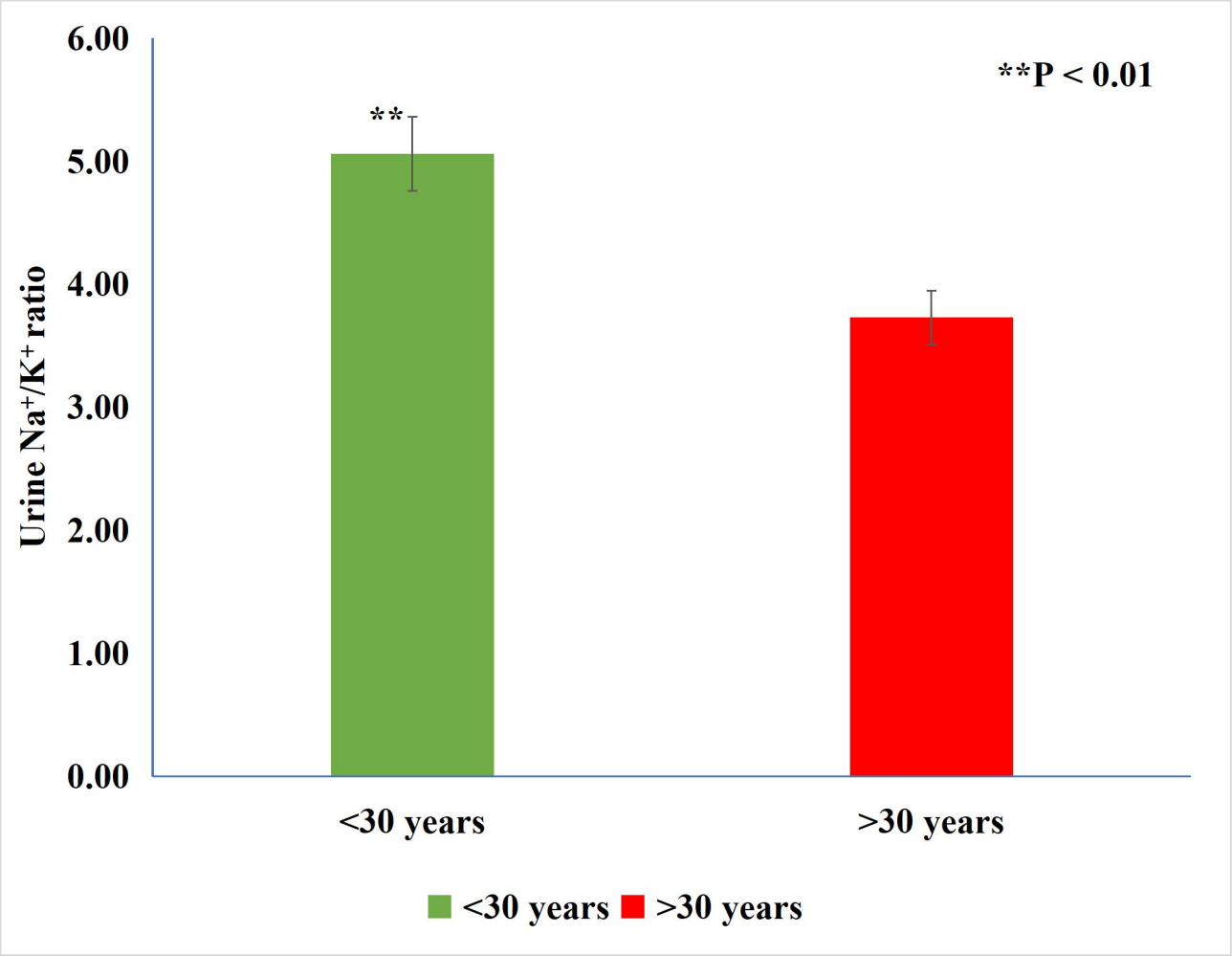

Fig. 4.104: Comparison of Urine Na^+/K^+ ratio of the Subjects age groups (< 30 and >30 years). There was significantly greater urine Na^+/K^+ ratio in the younger age group ($P < 0.01$).

Table 4.18: T-test of variations in parameters between male and female subjects

Parameters	Gender	N	Mean ± Sem	t	P-Value.
WEIGHT (kg)	Male	121	70.16±1.28	2.22	**0.03
	Female	122	65.88±1.39		
HEIGHT (meters)	Male	121	1.79±0.01	12.45	**0.00
	Female	122	1.68±0.01		
BMI	Male	121	23.33 ± 0.46	-2.51	**0.01
	Female	122	21.79 ± 0.37		
RBG (%)	Male	121	93.64±1.42	-3.50	**0.00
	Female	122	102.03±1.81		
mCrCl.ml/min	Male	121	124.64±8.37	0.01	0.05*
	Female	121	124.52±6.28		
CG (ml/min)	Male	121	90.92±2.65	3.06	**0.001
	Female	122	80.68±2.11		
CKD-EPI.Cr.2021 (ml/min).	Male	121	88.64±1.95	4.14	**0.001
	Female	122	78.41±1.56		
MDRD.ml/min	Male	121	108.45±0.91	25.56	**0.001
	Female	122	81.47±0.6		

Key: ** P < 0.001, *P < 0.05, NS = Not Significant

mCrCl (ml/min/1.73 m ^{2}) measured creatinine clearance

CG (ml/min/1.73 m ^{2}) Cockcroft-Gault estimated GFR

CKD-EP!Cr 2021 (ml/min/1.73 m ^{2}) National Kidney Foundation Chronic Kidney Disease Epidemiology Creatinine2021 equation

MDRD equation (ml/min/1.73 m ^{2}) Modification of Diet in Renal Disease equation

Graphical presentation of T-test comparison of the GFR for male and female Subjects (fig.4.105 to 4.108)

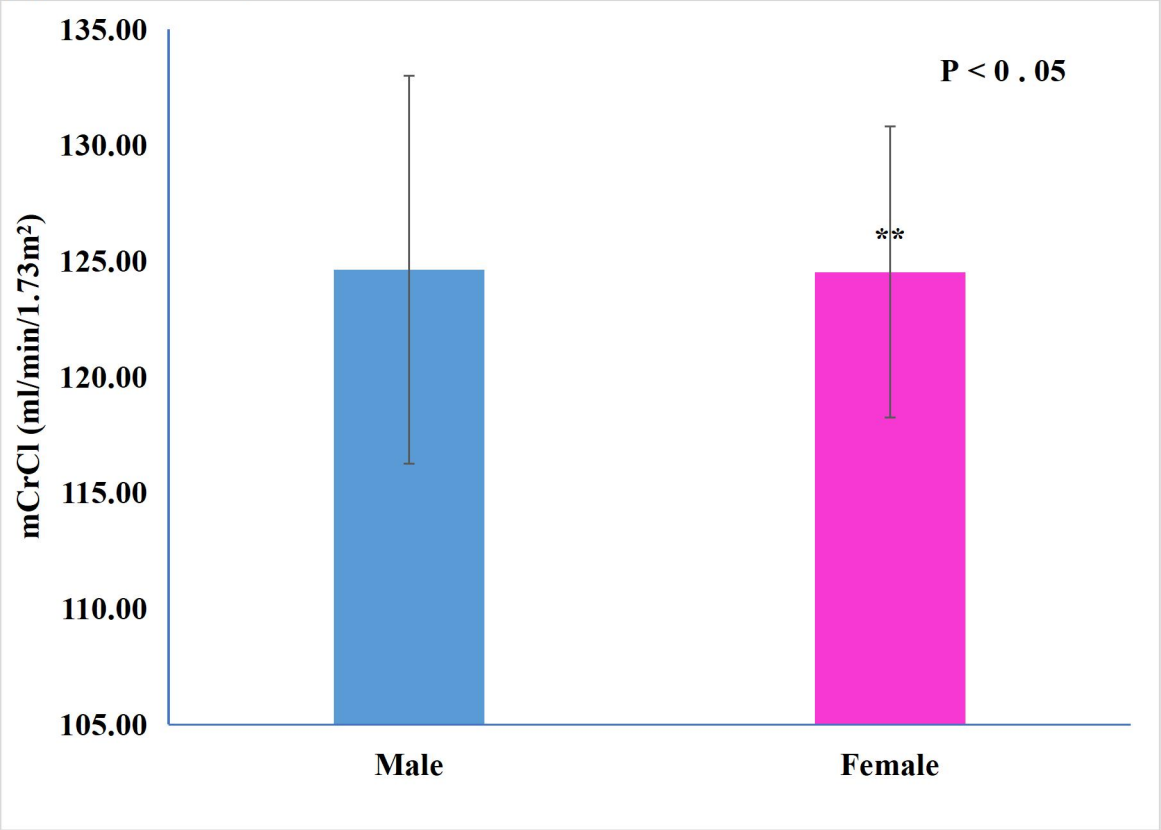

Fig. 4.105: Comparison of measured Creatinine Clearance (mCrCl in ml/min/1.73 m^2) of male and female subjects. The male Subjects had significantly higher mGFR than the females ($P < 0.05$).

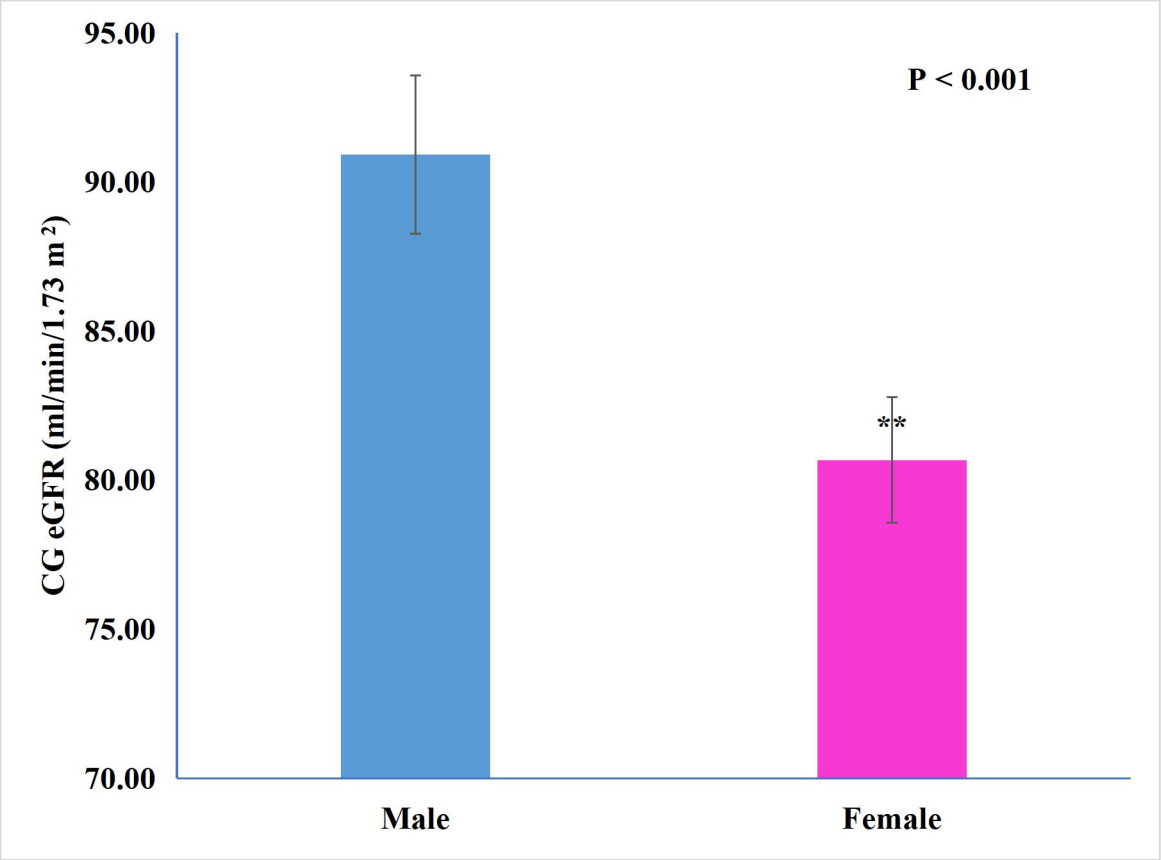

Fig. 4.106: Comparison of Cockcroft-Gault eGFR (CG in ml/min/1.73 m ^{2}) of the male and female subjects. The male Subjects had significantly higher eGFR than the females (P < 0.001).

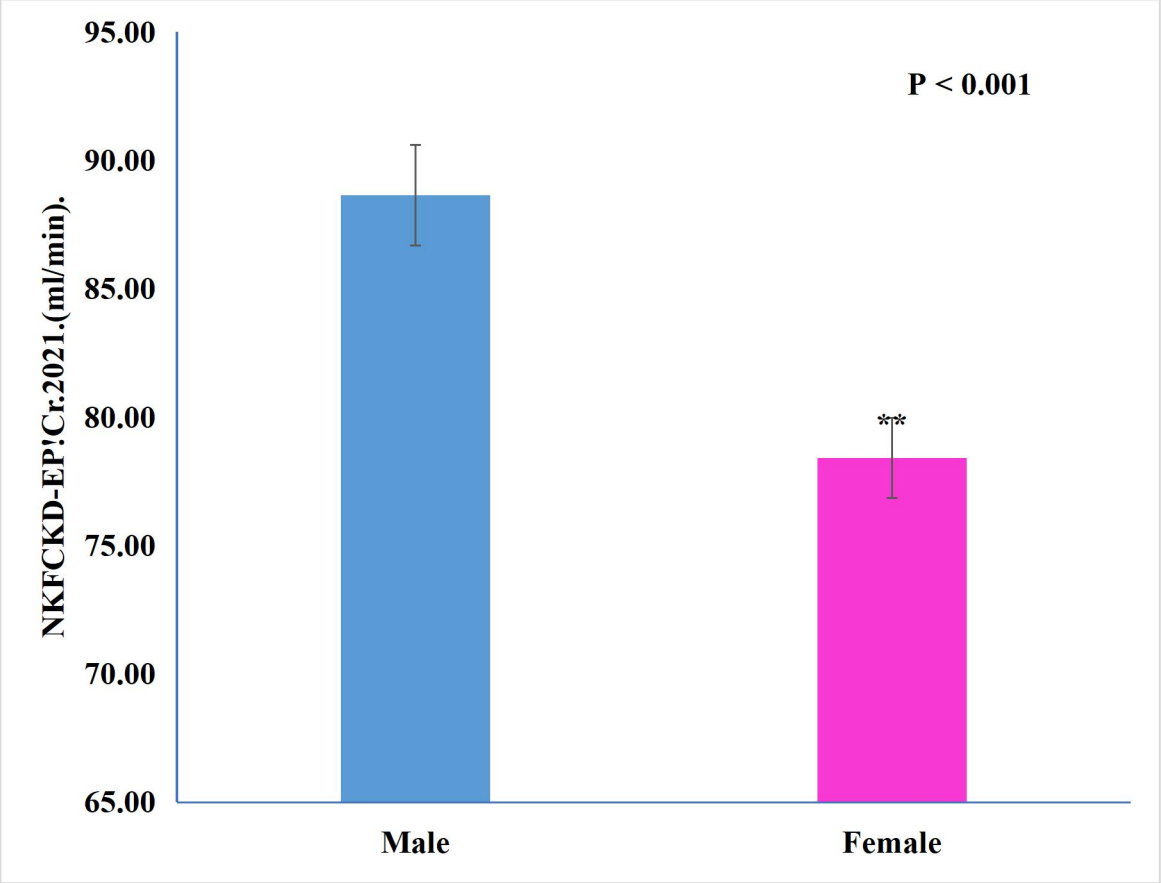

Fig. 4.107: Comparison of NKFCKD-EP!Cr 2021 eGFR (in ml/min/1.73 m^2) of male and female Subjects. The male subjects had significantly higher eGFR than the females (P < 0.001).

