Online Available at: https://www.sciplatform.com/index.php/wjb/article/view/1099

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746

WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY

Research Manuscript www.sciplatform.com

Comparative assessment of urea, creatinine, and blood urea nitrogen in male and female patients with different age groups of chronic kidney disease in Shaheed Benazir Abad, Sindh

[a] Haiqa Sami, [b] Imran Tarique, [a] Sehrish Arain, [a] Rida Mughal, [a] Iqra Majeed, [a] Rubia Saba, [a] Jaweria Rajput, [a] Manahil Altaf, [a] Lal Bux*

[a] Department of Genetics, Shaheed Benazir Bhutto University, Shaheed Benazir Abad 67450, Pakistan,
[b] Healthcare Biotechnology Department, Atta-ur-Rahman School of Applied Bio-Sciences (ASAB), National University of Sciences and Technology, Pakistan.

Authors' Contribution

Haiqa, S., T. Imran analyzed data, **A. Sehrish** performed GraphPad software, **M. Rida** collected blood samples, **M. Iqra** analyzed urea concentration, **S. Rubia** measured creatinine concentration, **R. Jaweria** confirmed blood urea nitrogen concentration, **A. Manahil** recorded the data, **L. Bux** analyzed data, and revised the manuscript.

*Corresponding Author's Email Address: lalbux@sbbusba.edu.pk

Review Process: Peer review

ABSTRACT

Chronic kidney disease (CKD) is a heterogeneous disorder that affects the structure and function of the kidney. The primary biochemical markers for diagnosis and evaluating stages of CKD include urea (Ur), blood nitrogen urea (BUN), and creatinine (Cr). In this study, we investigate three biochemical parameters such as Ur, BUN, and Cr levels in CKD patients' gender-vice with different age groups. Blood samples (n = 300) of males and females were collected from the Biochemistry Lab, Peoples University of Medical and Health Science, Shaheed Benazirabad during the 2023 year. Blood samples (5ml) were collected from the individuals through venipuncture and centrifuged for further analysis. Reagent 1 (2-Oxoglutarate ADP Urease GLDH and NADH) and Reagent 2 (tris), Reagent 1 (Picric acid) and Reagent 2 (Sodium hydroxide) were used for Ur and Cr analysis. Reagent A1 (Sodium salicylate, sodium nitroprusside, phosphate buffer) Reagent A2 (Urease) reagent B (Sodium hypochlorite, sodium hydroxide) reagent S (Glucose/Urea/Creatinine standard) used for BUN. Four age groups were studied in our research for both genders. The highest positive coefficient correlation was shown by Ur and BUN with 'r'=0.99 in this study. The statistical analysis showed a significantly higher average value of Ur, Cr, and BUN in males comparatively than in females with the age group of 61-80 years old. Whereas in the young age group (1-20 years old), female showed a significantly higher value than in the male sex group. Our study revealed a significant variation of Ur, Cr and BUN in male and female with different age groups, as male possessed higher muscle mass with increased dietary protein intakes than females. These biochemical markers aid in the proper assessment and are used for several routine analyses of CKD with different age groups of genders.

Keywords: Chronic kidney disease, urea, creatinine, blood urea nitrogen, age, sex.