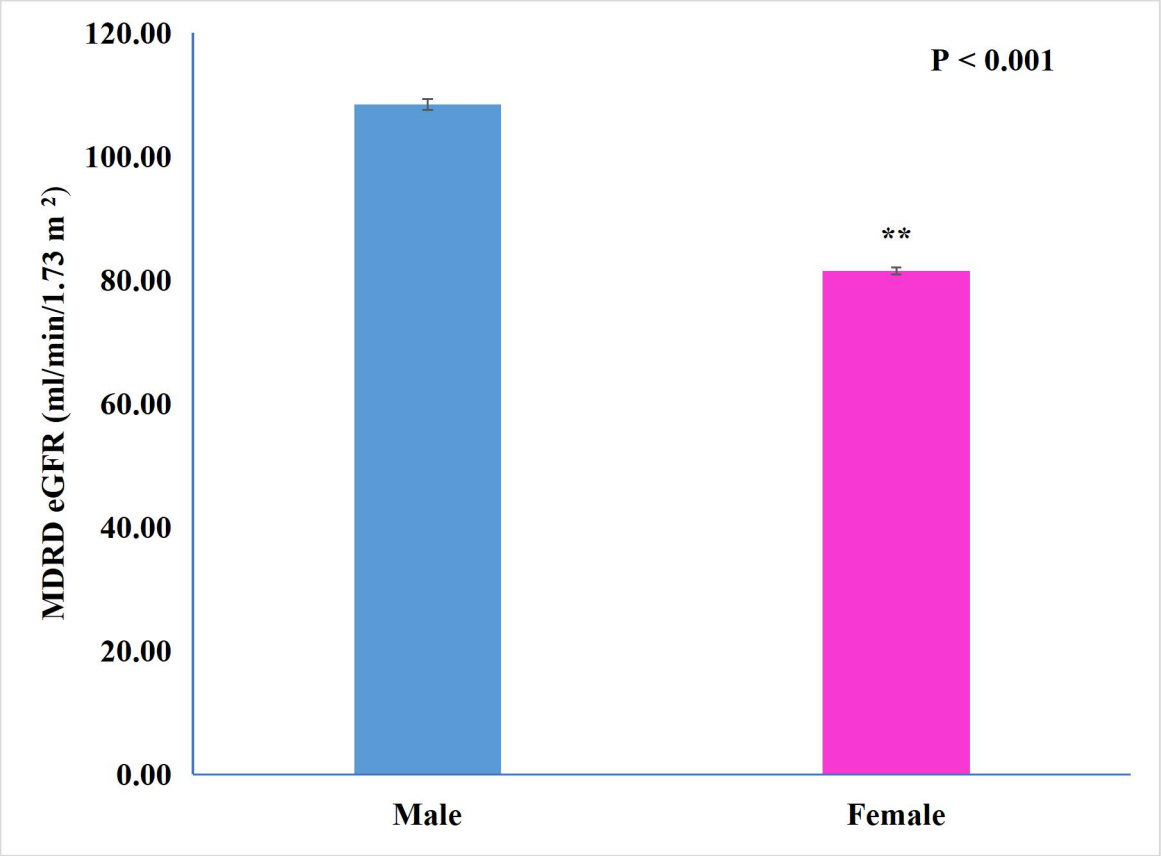

Fig. 4.108: Comparison of Modification of Diet in Renal Disease eGFR (in ml/min/1.73 m ^{2}) of the male and female subjects. The male subjects had significantly higher eGFR than the females ($P < 0.001$).

Comparison of Regression graphs of parameters for Male and Female Subjects (fig.4.109 to 4.124)

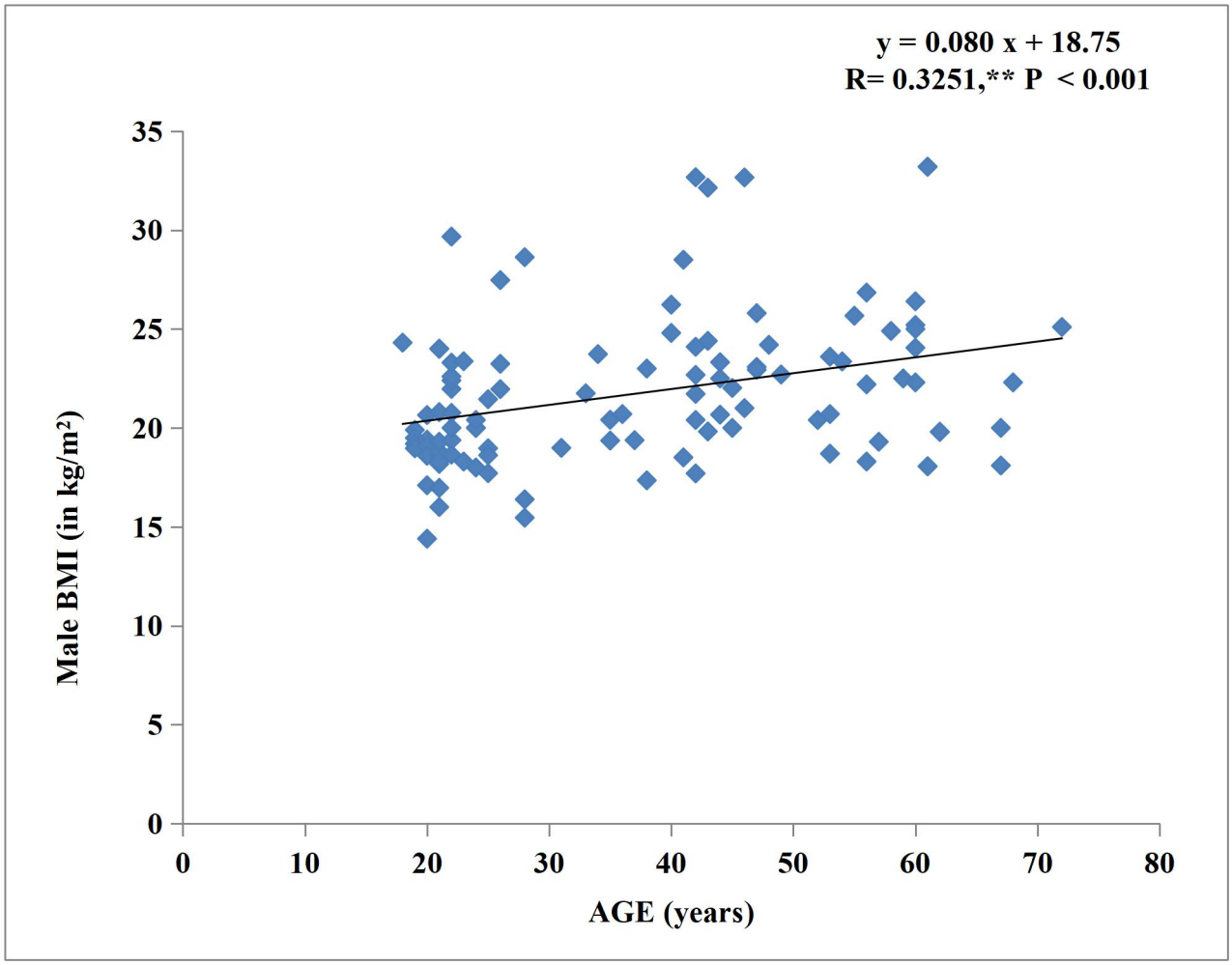

Fig. 4.109: Linear regression of BMI on Age (Years) for Males. There was significant increase in BMI as age increased ($P < 0.001$). The annual rate of increase was 0.08 kg/m ^{2} /yr. The rate of increase was 0.080 kg / m ^{2} /yr.

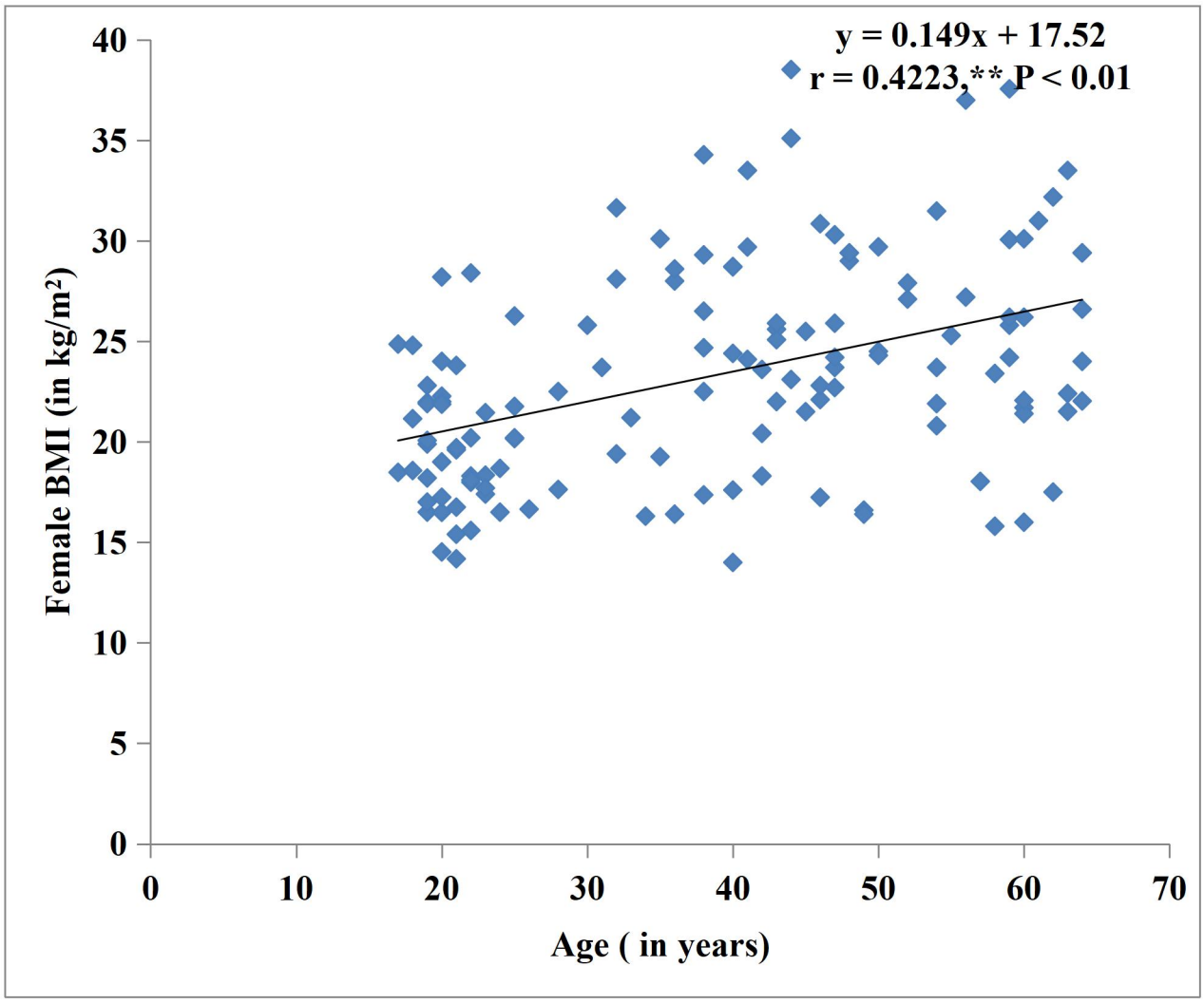

Fig. 4.110: Linear regression of BMI (in kg / m ^{2}) on Age (in years) for Females. There was significant annual rate of increase in BMI ($P < 0.01$). The rate of increase was 0.149 kg /m ^{2}/yr.

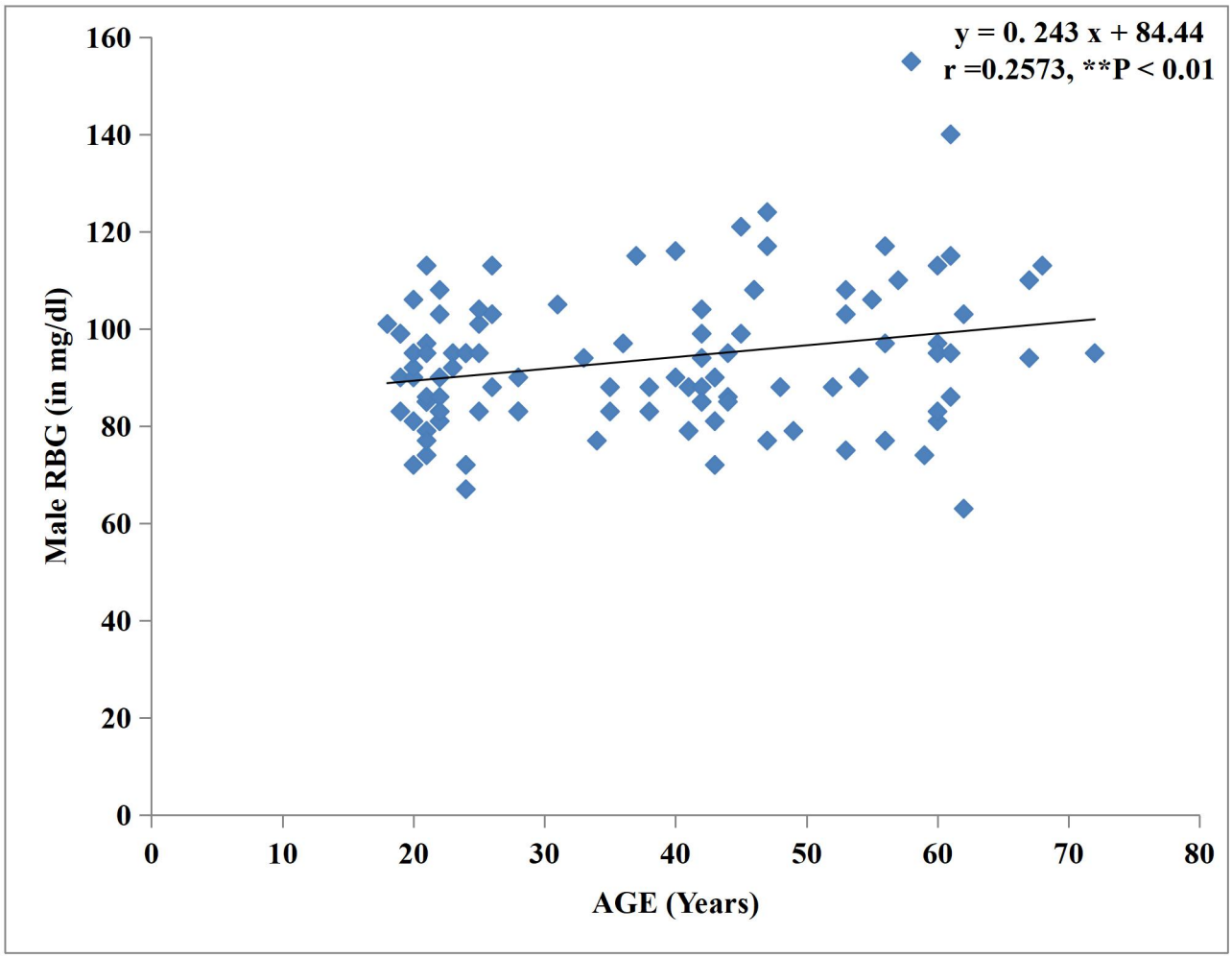

Fig. 4.111: Linear regression of Random Blood Glucose (in mg/dl) vs. Age (in years) for male Subjects. There was significant annual increase in RBG ($P < 0.01$). The rate of increase was 0.243 mg /dl / yr.