INTRODUCTION: Chronic kidney disease (CKD) has been identified as a public health issue worldwide and more than 10 million people have suffered (Kovesdy, 2022). It was estimated that CKD affects 13.4% (843.6 million people) of the world's population, whereas 10% of the adult population already suffering from this issue worldwide (Kalantar-Zadeh *et al.*, 2021). According to assessments of blood creatinine levels in various populations, the prevalence of CKD is 16.8% in the USA (Jones *et al.*, 2002) whereas the estimated prevalence of CKD ranges between 7.0-34.3% was reported in Asia (Liyanage et al., 2022), while recently in Pakistan the prevalence of CKD was 12.5% to 31.2% reported (Ahmed *et al.*, 2022). Due to the lack of Pakistan's central renal registry system, independent studies showed that 15% to 20% of people are affected by CKD (Rehman and Khan, 2020). Biochemical markers for renal activity including serum urea and creatinine levels are used to assess CKD status and renal status in susceptible diabetic and hypertensive individuals (Pundir *et al.*, 2019). The creatinine and urea levels were increased with the progress in kidney damage; their measurement in serum aids in determining the glomerular filtration rate, which is then followed by renal function (Pfaffe *et al.*, 2011). Since 2% of the body's creatine is believed to be turned into creatinine every day, the amount of creatinine produced daily is pretty constant (male: 20 to 25 mg/kg/day; female: 15 to 20 mg/kg/day) (Schafer *et al.*, 2014).

According to Peng *et al.* (2013) and Venkatapathy *et al.* (2014), urea (Ur) is a byproduct of dietary protein that the kidneys also filter into urine. It is commonly used as a substitute indicator of the severity of CKD and the sufficiency of dialysis. The liver's enzymes change ammonia into Ur. Healthy kidneys eliminate urea nitrogen from the blood, but when renal failure occurs, the amount of Ur in the blood rises (Junejo *et al.*, 2011). For a very long time, urea has been thought of as a relatively safe and inert substance. Previous investigations showed that urea has direct and indirect uraemic toxins (Massy *et al.*, 2016). In the kidneys, the amino acids arginine, glycine, and methionine are transaminated to produce creatinine (Cr). This creatine is phosphorylated into phosphocreatine in the brain and skeletal muscle as it circulates throughout the body. The majority of creatinine is produced in the muscles. As a result, the plasma Cr concentration was influenced by the patient's muscle mass (Finney *et al.*, 2000). Cr is produced by muscles and subsequently removed by the kidneys together with other wastes and the balance between renal excretion and creatinine production controls the level of creatinine in serum (Salazar, 2014). Protein metabolism in the urea cycle results in the production of blood urea nitrogen in the liver. Approximately 85% of the urea is eliminated by the kidneys, with the remaining 5% being passed by the digestive system. Most clinical parameters used to calculate renal function depend on the level of urea in the serum. It is helpful in the differential diagnosis of acute renal failure and conditions that are pre-renal and have an elevated blood urea nitrogen-creatinine ratio (Rosner & Bolton, 2006). To distinguish between pre-renal and renal reasons when the BUN is elevated, one might look at the ratio of BUN to creatinine. The pre-renal disease has a ratio of about 20:1, whereas intrinsic renal disease has a ratio of about 10:1. According to Gowda et al. (2010), upper GI haemorrhage is associated with a BUN-to-creatinine ratio that was abnormally high often larger than 30:1 ratio.

OBJECTIVES: In this study, the level of Ur, Cr, and BUN in patients of different ages and sexes were assessed, who were affected by CKD and calculated the correlation coefficient between urea, creatinine, and blood urea nitrogen in the CKD patients.

MATERIAL AND METHODS: This study was conducted in the Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University Shaheed Benazir Abad (SBBUSBA) with the collaboration of Biochemistry Lab, Peoples University of Medical and Health Sciences (PUMHS), Shaheed Benazir Abad in 2023. Sample data was collected from both normal and CKD patients through lab reports. Samples were collected from the patients by intra venous puncture for urea (Ur; in mg/dl), blood urea nitrogen (BUN; in mg/dl), and creatinine (Cr; in mg/dl). Standard reference ranges were used for evaluating Ur, Cr., and BUN (Walker *et al.*, 1990). The study was carried out with the permission of the Molecular Biology and Genetics Department, Shaheed Benazir Bhutto University, Shaheed Benazir Abad.

Blood sample analysis for urea: Five milliliters of blood samples were collected from the individuals through venipuncture and centrifuged for further analysis of Urea by following the protocol of the biochemistry analyzer (Micro lab 300-Netherland) in the laboratory. Reagent R1 (2-Oxoglutrate ADP (Adenosine diphosphate) Urease GLDH (glutamate dehydrogenase) and NADH (Nicotinamide adenine dinucleotide)) and R2 (Tris) were used for urea analysis. A total of 800 µl R1 reagent in the test tube with 10 µL sample and 200 µL Reagent 2 (tris) were used and incubated at 0-5 min. Finally, absorbance at 340 nm was measured and with the control, distilled water instead of a sample with formula.

$$Urea\ (mg/dL) = \text{Average absorbance of sample} * \text{con. Std/cal[mg/dL]} / Average\ standard/calibration$$

Blood sample analysis for creatinine: Blood samples (5mL) were collected from the individuals through venipuncture and

 Digital Object Identifier: https://doi.org/10.33865/wjb.008.02.1099

centrifuged for further analysis of Creatinine by following the protocol of the biochemistry analyzer (Micro lab 300) in the laboratory. The sample (50 µL) or standard (50 µL) or distilled water for blank in test tube pour 1000 µl Reagent R1 and mix it. Incubate for 0 - 5 min then add 250 µL Reagent R2. After 60 sec., read absorbance A1; after another 120 sec., read absorbance A2.

Creatinine [mg/Dl] = absorbance of sample * con. Std/cal[mg/dL] * 50/Average standard/calibration

Blood sample analysis for blood urea nitrogen: Blood urea nitrogen was estimated by Berthelot's method (Berthelot, 1859). Urease enzyme is applied to convert urea to NH3 and CO2. The ammonia is then converted into the blue compound carboxy endo phenol. This blue chemical's intensity was proportional to the amount of urea in the blood.

Statistical analysis: GraphPad Prism software version 9.0 was used to analyze the data. For the comparison of different age groups, a one-way analysis of variance (ANOVA) was carried out. Pearson's correlation coefficient analysis was conducted among urea, creatinine, and blood urea nitrogen. Data was presented as mean and standard error mean with a graphical presentation using GraphPad Prism.

RESULTS: In this study, blood samples from a total of 300 individuals (aged between 1 to 80 years) 58.30% males and 41.60% females were collected and used in the Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazir Abad, Pakistan. Samples were further divided into age groups such as 1-20, 21-40, 41-60, and 61-80. The basic statistics (mean ± SEM) of urea, creatinine and blood urea nitrogen for males and females were shown in tables 1 and 2, respectively. The mean values were 26.92, 49.54, 48.45, and 72.27 in the 1 to 20, 21 to 40, 41 to 60, and 61 to 80 age groups, respectively, for urea in males (table 1). However, in females the mean values were 35.13, 47.88, 44.23, and 68.90 in 1 to 20, 21 to 40, 41 to 60, and 61 to 80 age groups, respectively (table 2). The average of all studied parameters was extremely elevated in both males and females and showed the progression of CKD in aged persons. Comparison and variations in urea for different age groups of males and females were shown in figure 1 and 3.

Sr. No		Reference Range mg/dl	Age groups			
			1 to 20 (mean ± SD)	21 to 40 (mean ± SD)	41 to 60 (mean ± SD)	61 to 80 (mean ± SD)
1	Ur	10 to 50	26.92 ± 5.81	49.54 ± 39.79	48.45 ± 28.37	72.27 ± 53.51
2	Cr	0.17 to 1.20	0.73 ± 0.21	1.95 ± 2.40	1.47 ± 0.93	2.07 ± 1.47
3	BUN	6 to 20	12.58 ± 2.72	23.15 ± 18.59	22.64 ± 13.25	33.76 ± 25.02

Table 1: Descriptive statistics values for Urea, Creatinine, and Blood Urea Nitrogen in males. Ur, Cr, and BUN represent Urea, Creatinine, and Blood Urea Nitrogen, respectively