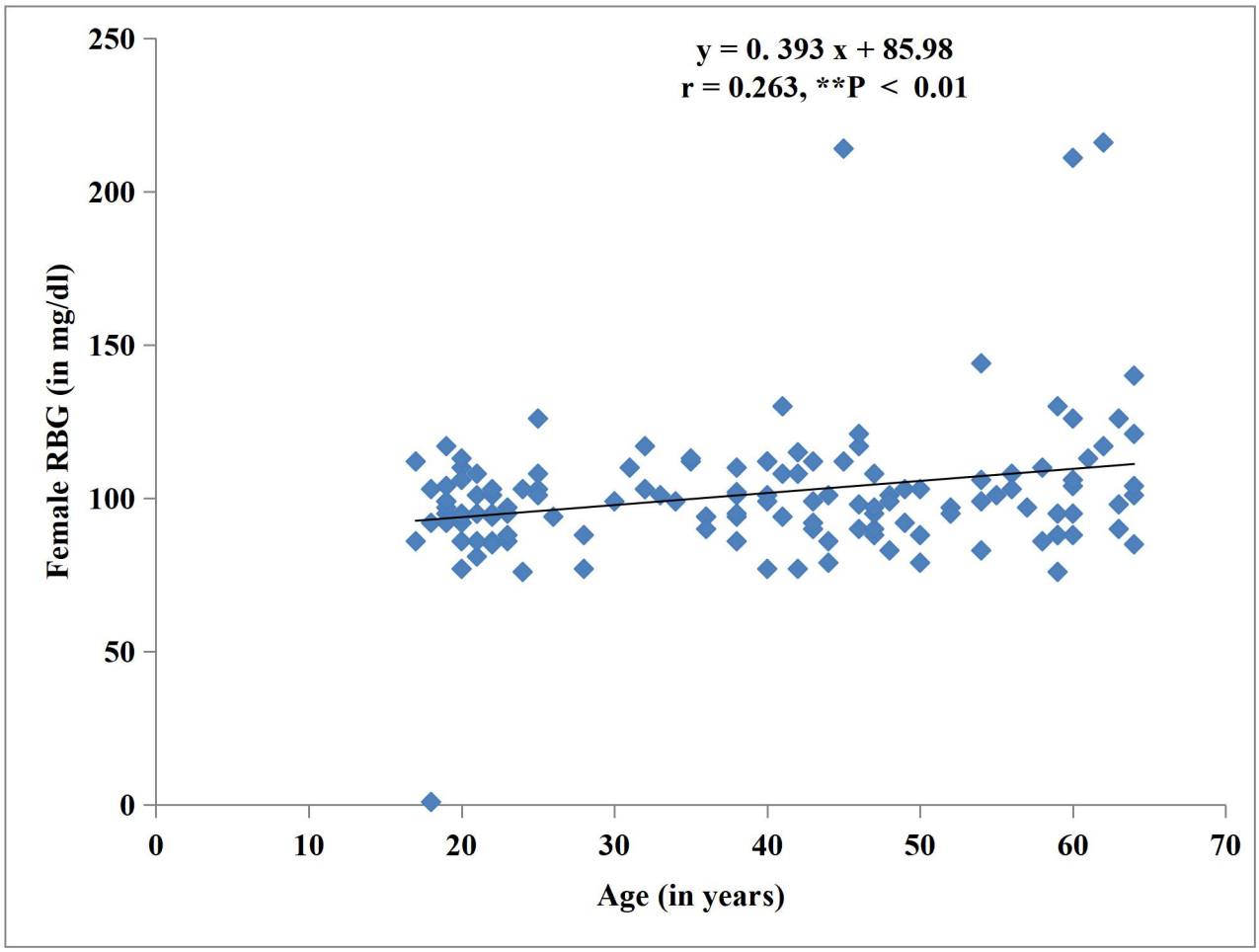

Fig. 4.112: Linear regression of Random Blood Glucose (in mg/dl) vs. Age (in years) for female Subjects. There was significant annual increase in RBG ($P < 0.01$). The rate of increase was 0.393 mg / dl / yr.

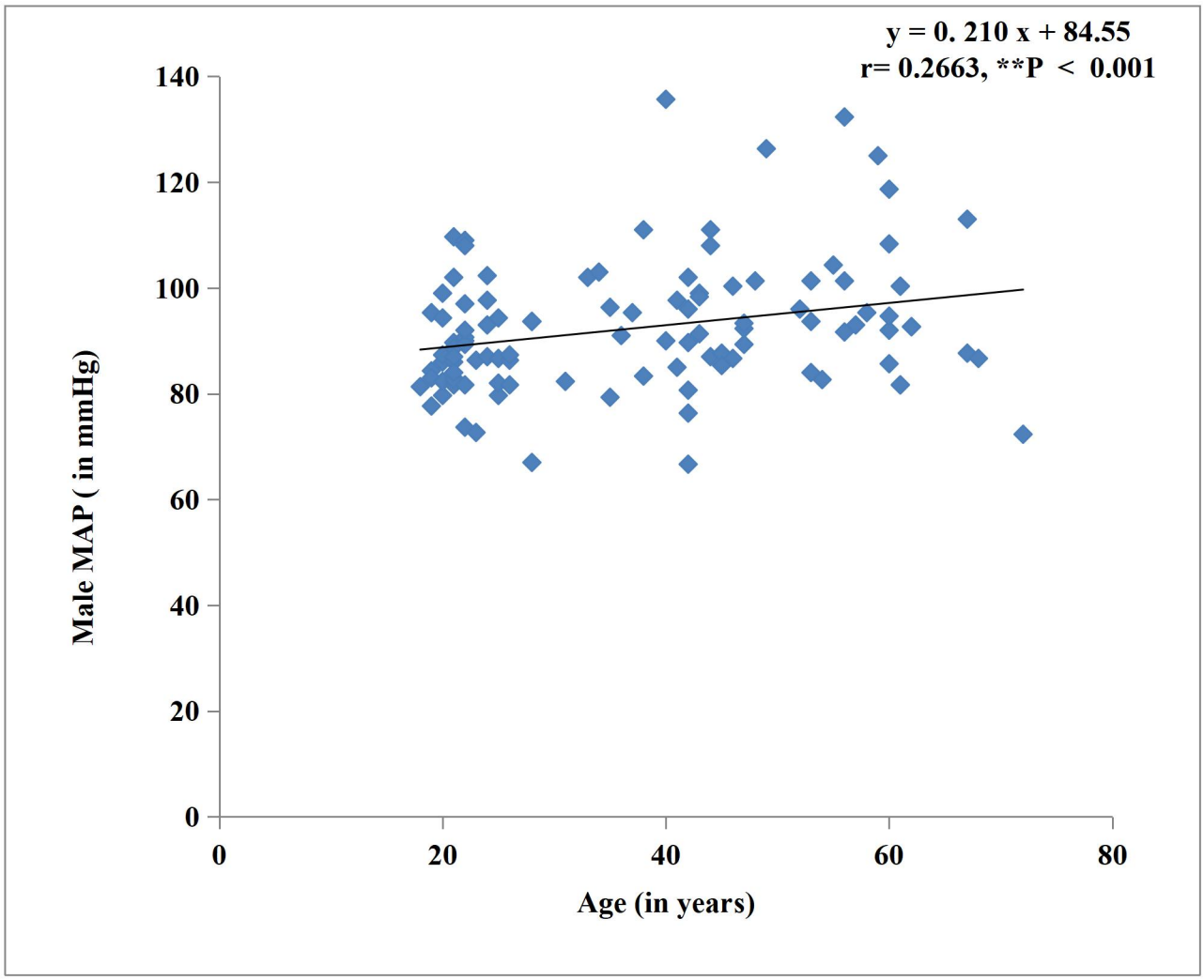

Fig. 4.113: Linear regression of Mean Arterial Pressure (in mmHg) vs. Age (in years) for Male Subjects. There was significant annual increase in MAP (in mmHg). The rate of increase was 0.210 mmHg /yr.

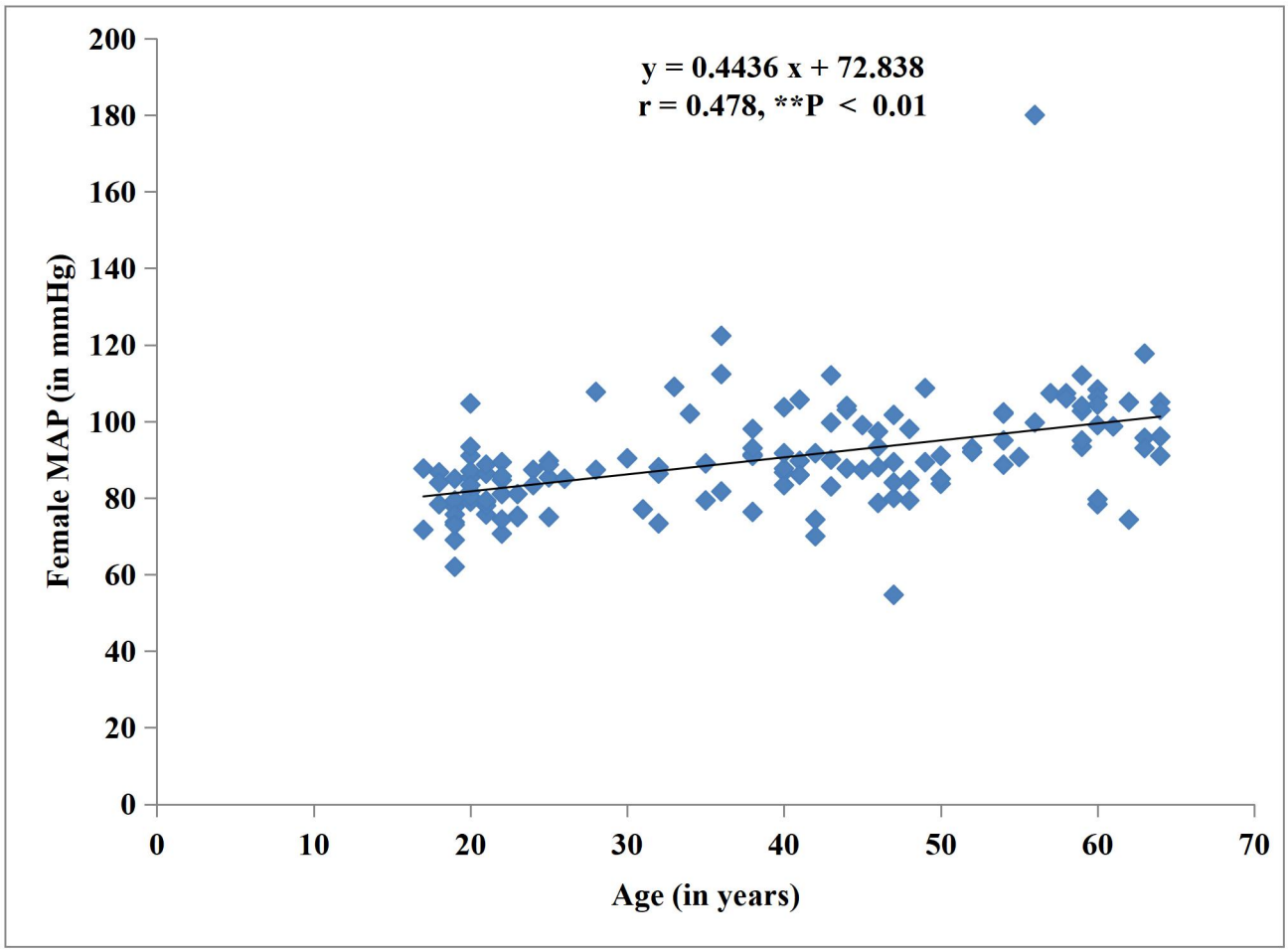

Fig. 4.114: Linear Regression of Mean Arterial Pressure (in mmHg) vs. Age (in years) for Female Subjects. There was significant annual increase in MAP ($P < 0.01$). The rate of increase was 0.444 mmHg / yr.

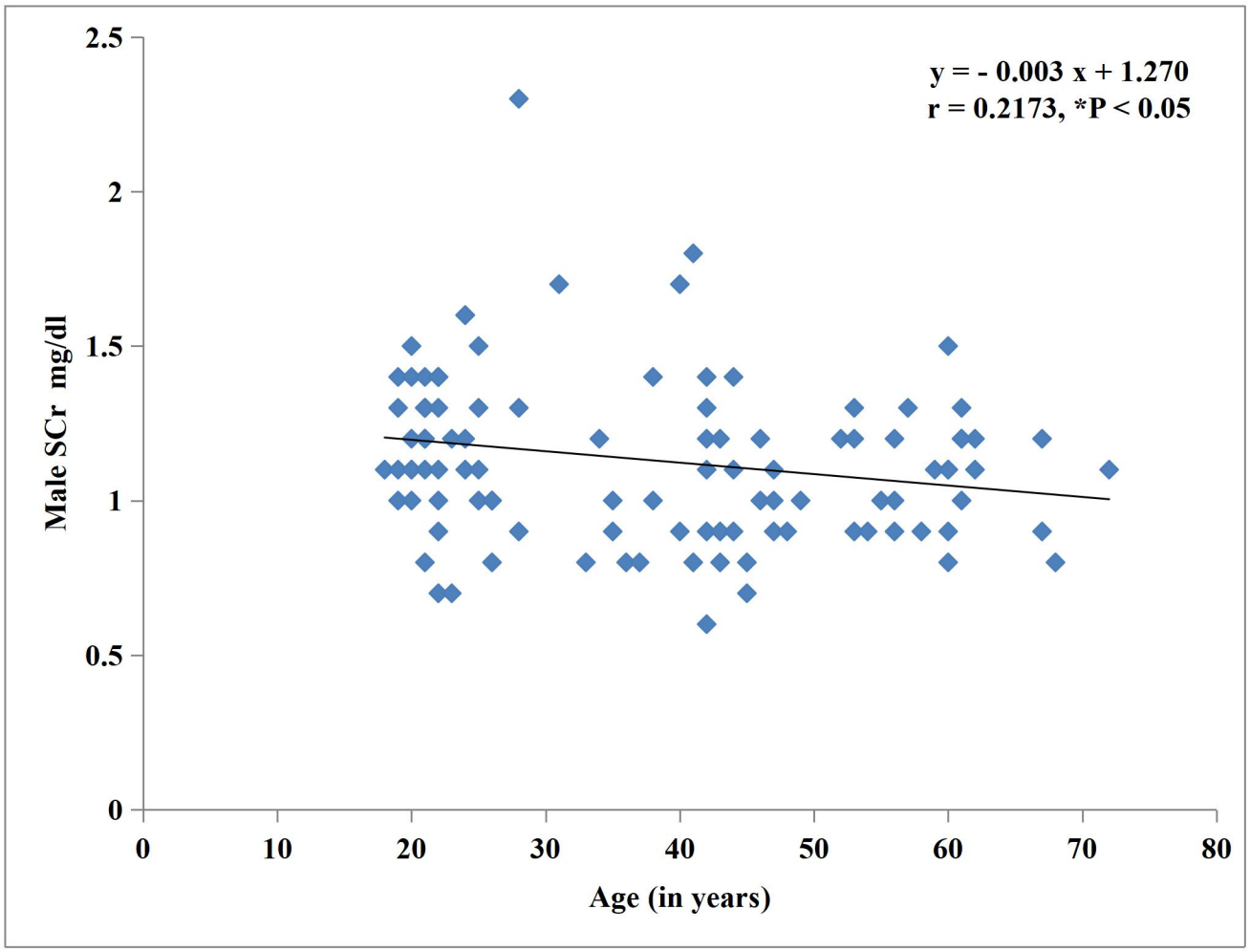

Fig. 4.115: Linear Regression of Serum Creatinine (in mg /dl) vs. Age (in years) for Male Subjects. There was significant annual decrease in SCr as age increased ($P < 0.05$). The rate of decrease was 0.003 mg / dl / yr.

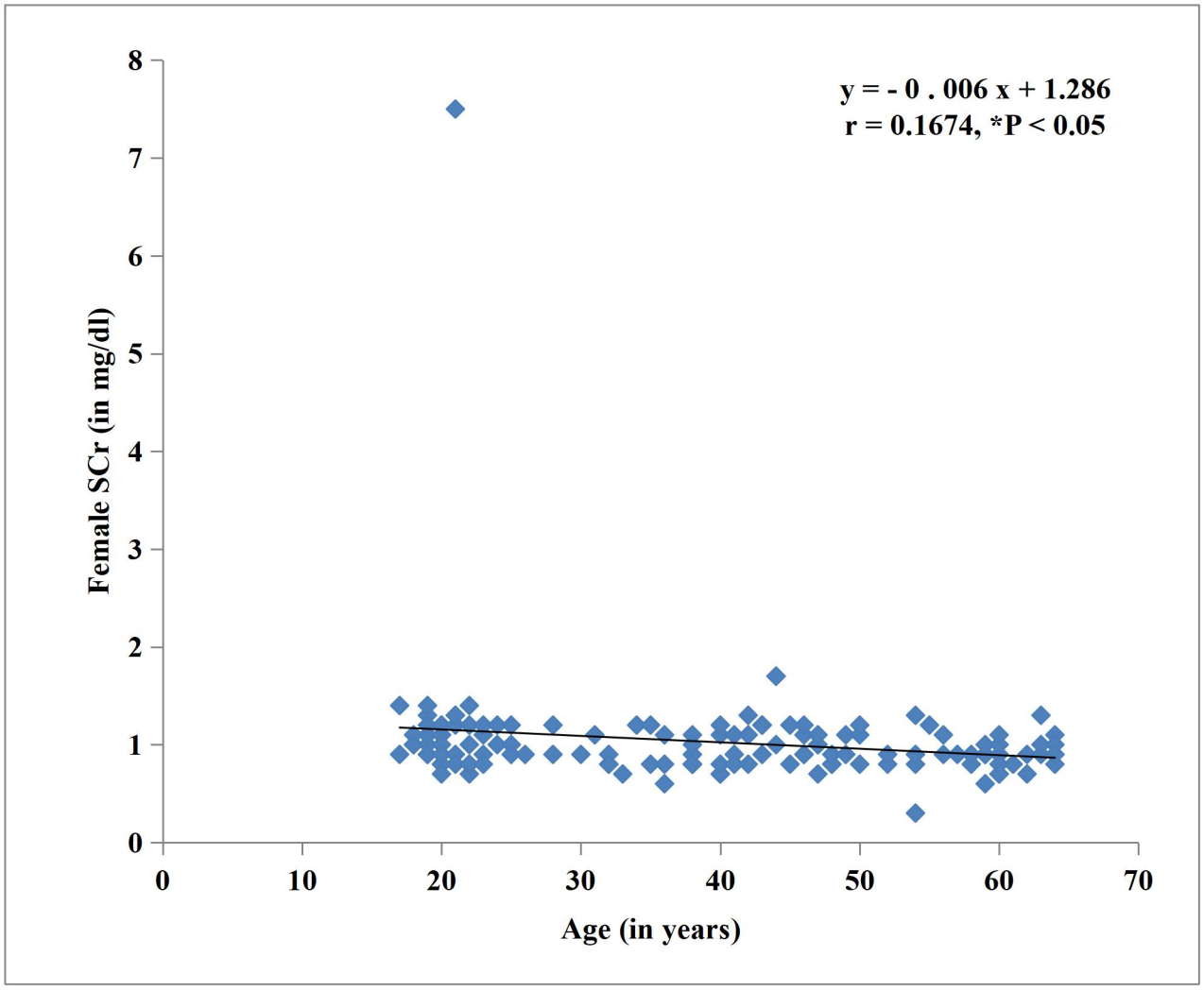

Fig. 4.116: Linear Regression of Serum Creatinine (in mg / dl) on Age (in years) for Female Subjects. There was significant annual decrease in SCr ($P < 0.05$). The rate of decrease was 0.006 mg / dl / yr.