Sr. No		Reference Range mg/dl	Age groups			
			1 to 20 (mean ± SD)	21 to 40 (mean ± SD)	41 to 60 (mean ± SD)	61 to 80 (mean ± SD)
1	Ur	10 to 50	35.13 ± 20.78	47.88 ± 44.31	44.23 ± 26.63	68.90 ± 41.56
2	Cr	0.17 to 0.90	0.99 ± 0.87	1.27 ± 1.23	1.16 ± 0.52	2.18 ± 1.72
3	BUN	6 to 20	16.36 ± 9.66	22.38 ± 20.71	20.36 ± 12.22	32.20 ± 19.42

Table 2: Descriptive statistics values for Urea, Creatinine, and Blood Urea Nitrogen in females Ur, Cr, and BUN represent Urea, Creatinine, and Blood Urea Nitrogen, respectively.

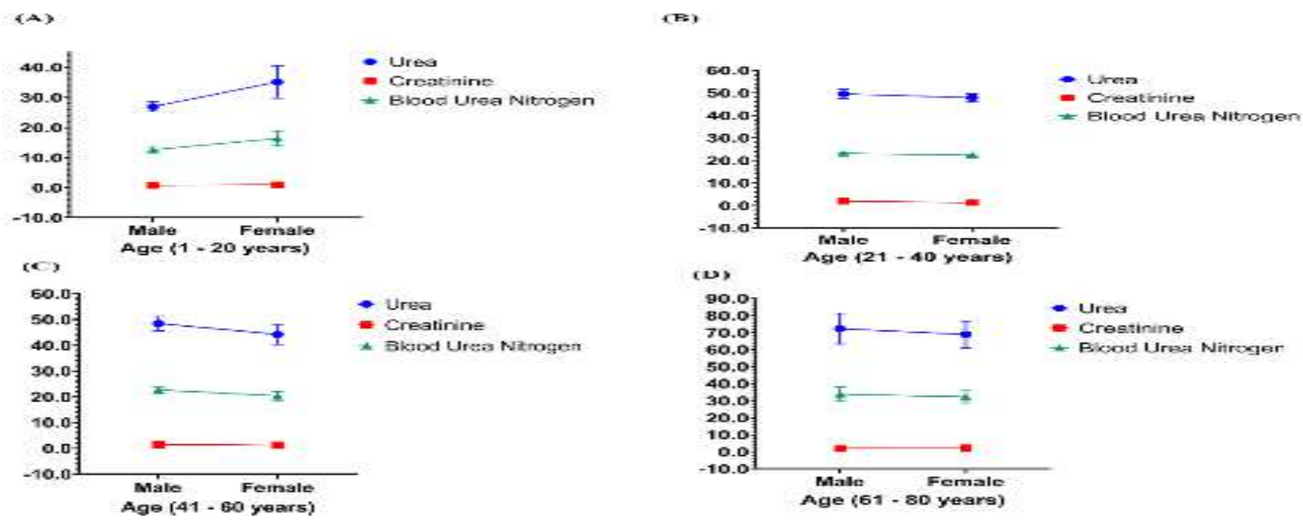

Figure 1: Age and gender-wise comparison for urea, creatinine, and blood urea nitrogen. Average values for urea, creatinine, and blood urea nitrogen in male and female age groups, 21-40, 41-60, and 61-80 in A, B, C, and D, respectively.

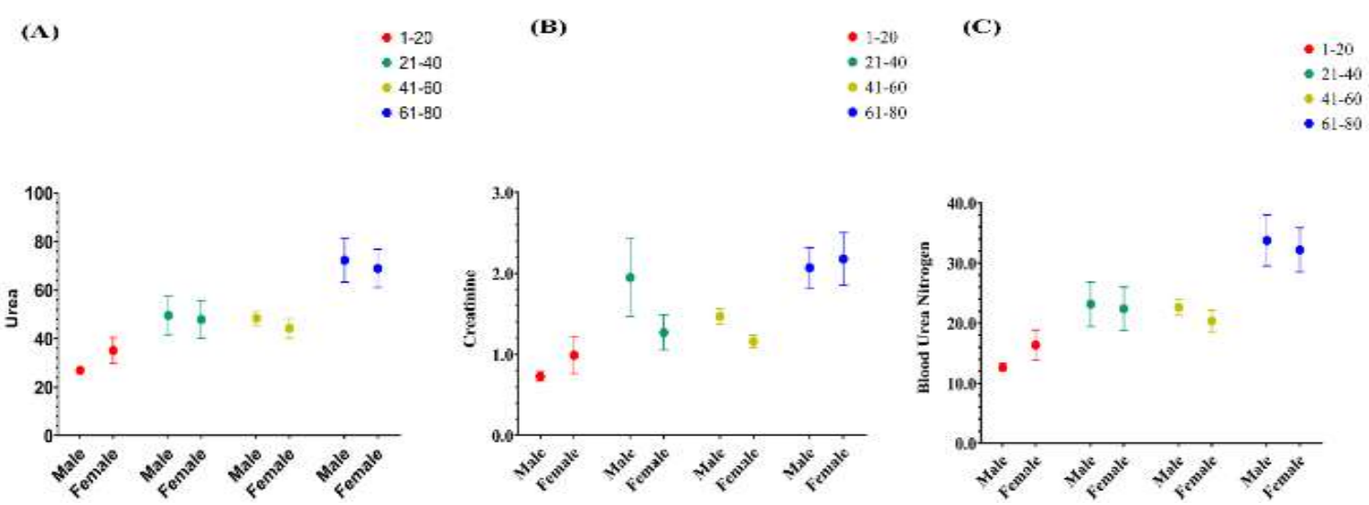

Figure 2: Comparison of average values of urea (A), creatinine (B), and blood urea nitrogen (C) in males and females of different age groups.