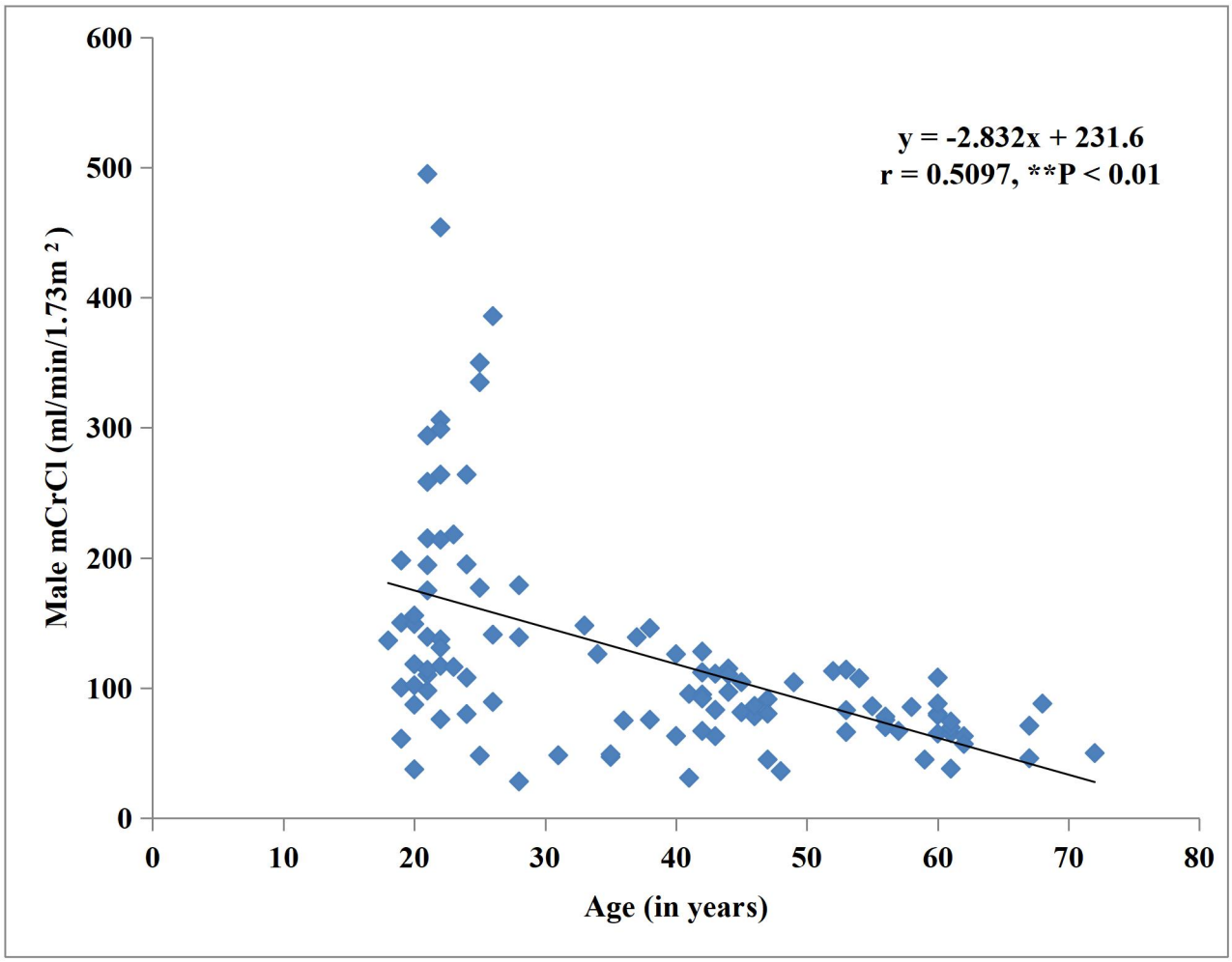

Fig. 4.117: Linear Regression of measured Creatinine Clearance (in ml/min/1.73m ^{2}) vs. Age (Years) for male Subjects. There was significant annual decline in mCrCl ($P < 0.01$). The rate of decline was 2.832 ml / min/ 1.73m2 / yr.

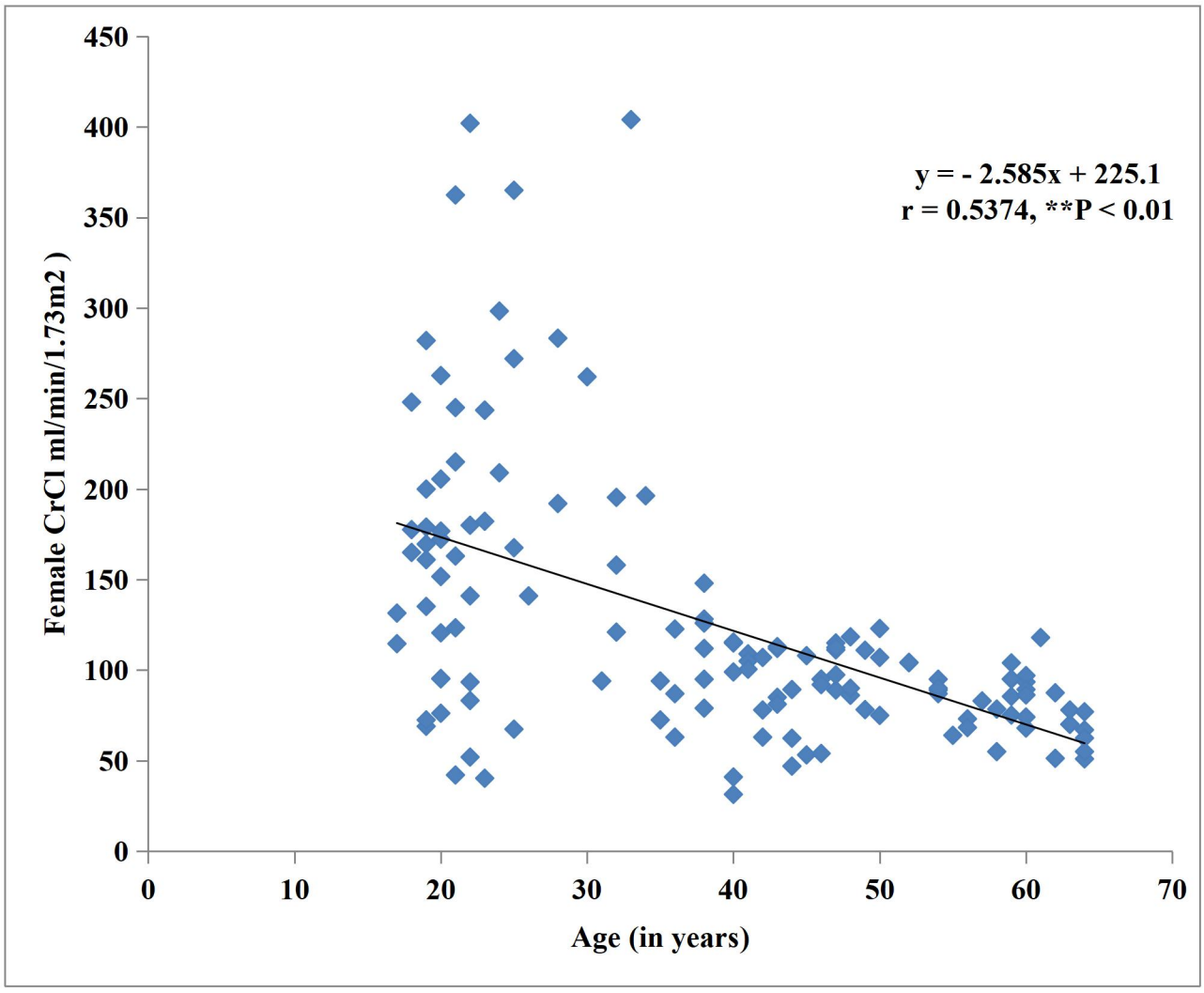

Fig. 4.118: Linear Regression of measured Creatinine Clearance (in ml/min/1.73 m ^{2}) vs. Age (in years) for female Subjects. There was significant annual decline in mCrCl ($P < 0.01$). The rate of decline was 2.585 ml / min / 1.73m^2/yr.

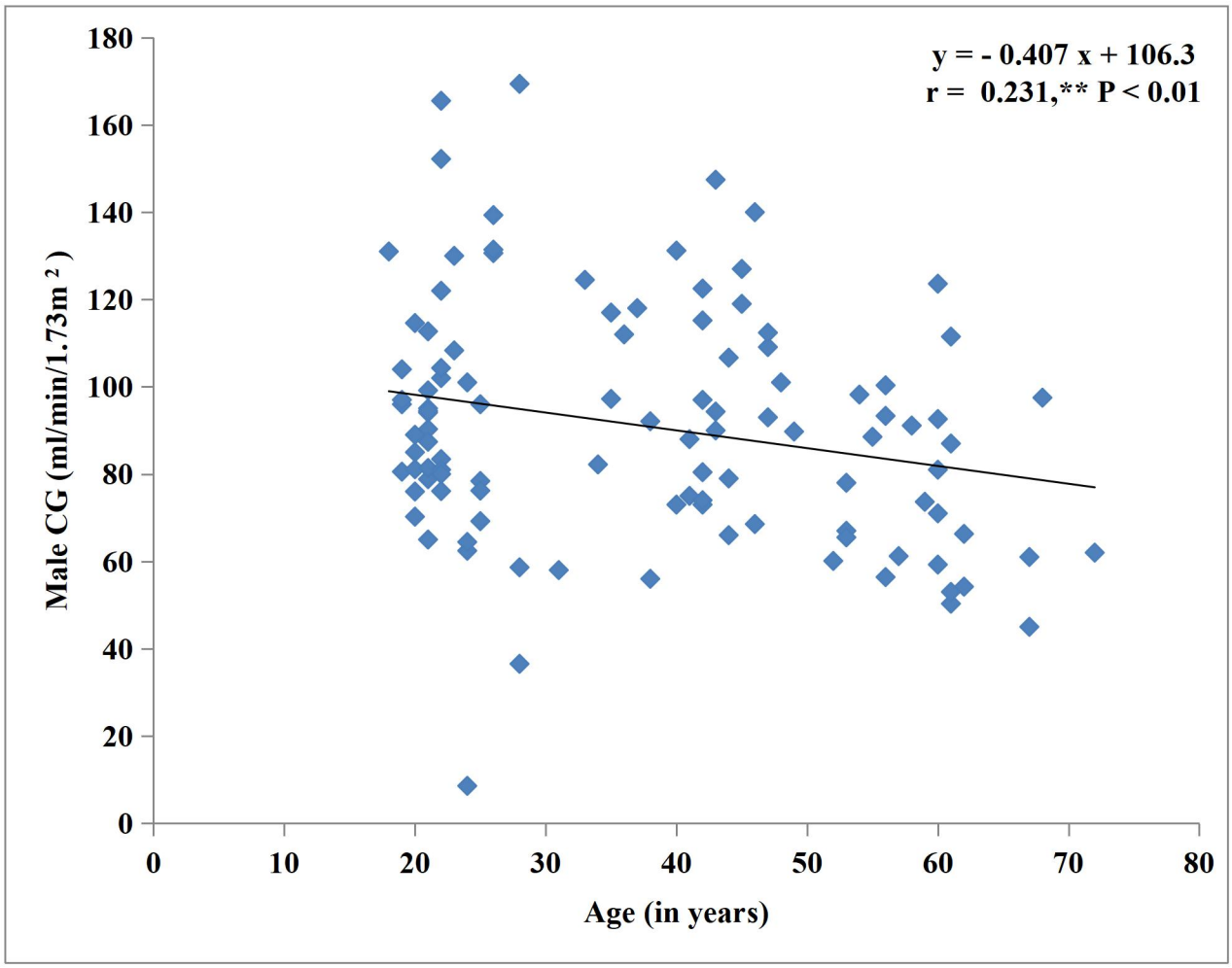

Fig. 4.119: Linear Regression of Cockroft-Gault eGFR (in ml/min/1.73m^2) vs Age (in years) for male Subjects. There was significant annual decline in CG eGFR ($P < 0.01$). The rate of decline was 0.41 ml / min/ 1.73m^2 / yr.

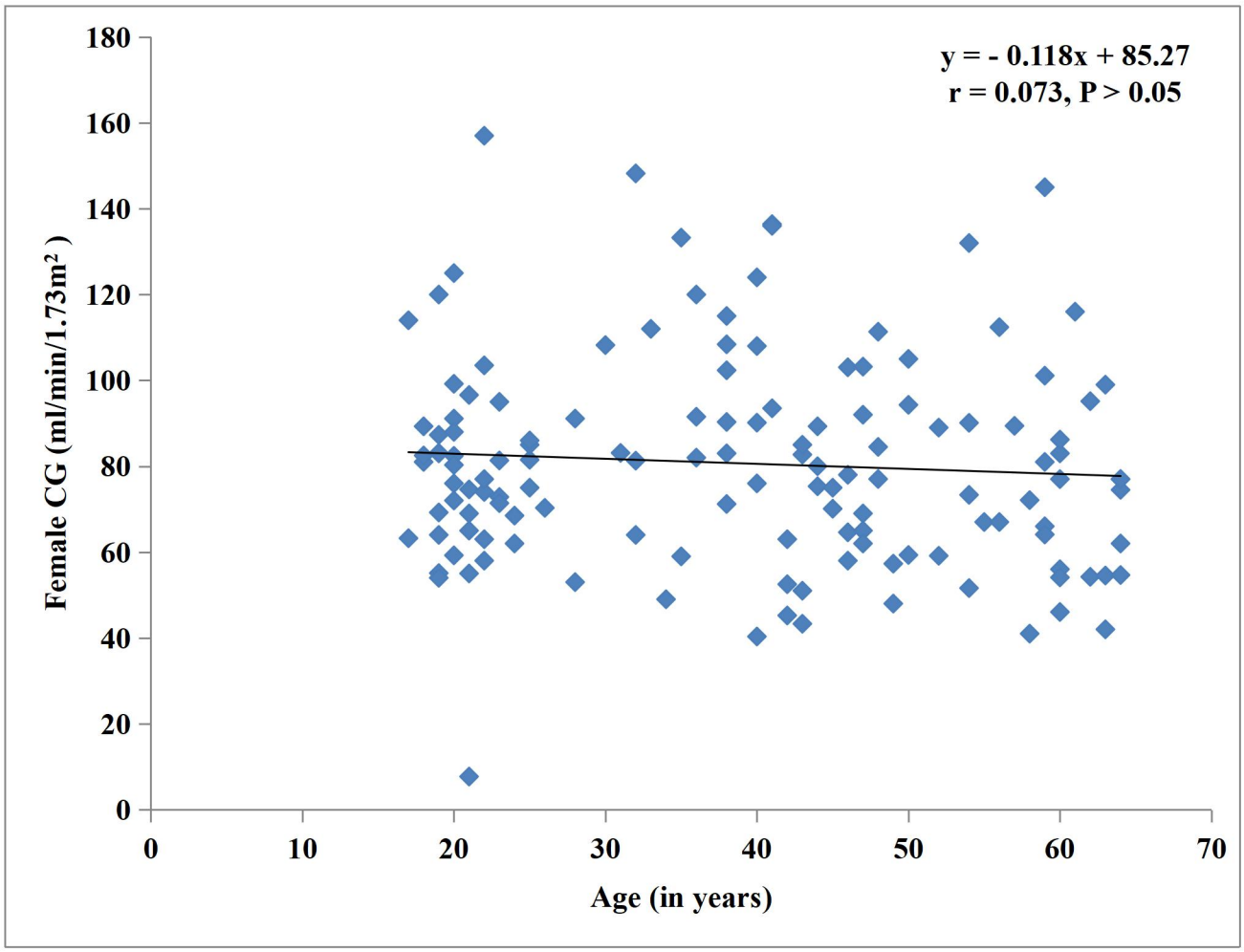

Fig. 4.120: Linear regression of Cockroft-Gault eGFR (in ml/min/1.73m ^{2}) vs. Age (in years) for female Subjects. The annual decline in CG eGFR was not significant (P > 0.05). The rate of decline was 0.118 ml /min /1.73m^2/yr.

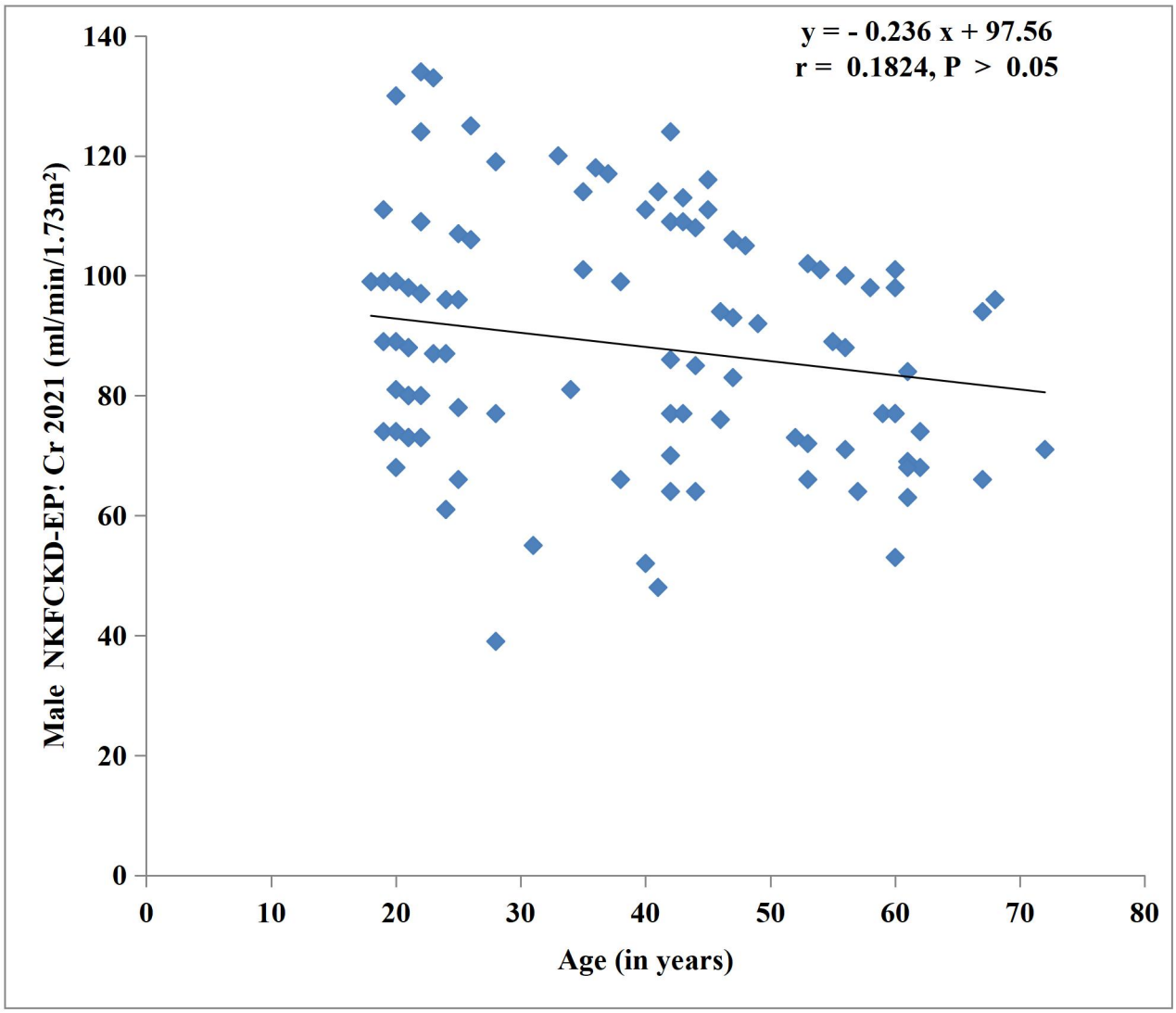

Fig. 4.121: Linear Regression of National Kidney Foundation Chronic Kidney Disease-Epidemiology Creatinine 2021 eGFR equation vs. Age (in years) for male Subjects. The decline in GFR was not significant ($P > 0.05$). The rate of decline was 0.236 ml / min / 1.73 m^2/yr.

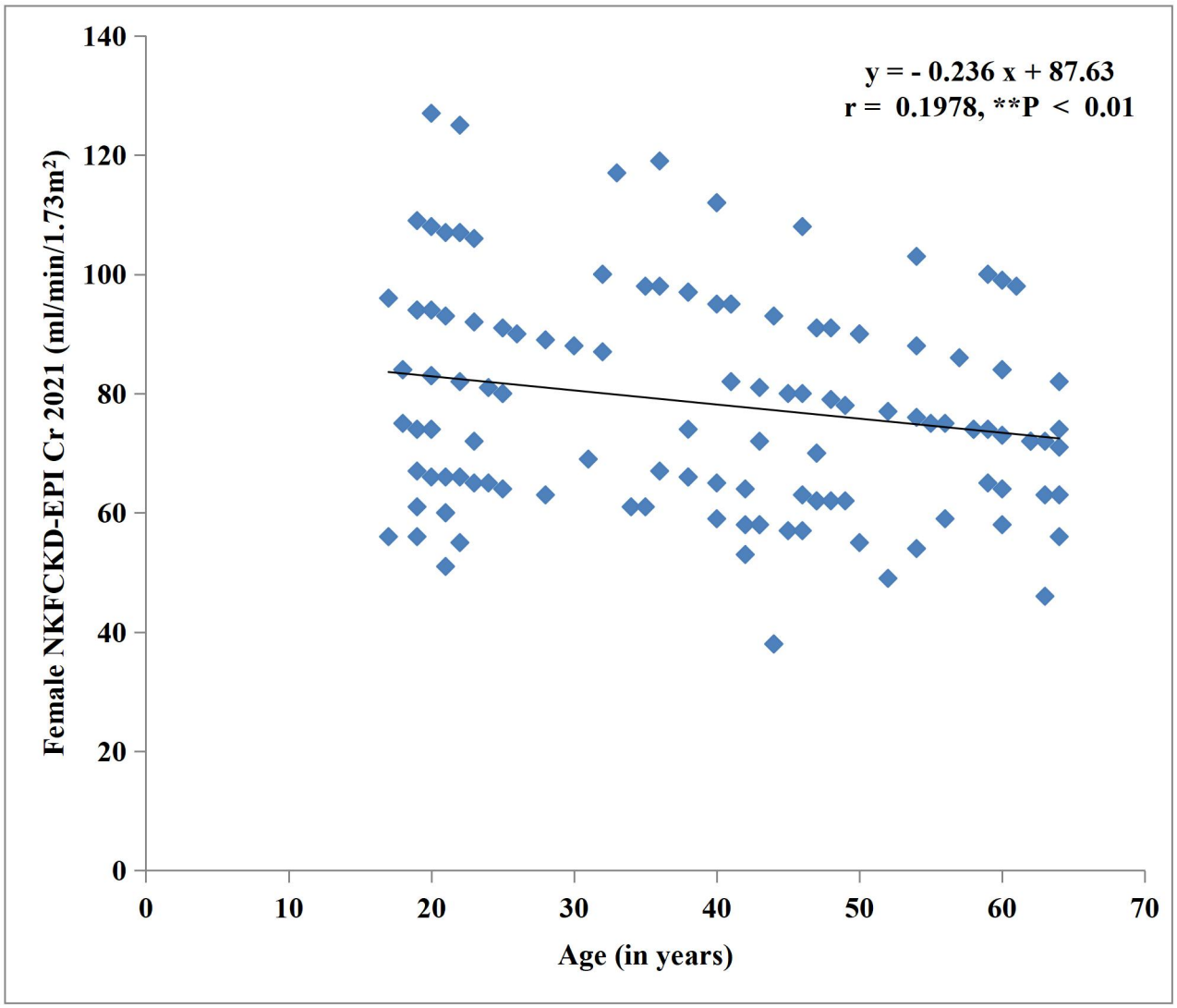

Fig. 4.122: Linear Regression of National Kidney Foundation Chronic Kidney Disease-Epidemiology Creatinine 2021 eGFR equation vs. Age (in years) for female Subjects. The decline in GFR was significant ($P < 0.05$). The rate of decline was 0.236 ml / min / 1.73 m^2/yr.

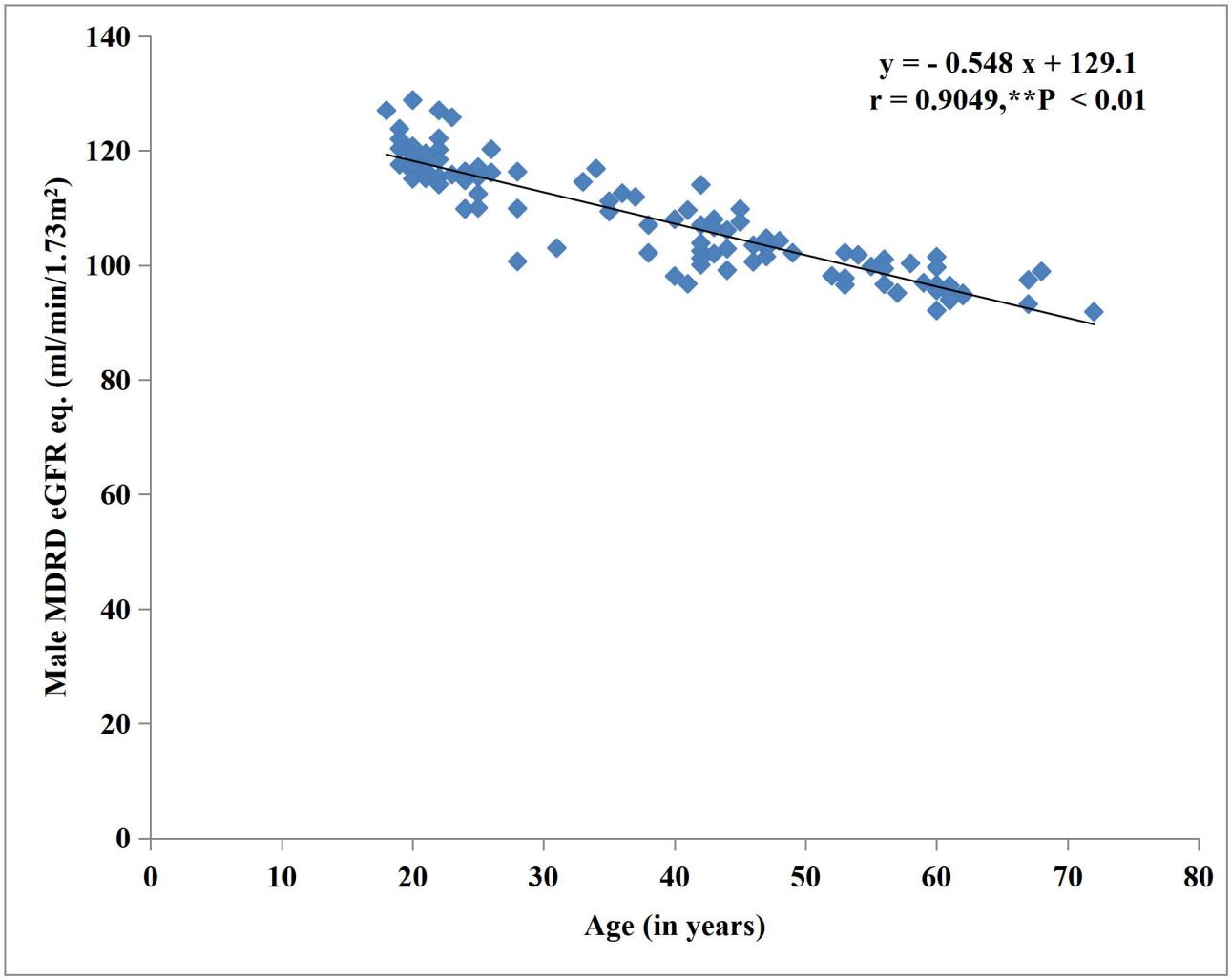

Fig. 4.123: Linear Regression of Modification of Diet in Renal Disease eGFR equation vs. Age (in years) for male Subjects. The decline in GFR was significant ($P < 0.05$). The rate of decline was 0.548 ml / min / 1.73 m^2/yr.

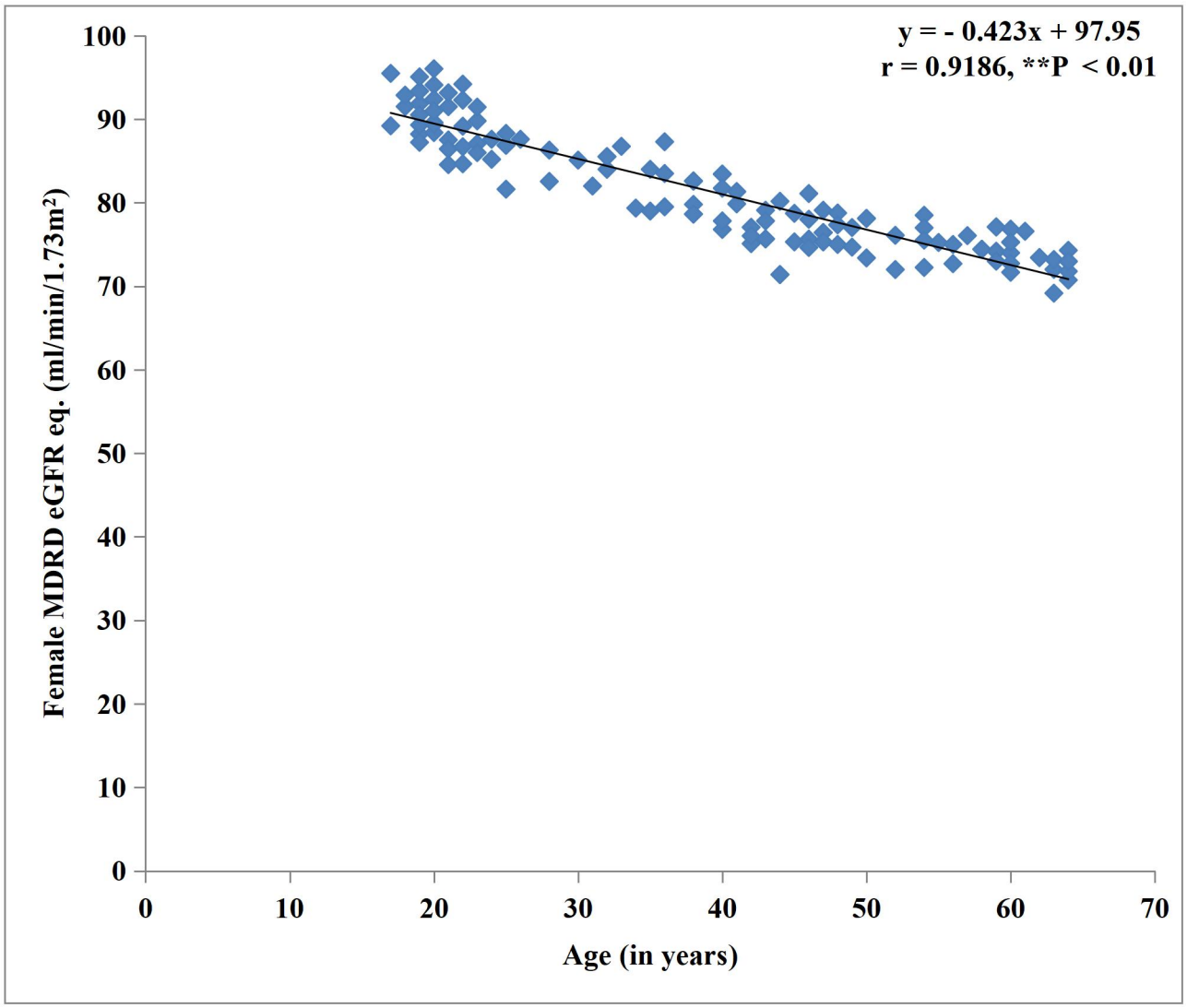

Fig. 4.124: Linear Regression of Modification of Diet in Renal Disease eGFR equation vs. Age (in years) for female Subjects. The decline in GFR was not significant ($P < 0.05$). The rate of decline was 0.432 ml / min / 1.73 m^2/yr.