 Digital Object Identifier: https://doi.org/10.33865/wjb.008.02.1099

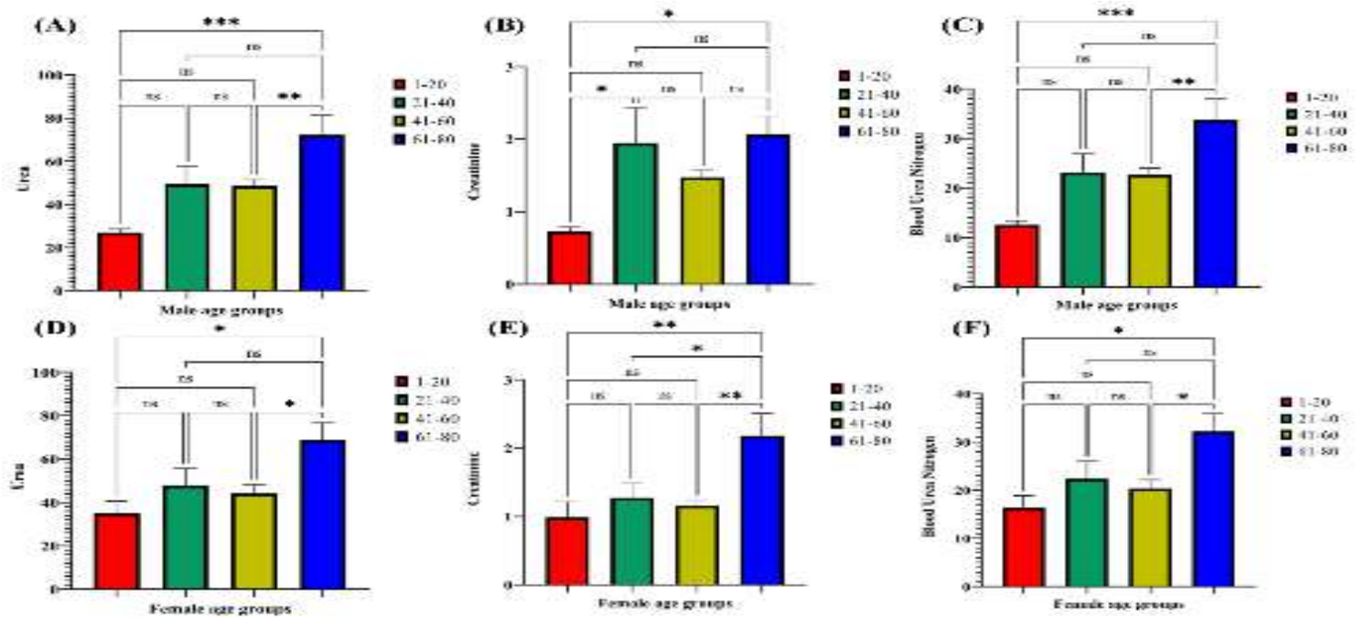

Figure 3: Analysis of urea, creatinine, and blood urea nitrogen in male and female age groups. A, B, and C for Urea, Creatinine, and Blood Urea Nitrogen, respectively, in males; D, E, and F for Urea, Creatinine, and Blood Urea Nitrogen, respectively, in females. *, ** and *** denotes p value between < 0.05, < 0.01, < 0.001, respectively.

Analysis revealed that Urea was significantly higher during the early age group (1-20 years) in males than in females comparatively in other age groups (figure 1). Further the average values of Urea were consistently higher in all male age groups than in female (figure 2A). Overall analysis of variance depicts the highest significance level at standard *p*-value (0.001) between age groups 1-20 and 61-80 in females (figure 3A,C). Creatinine results of different age groups of males and females were shown in figure 1, 2, 3B and 3E. The analysis found that Urea levels were considerably higher in males throughout the age group 21-40 years, 41-60, and 61-80 than in females in comparison to subsequent age groups (figure 1). Average creatinine levels were consistently greater in males than in females only in the 21-40 and 41-60 years of age group (figure 2B). The analysis of variance was carried out for all age groups in which the highest level of significance (*p*-value 0.01) was shown by age groups 1-20 and 61-80 in females (figure 3E). Figures 1 and 2 demonstrate a comparison and variation in BUN for different age groups of males and females. BUN levels were significantly higher in males age groups in 21-40,41-60 than in females (figure 1). Average BUN levels in males were consistently higher than in females across all age groups except the young age of 1-20 years (figure 2C). The analysis of variance reveals the greatest level of significance (0.001) between age groups 1-20 and 61-80 in males (figure 3).

The coefficient correlation between blood biochemical parameters was evaluated which showed a strong positive correlation between urea and BUN with a value 'r'=0.99 (table 3 & 4).

	U	Cr	BUN
U	1		
Cr	0.79	1	
BUN	0.99	0.79	1

Table 3: Pearson's correlation coefficient between urea, creatinine, and blood urea nitrogen. Ur, Cr, and BUN represent Urea, Creatinine, and Blood Urea Nitrogen, respectively.

	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	912.80	2	456.40	F (2, 3) = 33.49	0.0089
Residual (within columns)	40.88	3	13.63		
Total	953.70	5			

Table 4: One-way analysis of variance (ANOVA) for Urea, Creatinine and Blood Urea Nitrogen

Males physically possessed more muscular mass which required higher protein that resulted in increased urea. Moreover, BUN only reflects the nitrogen content of urea, whereas urea is twice that of BUN. However, both are indicative of healthy kidney functions.