Table 4.19: Summary of Regressions (increase or decline) in Parameters for Male and Female Subjects (fig. 116 to 131)

Parameters	**Male**	**Female**
BMI (kg/m^2)	0.080 ($P < 0.001$)	0.149 ($P < 0.01$)
RBG (mg/dl)	0.243 ($P < 0.01$)	0.393 ($P < 0.01$)
MAP (mmHg)	0.444 ($P < 0.001$)	0.210 ($P < 0.01$)
SCr (mg/dl)	- 0.006 ($P < 0.05$)	- 0.003 ($P < 0.05$)
mCrCl (ml/min/1.73m ^{2})	- 2.832 ($P < 0.01$)	- 2.585 ($P < 0.05$)
CG eGFR eq. (ml/min/1.73m ^{2})	- 0.407 ($P < 0.01$)	- 0.118 ($P > 0.05$)
NKF CKD-EP!Cr2021 eGFR eq. (ml/min/1.73m ^{2})	- 0.236 ($P < 0.05$)	- 0.204 ($P < 0.05$)
MDRD eGFR eq. (ml/min/1.73m ^{2})	- 0.548 ($P < 0.01$)	- 0.423 ($P < 0.01$)

$P < 0.05$, $P < 0.01$ and $P < 0.001$ indicate significant change

$P > 0.05$ indicate no significant change

The values with negative signs indicated annual rates of decline or decrease in parameter. The values without negative signs indicated annual rates of increase in parameter.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Preamble

This research thesis focused primarily on Age Determined Changes in Glomerular Filtration Rate (GFR) among Black Ethnic Normotensive and Hypertensive Adult Nigerians in order to discover the Age Determined Annual Rate of Decline (ADARD) in GFR. This was a cross-sectional study in order to capture renal health events in this set of apparently healthy two hundred and seventy adult male and female Nigerians (of Benin City as a sample in Edo state) within the period. The age range of the subjects was 18 to 70 years. Seven GFR formulas variously as applied in previous studies were used in this study in order to avoid bias for a particular one. This was necessary for comparison in order to identify a fair clinical tool for our population. The influences of BMI, urine albumin excretion and urine sodium potassium ratio on glomerular filtration rates were also examine The parameters examined were plotted as regression graphs against age within the groups, Student's T-test was used to compare differences between groups and correlation matrix was used to identify the dependence of age against all the parameters among the population. The P-value of less than 0.05 was accepted as high probability of significance.

5.2 Relationship between Gender, Age and BMI

The graphs in figs 4.3 and 4.4 shows significant increases in BMI with age in both males and females up to 50 years especially among females ($P < 0.01$). Body Mass Index generally increases from adolescent age, peaks between 50 to 69 years and declines after 70 years of age in both males and females which are considered as normal process of growth (Deborah *et al*., 2007).

The observations in this study were similar. These changes associated with age and BMI were also observed with other physiological features such as increase in blood pressure (MAP) and ADARD in GFR. BMI correlated significantly positively with MAP but negatively with MDRD eGFR.

5.3 Changes in MAP with Age

In this study there was significant increase in Mean Arterial Pressure (MAP) with age ($P < 0.001$). Similarly SBP and DBP also increased significantly with age. This was similar with the findings of the Framingham Heart Study in which physiologic changes associated with increasing age also increase SBP, DBP, MAP and Pulse Pressure (Mahmood et al., 2014). These are related to arterial changes, as aging results in the narrowing of the vessel lumen and stiffening of the blood vessel walls through a process known as atherosclerosis which increases peripheral resistance (Mahmood et al., 2014). In this study the MAP correlated negatively but not significantly with NKFCKD-EP!Cr eGFR and MDRD eGFR. MAP correlated positively significantly with BMI ($P < 0.01$), UrAlb ($P < 0.01$) and UrAlb / UCR ($P < 0.05$). Obviously urine protein (as albumin) excretion increased significantly with Age, BMI and MAP. Certainly BMI correlated significantly and positively with increase in blood pressure and hypertension (Song et al., 2023). Progressively increasing BMI and obesity contributes to 60–70% of hypertension cases, with the obese population facing a 3–4 times higher risk than individuals with a normal weight (Weng C et al 2017). It was not on record that urine protein excretion or UrAlb/UCR increases with Age and BMI except in this Nigerian study.

5.4 Changes in Age with ESR

The purpose of including assessment of Erythrocyte Sedimentation Rate (ESR) was to show

that Subjects were generally healthy. In this Study, the mean ESR was within normal range (6.01 ± 0.3 mm/Hr.) although there was increase in ESR with age in both gender but remained generally within normal limits. This suggested that most of the subjects were apparently healthy (may not have had obvious acute or chronic inflammatory disease) during the period. The ESR is a non-specific indicator of acute or chronic inflammatory disease if the values are high although normal in most subjects (Gillum, 1993). A normal ESR may not totally exclude organic disease as mildly elevated ESR of 20-30 mm/hour may have no consequence but levels above 100 mm/hour are significant and indicate presence of a disease (Erikssen et al.,2024). The normal ESR values are usually slightly higher in females (Eastham, 1954). It is a useful indicator of the presence and intensity of an inflammatory process but is not diagnostic of a particular disease. Erythrocyte Sedimentation Rate increases with age due to increase in blood fibrinogen concentration (Alende-Castro V et al. 2019). A raised ESR can also be a marker for coronary heart disease including risk of death (Erikssen et al.,2024). Other indicators stable health in these Subjects were normal values of blood hemoglobin concentration (13.02 ± 0.13), hematocrit (40.3 ± 0.54%), platelet count (217 ± 8.2 cells/mm^3) and Procalcitonin [(0.31 ± 0.04 ug/L) which is a protein biomarker of bacterial infection].

5.5 Age Related Decline in GFR

Decline in Glomerular Filtration Rate with Age was initially reported by Davies and Shock in 1950 (Davies and Shock, 1950). This observation was confirmed with measured Creatinine Clearance (mCrCl) although the decrease in urine Cr excretion with age was less well understood (Freidman et al, 1972). Serum creatinine decreased with age but remained significantly lower in females than males due to lower muscle mass. It is dangerous to use values of SCr alone to calculate dosing for toxic drugs because a 70 years old man may have the same serum creatinine

with a 25 years old male although only half the CrCl (Freidman et al, 1972). In this study various rates of changes in renal indices with age were recorded for; all subjects, males, females and age groups (< 30 and ≥ 30 years). Urine flow rate, urine Cr, SCr and GFR with various GFR methods decreased progressively and significantly with age ($P < 0.01$) in fig, 4.14 to 4.25.

5.6 Relationship between Age, urine flow rate/min, SCr and mCrCl

Summaries of these parameters were presented in tables 4.4 and 4.5. The results of this study showed that with increasing age there were progressive reductions in urine flow rate, serum creatinine and measured creatinine clearance (mCrCl). Age-associated losses of kidney functions have been recognized for decades. This was attributed to progressive loss of nephron number (due to nephrosclerosis plate 1), decrease in glomerular filtration rate and renal blood flow due to renal arteriosclerosis (Jessica, 2010). In this study, the rate of loss of mGFR was derived from the regression equation $y = -2.681 x + 227.7$ in fig. 4.25. This showed a gradient of -2.681 as the rate of annual decrease of mCrCl or 2.681ml/min/1.73m ^{2} / yr. This rate of decline was considered significantly very high (by the investigators) for apparently healthy population when compared with previously reported "gold standard' inulin based value of about 1.0 ml / min/ 1.73m ^{2} / yr among a similar Caucasian population (Morrissey and Yango, 2006). The mCrCl formula (Ucr. x V/ Pcr.) has been noted to over-estimate GFR due to renal tubular creatinine secretion into urine which accounts for about 10% (Joseph, 2012). There were further conversion of creatine to creatinine in stored urine samples and inaccurate timed urine collections (Pierre and Andrew, 2015). The mCrCl in this study may not be absolved from these faults because of timed urine collection. Other various creatinine based eGFR methods used in this study depended on single serum samples for creatinine assay for calculation of eGFR (without timed urine collection) were hoped to improve accuracy. Various rates of progressive reduction in

GFR were found with the estimated or calculated GFR (eGFR) formula as these formulas have their peculiar characteristics.

5.7 The Modification of Diet in Renal Disease eGFR equation

The MDRD study equation was developed in 1999 with the use of data from patients with chronic kidney disease. It estimates GFR adjusted for body-surface area, considered race as either black or not and reflected a higher average SCr level in blacks (due to higher muscle mass). It was noted to be more accurate than either the use of the Cockcroft–Gault equation or measured creatinine clearance (Levey, et al, 2006). This equation requires plasma or serum sample for creatinine assay to calculate eGFR as follows;

MDRD eGFR = $186 \times (SCr)^{-0.154} \times (\text{Age in years})^{-0.203} \times (0.742 \text{ if female}) \times (1.21 \text{ if black})$.

As illustrated in figs. 4.18, 4.19, 4.22 and 4.23 there were significant age and sex dependent decreases in SCr which was responsible in changing eGFR. The MDRD equation considers age, sex and racial differences which were not considered by the measured CrCl formula. Across all age groups there were regular significant differences in eGFR which was not found in other eGFR formulas ($P < 0.01$). The regression equation $Y = - 0.503x + 112.7$ (regression coefficient = 0.4934) showed the gradient as rate of decline in eGFR at 0.503ml/min/1.73m ^{2} /year which was significant ($P < 0.01$). The eGFR in males were symmetrically significantly higher than in females which was a finding peculiar to the MDRD eGFR in this study. There was a clear zone of separation between males (upper zone) and females (lower zone) in figs 4.22 (regression graph) and 4.23 (Anova graph) which was a feature peculiar with MDRD and was not found with the other GFR formulas or in literature. This important finding among population of males and females was a pointer that MDRD equation may be a better or fair eGFR equation for our

population. Specifically in this study the MDRD eGFR correlated negatively significantly with; Age ($P < 0.01$), BMI ($P < 0.01$), PR ($P < 0.01$), DBP ($P < 0.01$), RBG ($P < 0.01$), UrAlb/CR ($P < 0.01$) and UrNa+/K+ ($P < 0.01$).

5.8 National Kidney Foundation Chronic Kidney Disease Epidemiology Collaboration Creatinine equation

The NKF CKD-EP! Cr 2021equation (Inker et al, 2021) also revealed that there was significant age dependent progressive decline in eGFR. In this study the regression equation $Y = - 0.246x + 92.40$ (with coefficient of regression = 0.1916) showed that annual decline in GFR was at the rate of 0.246ml/min/1.73m^2 /year which was significant ($P < 0.01$) as shown in fig 4.21. This equation required serum creatinine, age and sex. Due to the complexity of the equation, an online calculator was used to ensure accuracy.

eGFR = 142 x min (standardized S_{cr}/K, 1)$^{\alpha}$ x max(standardized S_{cr}/K, 1) $^{- 1.200}$ x 0.9938 ^{Age} x 1.012 [if female]. Race was excluded after validation of its 2012 version and believed to be more suitable than mCrCl and other eGFR equations as it was developed with data from CKD stable population (inker et al 2021). The males generally had higher eGFR but not significant across all age groups. In this study NKFCKD-EP!Cr correlated negatively significantly with; Age ($P < 0.01$), UrAlb ($P < 0.05$), SCr ($P < 0.01$), Urine Flow rate ($P < 0.01$) but not significantly with BMI, PR, SBP, DBP and MAP.

5.9 Cockroft-Gault eGFR equation

This was developed in 1973 but published in 1976 (Cockcroft and Gault, 1976) and was noted to be only useful for research purposes because results were not standardized for initial creatinine values and body surface area. It gradually gained acceptance due to the ease of calculation both

in clinical use and dosing for kidney excretable toxic drugs (Scappaticci and Regal, 2017) and (Ferreira et al., 2016). In this study, there was consistently higher eGFR in the males than the females in most of the Age groups but more significant in the middle Age years ($P < 0.01$).

The CG eGFR = (140 − Age yrs) × weight (in kg) × (0.85 if female) / (72 × SCr in mg/dL). This equation considered age, weight, sex and SCr as factors for eGFR. The regression equation showed eGFR (Y) as : $Y = - 0.258x + 95.14$ with regression coefficient of 0.1509. The gradient was 0.258 represents the gradient of the graph in fig. 4.20. The annual rate of decline in eGFR was 0.258ml/min/1.73m2/year which was significant ($P < 0.05$). Cockcroft- Gault eGFR correlated negatively significantly with: Age ($P < 0.01$), SCr ($P < 0.01$), and Urine flow rate ($P < 0.01$) but not significantly with UrAlb /UCR, and DBP. This showed that these variables adversely affected GFR. The Cockcroft- Gault eGFR also correlated significantly positively with BMI ($P < 0.01$) which was not easily understood.

5.10 Cystatin C Based Equations

As earlier stated, Cystatin C is a 13-kDa cysteine proteinase inhibitor protein, produced by degeneration of all nucleated cells at a steady rate, freely filtered by the glomerular membrane with almost complete reabsorption and catabolism in the proximal tubular cells with insignificant excretion in urine in healthy kidneys (Stefanie et al, 2020). The usual mean value was noted as 0.62 – 1.15 mg/L (Murty et al., 2013). In this study, a relatively higher mean value of 1.21±0.07 (1.14 – 1.28 mg/L) was recorded in these apparently healthy subjects. It is a biomarker of CKD (Benoit et al., 2020). Various Cystatin C based eGFR equations have been used in assessment of GFR. Three serum Cystatin C based eGFR equations were used in this study. (a) The Simple Cystatin C formula (National kidney foundation 2002) as noted earlier was expressed as: eGFR = 100/serum Cystatin C (mg/L) = eGFR in ml/min/1.73m^2.

(b) NKF Cystatin C alone equation (Bevc et al, 2012) expressed as eGFR = 70.69 x (cystC) $^{-0.931}$ mL/min/1.73m^2

(c) NKF CKD-EP! (2021) Combined Creatinine-Cystatin C equation ((Inker *et al*, 2021)

$eGFR_{cr\text{-}cys} = 135 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-0.544} \times \min(S_{cys}/0.8, 1)^{-0.323} \times$

$\max(S_{cys}/0.8, 1)^{-0.778} \times 0.9961^{Age} \times 0.963$ [if female] (an online calculator was used)

5.11 Summary of results of the three Cystatin C based equations;

The regression equations for Cystatin C eGFR formula are;

Simple Cystatin C formula: Y = 0.225x + 94.28 (R = 0.0056, P > 0.05), eGFR increased at the rate of 0.225ml/min/year as seen in fig. 4.28.

NKF Cystatin C alone equation (2012): Y = 0.115x + 67.69 (R=0.045, P > 0.05), eGFR increased at the rate of 0.115ml/min/year as seen in fig. 4.29.

NKF CKD-EP! (2021) eGFRcr-cys equation: Y = - 0.134x + 85.67 (R=0.076, P > 0.05). The gradient is -0.134, eGFR decreased by 0.134ml/min/year as seen in fig. 4.31.

Among the above three equations, NKFCKD-EP!cr- cystc equation was considered better than the previous two because they over estimated eGFR and which was the reason for development of NKFCKD-EP! comb cr-cysc equation (Stefanie et al, 2020 and Inker et al 2021). Serum Cystatin C levels are generally known to be stable in healthy persons but increases with substantial decline in GFR (Stefanie et al, 2020) as noted in this study. In this study, it was therefore not surprising that annual decline of kidney function was not detected or not significant.

5.12 Human Urinary Alpha-1-microglobulin (HU α -1m)

This is a low molecular weight glycoprotein, mostly synthesized by the liver, exists free in plasma or bound to IgA and albumin (Moresco, 2013). The Free form (99.9%) is filtered by glomeruli and reabsorbed by proximal tubule except in renal tubular damage when increased quantities appear in urine (Kang, 2015). There was increased urinary quantity of this molecule in association with male gender and increased Age among apparently healthy individuals (Zhang *et al*. 2017). The normal urine value range is 20 – 42 mg/L but elevated values in CKD are associated with decreased survival (Oliva-Damaso *et al*, 2023). In this study the mean urine value among apparently healthy subjects was 0.882 ± 0.00564 ng / ml (or 882 ± 5.64 mg/L) as shown in table 4.7 which revealed higher value than the reference range which is a feature of renal tubular damage. There was significant Age determined decline in urine concentration of human alpha-1-microglobulin among these Subjects ($P < 0.01$) in fig. 4.32. This showed that the urine microglobulin was greater in the younger population but decreased with age. This was therefore peculiar to this study and may be race related or that there was persisting renal tubular damage in most of the Subjects. When compared with MDRD eGFR regression, there was no significant relationship with Hua-1m ($P > 0.05$) as shown in fig. 4.35. There was no similar report in our environment.

5.13 Urinary Monocyte Chemoattractant Protein-1 (Urine MCP-1)

It is a chemokine or cytokine that serves as a mediator of innate immunity and tissue inflammation especially in the kidneys (Liu *et a*l, 2024). It can predict early decline in GFR in microalbuminuria, Type1 and Type 2 DM nephropathy because the urinary concentration increases in these conditions (Gudeta *et al*, 2015). Normal range of urine MCP-1 is 0 – 800mg /L but in this study there was a very high mean urine value of 2.33 ± 0.05 ng. / ml (or 2330 ± 50

mg/L) shown in table 4.7. Significant age dependent annual increase in urinary MCP-1 (at the rate of 0.012 ng./ml/yr) was observed in the individuals ($P < 0.001$) as shown in fig. 4.33. This was associated with significant but negative age dependent decrease in eGFR. Comparison of regression of MDRD eGFR with Urine MCP-1 showed that increase in Urine MCP-1 was associated with decline in MDRD eGFR ($P < 0.05$). The eGFR declined at the rate of 5.24 ml/min/yr. These observed features of urine MCP-1 may suggest that there was always on-going inflammation in the kidneys of these apparently healthy participants which accounted for the age dependent decline in eGFR. This finding may be the first in our population as none was found in literature search.

5.14 Urine Albumin Excretion (Microalbuminuria)

Minimal albumin can normally be filtered by the glomeruli membrane and reabsorbed by the proximal tubular cells but increase quantities are filtered if progressive glomerular membrane podocyte effacement occurs (Murton *et al*., 2021). Microalbuminuria occurs if urine albumin: creatinine ratio (UACR) is > 3 to 30mg/mmol or > 30mg/g to 299mg/g, (Levy et al., 2003). UACR was usually assessed in diabetic CKD patients in order to stage the severity of CKD but has become important measurement in studies of all CKD (Murton *et al*., 2021). In this study urine albumin excretion and UACR were assessed in the participants with mean values of 10.92 ± 0.37 and 81.12 ± 3.58 respectively (shown in table 15). The UACR increased significantly across all ages of all the subjects at the rate of 1.4457mg/g/year ($P < 0.001$) as shown in fig 4.36. The proportion of subjects who had UACR ≥ 30mg/g was 90.5% (219/242). In this study, the annual increase in urine albumin excretion for all the subjects was significant (0.056 mg / dl/yr, $P < 0.05$ in fig 44). It was more significant in the males (0.079 mg/dl/yr, fig 45) than in the females (0.042 mg/dl/yr, $P > 0.05$ in fig 46) as shown in ($P < 0.05$) as was previously reported in

American blacks (David *et al*, 2013). The MDRD eGFR, mCrCl, UCR and Na+/K+ correlated significantly negatively with UACR ratio and Urine Albumin excretion ($P < 0.01$) as shown in Correlation table. This showed that UACR was significantly responsible for the decline in eGFR among these subjects and may have pre-dated decline in GFR. The urine albumin excretion and UACR correlate positively significantly with SBP, DBP and MAP ($P < 0.01$). There is relationship between microalbuminuria (or low grade albuminuria, 10 – 30 mg / g), hypertension and cardiovascular risk (de Souza *et al*., 2022). Albuminuria is associated with cardiovascular mortality among patients with diabetes, hypertension, chronic kidney disease, or heart failure and adults with few cardiovascular risk factors because it reflects widespread endothelial dysfunction (Claudel and Verma, 2025). Moderate sodium restriction diets reduce urinary albumin excretion and decrease the level of blood pressure, especially for patients with macro-albuminuria. Thus, it is necessary to strengthen the intervention and health education as well as to provide individualized dietary advice (Chen *et al*., 2022). In a meta-analysis, lower creatinine based eGFR, lower combined Serum Cr – Cyst C based eGFR, and high UACR were each associated with increased rates of adverse outcomes, including adverse kidney outcomes, cardiovascular diseases, and hospitalizations (Grams *et al*, 2023). The molecular mechanism for albuminuria in kidney disease has emerged from large scale morphometric and ultra-structural studies have pointed at abnormalities of slit diaphragm, foot process and glomerular basement membrane as causes of microalbuminuria and frank albuminuria (Butt *et al*, 2020). Evidence from the hypertensive Ren-2 transgenic rats TGR (mREN2)27 have demonstrated the integral functional involvements of excessive angiotensin-II activation of the AT1 receptor (AT1R) that leads to severe oxidative stress, inflammation, endothelial dysfunction manifesting as hypertension and albuminuria (Whaley-Connell *et al*, 2006).

5.15 Effects of Oxidative Stress and Total Antioxidant Capacity on Age and GFR

Oxidative stress is an imbalance between the body's generated reactive oxygen species (ROS) from the mitochondria during oxidative metabolism and the ability to inhibit their harmful effects by both endogenous and exogenous antioxidants (Andrea, 2024). ROS are natural by-products of cellular metabolism during energy production in the mitochondria but excessive accumulation in plasma and tissues can cause significant damage to cell membrane, DNA and tissues (Jarrett and Boulton, 2012). Examples of ROS are; O^{-}_{2} (superoxide radical), OH^{-} (hydroxyl radical) and H_2O_2 (hydrogen peroxide) which cause cell membrane lipid peroxidation to generate mostly MDA (malondialdehyde) in addition to other aldehyde electrophiles (Pryor and Stanley, 1975). Malondialdehyde (MDA) attack cell DNA to induce mutagenicity (Marnett 1999), neuro-degeneration (Patel and Chu, 2011), cardiovascular and diabetic (Andrea 2024), and chronic kidney disease (Mariana et al, 2020). MDA is the major Oxidative Stress Biomarker assayed in plasma and serum (Nair, 2008). Various endogenous antioxidant enzymes protect the cells from oxidative damage. These are superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), ceruloplasmin, and proteins such as metalothionins (Kusano and Ferrari, 2001). Instead of separate antioxidant enzyme assays, Miller et al. (1993) developed a test to measure the Total Antioxidant Capacity (Trolox equivalent antioxidant capacity" (TEAC) method) which was later modified by Erel (2004) to minimize the effect of uric acid. This study examined the relationship between age, Oxidative stress, Total Antioxidant capacity and GFR as summarized in table 4.9. It also examined the effects of Oxidative stress and Total Antioxidant capacity on GFR. In this study there was no significant age dependent increase in serum MDA ($P > 0.05$) in fig. 4.40. This probably suggested that there was systemic check on MDA generation. It was reported that there was age-

related build-up of ROS and MDA which overtime lead to cellular dysfunctions, tissue degeneration and the progressive decline in physiological functions associated with aging (Andrea 2024) and CKD (Mariana et al, 2020). Age associated increase in MDA occurs in both health, ill health and identified in serum and urine (Dimitrios et al, 2023). In this study there was significant increase TAC as age of the population increased ($P < 0.05$) in fig 4.41. This finding agrees with the report of Limberaki *et al* (2012) which showed lower serum TAC in younger people compared to middle aged and elderly. The relevance may suggest greater physiological response to oxidative stress or greater availability of exogenous antioxidant diet and supplements due to improved economy or awareness in the older age groups. There was association between the oxidative balance score (OBS) and eGFR according to National Health and Nutrition Survey (2007–2018 NHANES. Mingda *et al,* 2024). A higher OBS suggests greater exposures to antioxidants. Habitual intake of dietary antioxidants is associated with a lower risk of CKD (Parivash *et al*, 2020). In this study there was significant decrease in GFR associated with increase in MDA ($P < 0.05$) as shown in fig 4.42. There was also significant increase in GFR associated with TAC as shown by regression of MDRD eGFR and mCrCl against TAC respectively ($P < 0.01$) in figures 4.43 and 4.44.

5.16 Comparison of Renal Parameters between Hypertensive and Non-Hypertensive Subjects

The parameters compared as T-tests between hypertensive and non-hypertensive subjects were summarized in table 4.10. In this study, 21.1% of the subjects were hypertensive which differs from 2017 national average of 38.1% (Odili et al.,2020). It also differs from 28.9% Nigeria prevalence value of HTN among adults 30-79 years as stated by WHO (2023). The MAP of hypertensive subjects was significantly higher ($P < 0.001$) as shown if fig 4.48. The MDRD

eGFR revealed most significant decrease among hypertensive than non-hypertensives ($P < 0.05$) as shown in fig 4.49. The urine ALB and UrALB/CR (mg/g) were significantly increased in hypertensive than non-hypertensive ($P < 0.05$ and $P < 0.01$ respectively) as shown in fig 4.53 and 61respectively. This showed that albuminuria and hypertension were important factors for decline in GFR as demonstrated in Ren -2 transgenic rats (Whaley-Connell et al., 2006). There was significant reduction in urine creatinine excretion (UCR) among hypertensive than non-hypertensive subjects ($P < 0.001$) as shown in fig 4.55. Hypertension impaired urine UCR excretion. The eGFR for Cockcroft-Gault, NKFCKD-EP! and mCrCl were also reduced in hypertensive than non-hypertensive subjects but not significant ($P > 0.05$) as shown in fig 4.50, 4.51 and 4.52 respectively. The urine Na+/K+ was greater in non-hypertensive subjects but not statistically significant ($P > 0.05$). This may be responsible for their lower blood pressure as age negatively but not significantly correlated with MAP.

5.17 Age above 30yrs and decline in GFR

Although the GFR measurements were not the Gold-standard Inulin based in which GFR decline of 1ml/min/yr was found but it was obvious that rate of decline in GFR for subjects $\geq$ 30yrs was significantly greater than for subjects < 30 years and for all the participants (table 4.17). This was also associated with significant progressive increase in UACR ratio ($P \leq 0.001$) and MAP ($\leq$ 0.001) with age. This increase in UACR was greater in males and hypertensive than females and normotensive. Ninety percent (90.5%) of the subjects had UACr > 30mg/g which is the CKD definition level by KDIGO (Murton *et al.*, 2021). Diabetes Mellitus, Hypertension and Albuminuria are three key factors that cause decline in GFR and progression to CKD/ESRD (Murton *et al.,* 2021). In this study age was also an important contributory factor. The random

blood glucose (mean RBG 98.27±1.23) values of the subjects increased significantly ($P < 0.01$) across the age of participants for males and females as shown in fig 4.107 and 4.108 respectively.

5.18 Urine Sodium Potassium Ratio

A low urinary Na+/K+ ratio may relate with lower CKD development risk in adults with preserved kidney function (Young *et al.,* 2022). In this study, urine Na+/K+ declined significantly with increase in age ($P < 0.01$) and correlated negatively significantly with mCrCl ($p < 0.05$) and MDRD eGFR ($P < 0.01$). This decline in urine Na+/K+ was more significant in the females than the males ($P < 0.01$). Possibly, females excrete more salt in their urine which may be responsible for their lower mean arterial blood pressure than the males.

5.19 Comparison of GFR between Male and Female subjects

The Student's T-test comparison for male and female GFR parameters (in tables 4.18 and figs. 4.105 to 4.108) showed that males have significantly higher mean GFR than females which was also supported by MDRD Anova graph (fig 4.23). These significant differences were noted in mean GFR values of four formulas as follows; mCrCl ($P < 0.05$), Cockcroft-Gault eGFR ($P < 0.001$), NKF CKD-EP!Cr eGFR 2021 ($P < 0.001$) and MDRD eGFR ($P < 0.001$). These formulas depend on body weight, BMI and SCr in addition to gender factor which are significantly higher in males (table 4.18). Further consideration implies that males have greater muscular mass than females due to higher plasma testosterone (Deborah et al., 2007). In this study males had significantly greater; BMI ($P < 0.01$), bodyweight ($P < 0.05$) and SCr ($P < 0.01$). Examination of regression graphs (in figs 4.109 to 4.124 and table 4.19) showed that ADARD in GFR occurred in both gender but was relatively greater in males than females for most of GFR formulas (mCrCl, CG eGFR, and MDRD eGFR). This finding could not be explained in this

study. Although there were consistent symmetrical and significant mean GFR differences in favor of males across all age-groups as shown by Anova ($P < 0.001$), the ADARD in GFR was less in females than males which means that males lost their GFR at a faster rate than females. This finding is peculiar in this study and first of its kind in Nigeria at least. The NKF CKD-EP!Cr 2021 eGFR equation also showed significant decline in GFR in males than females ($P < 0.05$) which was expected. The more significant increases in BMI and MAP with age in males will favor stepper decline in GFR. The BMI correlated positively significantly with Age and MAP but negatively significantly with MDRD eGFR. It has been reported that at baseline, mean measured GFR was significantly higher among men than women: 98.0 vs 90.0 mL/min/1.73 m^2. Men had a 25% steeper rate of annual decline in mean measured GFR than women, however: 1.20 vs 0.96 mL/min/1.73 m2 per year (Melsom *et al*., 2022).

5.20 Suitability of the eGFR formulae for clinical purposes

The suitability of GFR formula for clinical purposes was considered with the percentage of CKD (GFR < 60ml/min/1.73m ^{2}) detected by the seven GFR methods and UALB/CR (or UACR > 30mg/g) from 243 apparently healthy Subjects (in table 4.13 above). The MDRD eGFR showed zero detection for CKD in apparently healthy population while UACR showed 90.5% indication of CKD in same population. The NKF Cystatin C alone equation showed the highest detection rate for CKD detection in this population (29.9%) followed by Combined NKF CKD-EP!Cr-Cyst C 2021 equation (19.54%). The Cockcroft-Gault equation had 15.7% CKD detection rate followed by simple Cyst C equation with 13.8%. The measured CrCl detected 9.91% while the NKFCKD-EP!Cr 2021 equation detected rate was 8.7%. There are differences in sensitivity of the various GFR methods to detect CKD (i.e. GFR < 60ml/min/1.73m^2). Most of the formula likely overestimated CKD in this health stable population. A UACR value above 30mg/g is

considered the starting point for development and progress of CKD and usually combined with the GFR value for adequate interpretation of the clinical stage (Stevens *et al*., 2013). The UACR > 30mg/g set the background for presence and decline in GFR in most of the subjects but there is danger of placing healthy individuals into CKD category when they are not. A combination of UACR and MDRD are appropriate for CKD screening in health stable population before the use of NKFCKD-EP!Cr 2021 equation and others. The UACR correlated significantly negatively with MDRD eGFR ($P < 0.01$) and measured CrCl ($P < 0.05$) in appendix 1. Although measured CrCl is associated with drawbacks, of accurate urine volume collection and unstable creatinine in stored urine efforts should be made to improve the results because it is near real time as gender or racial differences are not incorporated.