DISCUSSION: Chronic kidney disease is considered a life-threatening disease. It is defined as a gradual decrease in kidney function (Venkatapathy *et al.*, 2014). Routine tests for urea, creatinine, and blood urea nitrogen were used to check kidney function. These parameters are widely accepted for the diagnosis of CKD (Pandya *et al.*, 2016). Urea, creatinine, and BUN values are increased with the advancement of age because CKD is more prevalent in older ages due to hypertension (Liew *et al.*, 2008). An increased amount of urea, creatinine, and blood urea nitrogen in the blood caused many complications in the normal working of the kidneys. Current study, we find that the values of Ur, Cr, and BUN are useful for assessing the normal functions of the kidney. Kidney functioning tests including urea, serum creatinine, and blood urea nitrogen play an important role in the early detection of renal diseases (Yang *et al.*, 2020). These are the most commonly used, which were routinely applied in every diagnostic laboratory for renal function monitoring (Yeşil *et al.*, 2015). Kidney function loss leads to an increased amount of Ur, Cr, and BUN in serum analysis. In this study correlation analysis was conducted between urea, creatinine, and BUN in which a positive correlation was seen between urea and BUN. The study found that if serum urea and creatinine levels rise, correspondingly increase salivary urea and creatinine levels, and vice versa (Pandya *et al.*, 2016). Previous studies showed that urea at higher levels in CKD patients and indicated a minor obstruction in urea excretion in kidney disease patients, as well as a loss in renal function (Pandya *et al.*, 2016). Correspondingly, higher urea levels were observed in aged males in this study. Serum creatinine level helps for checking renal functioning (Pundir *et al.*, 2019). Adler *et al.* (2003) showed that creatinine's higher concentration in males is due to its storage as a waste product in muscle mass and the existence of greater muscle mass in males than females which supports our study. The mean of creatinine was observed higher in females. Thus, serum creatinine is used for monitoring disease progression (Wagle, 2010). Blood urea nitrogen is a test that measures the nitrogen component of serum urea. BUN levels are inversely related to impairment of renal function. BUN-level assessment helps in the proper diagnosis of renal outcomes. A higher BUN level is an indication of CKD (Seki *et al.*, 2019). In this study, higher BUN level was observed in almost every age group of male and elevated value were observed in the 61-80 age group of females. (Gounden *et al.*, 2018) Studied those reductions in renal clearance were observed when the BUN level increases. BUN averages were also observed in higher concentration in males than the females in this study..

CONCLUSION: In conclusion, with the advancement of age there is an increase levels of Ur, Cr and BUN in male than female groups. Moreover, a significant correlation of Ur and BUN in male than female is indicative of higher muscular mass which requires great protein with lot of body support which in turn be eliminated from the body in form of Ur, Cr, and BUN. These blood parameters assessments are used for several routine analyses and add up values in the proper diagnosis of CKD, and also used in the population of the SBA district.

CONFLICT OF INTEREST: Authors have no conflict of interest.

ACKNOWLEDGEMENT: This work was financially supported by the Genetics Department, Shaheed Benazir Bhutto University, Shaheed

Benazir Abad with collaboration of the Biochemistry Lab, Peoples University of Medical and Health Science, Shaheed Benazirabad

REFERENCES: Adler, A. I., R. J. Stevens, S. E. Manley, R. W. Bilous, C. A. Cull and R. R. Holman, 2003. Development and progression of nephropathy in type 2 diabetes: The united kingdom prospective diabetes study (ukpds 64). Kidney international, 63(1): 225-232.

Ahmed, J., S. Azhar, N. ul Haq, S. Hussain, A. Stájer, E. Urbán, M. Gajdács and S. Jamshed, 2022. Awareness of chronic kidney disease, medication, and laboratory investigation among nephrology and urology patients of quetta, pakistan. International journal of environmental research public health, 19(9): 5015.

Finney, H., D. J. Newman and C. P. Price, 2000. Adult reference ranges for serum cystatin c, creatinine and predicted creatinine clearance. Annals of clinical biochemistry, 37(1): 49-59.

Gounden, V., H. Bhatt and I. Jialal, 2018. Renal function tests.

Jones, C. A., M. E. Francis, M. S. Eberhardt, B. Chavers, J. Coresh, M. Engelgau, J. W. Kusek, D. Byrd-Holt, K. V. Narayan and W. H. Herman, 2002. Microalbuminuria in the us population: Third national health and nutrition examination survey. American journal of kidney diseases, 39(3): 445-459.