5.21 The Age Determined Annual Rate of Decline in GFR (ADARD) in this Study

The regression equations and graphs for different formulas revealed various gradients or ADARDs for age groups < 30 years, 30-70 years and all the subjects 18-70+ (table 4.16). The ADARDs for age < 30 years were not significant but were significant for 30-70 years ($P < 0.05$). The ADARDs were most significant for all the population in the 18-70+ years ($P < 0.01$). The various rates for the formulas in this group are; (a) Cockroft-Gault equation = 0.26 ml/min/1.73m^2/yr ($P < 0.05$), (b) NKF CKD-EP! Cr 2021 eq = 0.25 ml/min/1.73m^2/yr ($P < 0.01$), (c) MDRD eGFR eq = 0.503 ml/min/1.73m^2/yr ($P < 0.01$) and measured CrCl = 2.7 ml/min/1.73m^2/yr ($P < 0.01$). In this study the MDRD eq showed great consistency in symmetry of the graphs by clear demarcation of male against female values was new in this study. It also correlated reasonably with important parameters as expected. This study established that the ADARD in GFR among this group is 0.503 ml/min/1.73m^2/yr which was very significant. The MDRD eq incorporated race factor for blacks, which is not found in Cockroft-Gault eq, NKF

CKD-EP!Cr2021 eq and CrCl. The results of CrCl appear exaggerated due to the known disadvantages earlier mentioned. The value of 2.68 ml/min/1.73m^2/yr is too much and not close to other values reported above and other countries of America and Europe. The MDRD value for ADARD of 0.503 ml/min/1.73m^2/yr chosen from this research compares favorably with other Age Determined Annual Rate of Decline in GFR reported in other countries as earlier noted. The first systematic review (between 1958 and 2021) that investigated the longitudinal decline in kidney function with age in healthy individuals involved data of 12 reports from 8 countries, the normal decline in GFR was between 0.37 and 1.07 mL/min/1.73 m^2/year in healthy adults (Guppy *et al.,* 2024).

5.22 Conclusions

The outcome of this research has proven that there was significant age-determined decline in glomerular filtration rate (assessed by both measured creatinine clearance and estimated glomerular filtration formulas. The mean glomerular filtration rate and the rate of decline varied with the different formulas. The rate of decline in glomerular filtration rate was mostly dependent on proteinuria. In assessment of glomerular filtration rate the choice of MDRD or measured creatinine clearance in addition to UACR should be used.

5.23 Findings

The findings from this study exceeded the 10 set objectives listed in **chapter 1.5 page 7** and are listed as follows:

1. Mean GFR for various formulae were; (a) mCrCL 124.86 ± 5.09 (b) CG 85.22 ± 1.69 (c) NKF CKD-EP!Cr 2021, 82.95 ± 1.27 (d) NKF CKD-EP!cyst c 2021, 72.90 ± 3.88 (e) NKF CKD-EP! cr-cyst C 2021, 79.62 ± 2.64 (f) MDRD 93.44 ± 1.01.

2. The ADARD (in ml/min/year) in GFR were significant (P<0.01); (a) mCrCL 3.64 (b) Cockroft-Gault 0.7501, (c) NKF CKD-EP!Cr 2021, 0.4398 (d) MDRD 0.503.
3. Only the NKF CKD-EP!Cr 2021 eGFR formula showed significant ADARD in GFR in females than males ($P < 0.01$).
4. Suggested preferable mGFR or eGFR clinical tools were (a) MDRD and (b) CrCl because their means are closer to normal of 125 ml/min/1.73 m^2 and have the lowest CKD detection in this health stable group of subjects.
5. Proportion of hypertensive is 21.1% (51/191) while mean GFR is significantly lower in hypertensive than normotensive subjects (MDRD, $P < 0.05$).
6. The mUACR in the subjects was 81.12 ± 3.58 (greater than normal value of 30 mg/g). Thus, 90.5% had UACR>30 mg/g while the age determined annual rate of increase was 1.446 mg/g.
7. Hypertensive subjects have significantly higher mUACR than normotensive subjects, 98.14 ± 9.29 vs. 76.6 ± 3.74 ($P < 0.01$).
8. There is significant age determined increase in urine MCP-1 ($P < 0.001$) that is associated with significant ADARD in GFR (using MDRD, $P < 0.05$). There is significant age associated decrease in HU -α -1m ($P < 0.01$) which has no significant effect on GFR.
9. There is significant age associated increase in TAC ($P < 0.05$) which is associated with significant increase in GFR ($P < 0.01$). There is also an insignificant age associated increase in MDA but with insignificant decrease in GFR.
10. There is age determined decline in urine Na^+/K^+ ratio ($P < 0.01$) which is more significant in females than males ($P < 0.01$) and suggests greater salt excretion in females. It correlates significantly negatively with mCrCl ($P < 0.05$) and MDRD ($P < 0.01$).

Other findings include:

(11) There is greater BMI increase with age in both males and females but more in males ($P < 0.001$) than females ($P < 0.01$).

(12) There is significant MAP increase with age in this study ($P < 0.001$) but greater in males ($P < 0.001$) than females ($P < 0.05$).

(13) Serum creatinine decreased significantly with age ($P < 0.01$) but remained generally lower in females than males although the rate of decline was similar in both gender ($P < 0.05$).

(14) There is significant Random Blood Glucose increase with Age ($P < 0.001$) at similar rate in males and females ($P < 0.01$).

(15) There is progressive significant decrease in Urine flow rate and urine Cr, with age ($P < 0.01$).

(16) Measured CrCl show the most rate of decline in GFR both in the population of subjects and subjects > 30years, 2.681 vs 3.689 mls/min/1.73m ^{2}/year, respectively ($P < 0.01$).

(17) MDRD eGFR results are most symmetrical among males and females across all age groups. The decline is significant ($P < 0.01$) with zero CKD detection among this apparently healthy population. The rate of decline in eGFR are similar between males and females ($P < 0.01$) and different between ages above 30yrs and overall population, 0.285 vs 0.503 mls/min/1.73m2/year respectively ($P < 0.01$).

(18) Comparing the regression of MDRD eGFR with Urine MCP-1 show that increase in Urine MCP-1 is associated with decline in MDRD eGFR ($P < 0.05$). Thus increase in Urine MCP-1 is inversely related to GFR.

(19) Increase in serum MDA (Oxidative stress marker) decreases GFR while increase in serum TAC increases GFR as shown by MDRD eGFR and mCrCl ($P < 0.01$).

(20) Male subjects had significantly higher GFR than females as assessed by mCrCl ($P < 0.05$), Cockcroft-Gault eGFR ($P < 0.001$), NKF CKD-EP!Cr 2021eGFR ($P < 0.001$) and MDRD eGFR ($P < 0.001$).

(21) In this study albuminuria as UACR increased significantly across all ages at the rate of 1.4457mg/g/year ($P < 0.001$).

(22) MDRD, CKD-EP!, mCrCl and CG correlated significantly negatively with UACR and Urine Albumin excretion ($P < 0.01$). This showed that UACR was significantly responsible for the decline in GFR.

(23) There is very significant reduction in urine creatinine excretion among Hypertensive than non-Hypertensive subjects ($P < 0.01$).

5.24 Possible Physiological Mechanisms

Age correlated positively (directly) and significantly with RBG ($P < 0.001$), similarly for BMI ($P < 0.001$) and MAP ($P < 0.001$). This means that as these individuals progressed in Age there will gradual increase in bodyweight which is associated with progressive loss of insulin sensitivity and development of hypertension. This finding depended specifically on fat mass, muscle mass and gender because similar changes were note in males and females. Increasing Age and MAP were associated with increase in UACR ($P < 0.01$) and decrease in UCR ($P < 0.01$). Obviously deterioration in glomerular filtration barrier led to progressive increase in UACR (90.5%) predated increase in MAP but was made worse by Hypertension (21.074%). Increase in Age significantly decreased serum and urine creatinine ($P < 0.01$) which are important in preservation

of kidney function in apparently healthy population. The focus of this research was to find out if Age determined changes in GFR among Normotensive and Hypertensive Subjects. Significant Increase in rates of decline in GFR were associated with different formulae; CG (0.7501ml/min/yr, $P < 0.05$), NKF CKD-EP!cr (0.4398ml/min/yr, $P < 0.01$), MDRD (0.503ml/min/yr, $P < 0.01$), mCrCL (3.639ml/min/yr, $P < 0.01$) . Age, Renal arteriosclerosis and Proteinuria were the key culprits for these changes. The components of albuminuria hypertension were important factors for decline in GFR as demonstrated in Ren -2 transgenic rats because of increased activation of renin angiotensin II aldosterone activity (Whaley-Connell et al., 2006).

5.25 Contributions to Knowledge

(1) This study established the age determined annual rate of decline (ADARD) in GFR in our indigenous population.

(2) This study identified MDRD equation as the best eGFR for health stable black population.

(3) It also established that the rate of decline in GFR in our local population increased greatly after the age of 30 years.

(4) Age, Albuminuria/Proteinuria (as UACR) and HTN are very important in progressive ADARD in GFR among the subjects under study.

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Appendix I; Correlation matrix table 1.

	AGE.yr	RBG.%	UrSGr	Urine.pH	Ucr mg/dl.	Urine.CRg/dl.(Ucr)	Ur.ALB.mg/dl	Ur.ALB/UCR	SCr.mg/dl.	CG.ml/min	EP!GCr.ml/min.	MDRD.ml/min..	UrNa/K	Ucr mg/dl.	Ur.flow..ml/min.	SCr.mg/dl
AGE.yrs	1															
RBG.%	.240**	1														
UrSGr	0.014	-0.061	1													
Urine.pH	-0.054	-0.015	.298**	1												
Ur.Cr mg/dl.	-.661**	-.166**	-0.029	0.005	1											
Ur.CR.g/dl.(UCR)	-.651**	-0.122	-0.028	-0.002	.984**	1										
Ur.ALB.mg/dl	.151*	0.047	0.044	0.014	-0.115	-0.115	1									
Ur.ALB/UCr	.399**	0.078	0.036	0.042	-.597**	-.605**	.756**	1								
SCr.mg/dl.	-.212**	-.187**	0.046	0.024	0.081	0.084	0.131*	.0.058	1							
CG.ml/min	-.151*	-0.001	0.067	0.037	0.099	0.081	0.081	-0.029	-.502**	1						
EP!Cr.ml/min.	-.192**	-0.037	-0.01	0.095	.162*	.146*	0.107	-0.045	-.744**	.699**	1					
MDRD.ml/min..	-.493**	-.281**	0.052	.154*	.355**	.337**	-0.074	-.204**	.165*	.351**	.480**	1				
UrNa/K.ratio	-.227**	-0.04	0.021	-0.062	.230**	.220**	0.109	-0.048	0.008	0.036	0.11	.183**	1			
UCR..mg/dl.	-.653**	-.155*	-0.03	-0.003	.983**	.967**	-0.123	-.587**	0.069	0.099	.164*	.338**	.235**	1		
Ur.flow..ml/min.	-.241**	-.166**	-0.024	.130*	0.017	0.041	0.02	0.049	.325**	-.133*	-.170**	.180**	0.014	-0.03	1	
SCr.mg/dl.	-.212**	-.187**	0.046	0.024	0.081	0.084	0.131*	0.058	1.000**	-.502**	-.744**	.165*	0.008	0.069	.325**	1
mCrCl.ml/min	-.520**	-.152*	-0.063	.128*	.607**	.611**	0.026	-.291*	-0.057	.150*	.245**	.281**	.156*	.593**	.622**	-0.057

** Correlation is significant at the 0.01 level (2-tailed) * Correlation is significant at the 0.05 level (2-tailed), N = 242

Age correlated positively significantly with; **RBG, *UrAlb, **UrAlb/UCR

Age correlated negatively significantly with; **UrCr, **UCR, **SCr, Ur*CG, **EP!Cr, **MDRD, **mCrCl, **Urflow, **UrNa/K,

UCR correlated positively significantly with; **EP!Cr, **MDRD, **mCrCl, **Na/K ratio and negatively with **UAlb/UCR

UrAlb/CR or UACR correlated negatively significantly with **UCR, **UrAlb, **MDRD, *mCrCl

SCr correlated negatively significantly with **RBG, **CG, **EP!Cr, *MDRD, *mCrCl and positively with *UrAlb

CG correlated negatively significantly with **Age, **SCr, **Urine flow but not significantly with UrAlb/UCR and positively with **EP!Cr, **MDRD, *mCrCl

CKD-EP!Cr correlated negatively significantly with *UAlb, **SCr, **Urine Flow and positively significantly with *UCr, **CG, **MDRD, mCrCl

MDRD correlated negatively significantly with **RBG, **UrAlb/CR, **UrNa/K and positively significantly with **UCR, *SCr, **CG,

**EP!Cr, **Urine flow, **mCrCl

UrNa+/K+ correlated positively significantly with **MDRD, **UCR, **mCrCl

mCrCl correlated negatively significantly with *RBG, **UrAlb/CR, SCr and positively significantly with *UrNa/K, **Urine flow, **UCr,

*CG, **EP!Cr, **MDRD

Appendix II; Correlation Matrix for the Parameters with BMI and MAP

	Age	HT	Wt	BMI	PR	SBP	DBP	MAP	RBG	UrSG	UrpH	UCr	SCr	mCrCl	UrAlb	UrAlB/CR	CG	EP!Cr	MDRD
Age yrs	1																		
Wt kg	0.265**	1																	
HT m	-0.238**	.357**	1																
BMI	0.387**	.863**	-0.099	1															
PR	-0.008	-0.074	-.194**	0.004	1														
SBP	.314**	.253**	0.071	.217**	-0.018	1													
DBP	.392**	.265**	-0.118	.332**	0.112	.794**	1												
MAP	.382**	.279**	-0.031	.300**	0.061	.929**	.955**	1											
RBG%	.240**	0.072	-.141*	.165*	.213**	0.021	-0.018	-0.002	1										
UrSGr	-0.101	0.074	0.12	0.009	-0.003	-.143*	-0.106	-0.121	0.017	1									
Urine pH	-0.068	-0.059	0.116	-0.101	-0.06	0.026	-0.032	-0.013	0.008	-.370**	1								
UCr	-0.03	0.002	-0.117	0.053	-0.057	-0.02	0.019	0.019	-0.023	0.076	-0.036	1							
SCr	-.212**	0.049	.333**	-0.111	-0.067	0.019	-0.024	-0.007	-.187**	0.044	0.011	-.157*	1						
mCrCl	.225**	0.083	-.140*	.145*	-0.064	0.084	.140*	0.122	-0.008	0.028	0.079	.314**	-.347**	1					
UrAlB	.151*	0.076	0.016	0.069	0.04	0.122	.185**	.170**	0.047	-0.092	-0.006	-0.042	-.131*	0.107	1				
UrALB/UCR	0.12	0.065	0.108	0.012	0.056	.161*	.162*	.164*	-0.022	-0.097	0.02	-.509**	-0.03	-0.099	.615**	1			
CG	-.151*	.593**	.312**	.462**	-0.084	0.06	-0.001	0.031	-0.001	0.118	0.02	0.09	-.502**	.161*	0.081	0.022	.161*	1	
EP!Cr	-.192**	-0.042	0.104	-0.097	-0.051	-0.023	-0.121	-0.079	-0.037	0.026	0.106	0.097	-.744**	.181**	0.107	0.033	.181**	.699**	1
MDRD	-.493**	-0.012	.609**	-.314**	-.237**	0.035	-.215**	-0.11	-.281**	0.107	.150*	-0.005	.165*	-.176**	-0.074	-0.011	-.176**	.351**	.480**
N	242	242	242	242	242	242	242	242	241	240	240	242	242	242	242	242	242	242	242

**Correlation is significant at P < 0.01 level (2-tailed). *Correlation is significant at P < 0.05 level (2-tailed)

AGE correlated negatively significantly with **SCr and positively significantly with **BMI, **SBP, **DBP, **MAP, **RBG

BMI correlated negatively significantly with **MDRD, EP!Cr and positively significantly with **SBP, **DBP, **MAP, **RBG, **mCrCl, **CG

MAP correlated negatively but not significantly with EP!Cr, MDRD and positively significantly with **BMI, **SBP, **DBP, **UrAlb, *UrAlb/CR

SBP correlated positively significantly with **Age, **BMI, **DBP, **MAP, *UrSGr, *UrAlb/CR

DBP correlated negatively with **MDRD, EP!Cr and positively significantly with **Age, **BMI, **SBP, **MAP, *mCrCl, **UrAlb, *UrAlb/CR

MDRD correlated negatively significantly with **Age, **BMI, **PR, **DBP

EP!Cr correlated negatively significantly with **Age, **SCr but not significantly with BMI, PR, MAP, SBP, DBP

CG (Cockroft-Gault eGFR) correlated significantly positively with BMI