Junejo, N., M. Khanif, K. Dharejo, A. Abdu and H. Abdul-Hamid, 2011. A field evaluation of coated urea with biodegradable materials and selected urease inhibitors. African journal of biotechnology, 10(85): 19729-19736.

Kalantar-Zadeh, K., T. H. Jafar, D. Nitsch, B. L. Neuen and V. Perkovic, 2021. Chronic kidney disease. The lancet, 398(10302): 786-802.

Kovesdy, C. P., 2022. Epidemiology of chronic kidney disease: An update 2022. Kidney international supplements, 12(1): 7-11.

Liew, G., P. Mitchell, T. Y. Wong, S. K. Iyengar and J. Wang, 2008. Ckd increases the risk of age-related macular degeneration. Journal of the american society of nephrology, 19(4): 806.

Massy, Z. A., C. Pietrement and F. Touré, 2016. Reconsidering the lack of urea toxicity in dialysis patients. In: Seminars in dialysis. Wiley online library: pp: 333-337.

Pandya, D., A. K. Nagrajappa and K. Ravi, 2016. Assessment and correlation of urea and creatinine levels in saliva and serum of patients with chronic kidney disease, diabetes and hypertension–a research study. Journal of clinical and diagnostic research, 10(10): ZC58.

Peng, C.-h., Y.-c. Xia, Y. Wu, Z.-f. Zhou, P. Cheng and P. Xiao, 2013. Influencing factors for saliva urea and its application in chronic kidney disease. Clinical biochemistry, 46(3): 275-277.

Pfaffe, T., J. Cooper-White, P. Beyerlein, K. Kostner and C. Punyadeera, 2011. Diagnostic potential of saliva: Current state and future applications. Clinical chemistry, 57(5): 675-687.

Pundir, C., S. Jakhar and V. Narwal, 2019. Determination of urea with special emphasis on biosensors: A review. Biosensors bioelectronics, 123: 36-50.

Rehman, I. U. and T. M. Khan, 2020. Epidemiology of chronic kidney diseases (ckd) in malaysia and pakistan, pathophysiology of ckd-associated pruritus and other ckd-associated dermatological disorders. Progress In microbes molecular biology and evolution, 3(1).

Schafer, C. A., J. J. Schafer, M. Yakob, P. Lima, P. Camargo and D. T. J. S. S. Wong, 2014. Saliva diagnostics: Utilizing oral fluids to determine health status. Saliva: Secretion functions, 24: 88-98.

Seki, M., M. Nakayama, T. Sakoh, R. Yoshitomi, A. Fukui, E. Katafuchi, S. Tsuda, T. Nakano, K. Tsuruya and T. Kitazono, 2019. Blood urea nitrogen is independently associated with renal outcomes in japanese patients with stage 3–5 chronic kidney disease: A prospective observational study. BMC Nephrology, 20: 1-10.

Venkatapathy, R., V. Govindarajan, N. Oza, S. Parameswaran, B. Pennagaram Dhanasekaran and K. V. Prashad, 2014. Salivary creatinine estimation as an alternative to serum creatinine in chronic kidney disease patients. International journal of nephrology, 2014.

Wagle, T. J., 2010. Genderwise comparison of serum creatinine and blood sugar levels in type 2 diabetic patients. Bombay hospital journal, 52(1): 64-68.

Walker, H. K., W. D. Hall and J. W. Hurst, 1990. Clinical methods: The history, physical, and laboratory examinations.

Yang, Q., Y. Lu, Y. Deng, J. Xu and X. Zhang, 2020. Homocysteine level is positively and independently associated with serum creatinine and urea nitrogen levels in old male patients with hypertension. Sci Rep, 10(1): 18050.

Yeşil, S., B. KARSlı, N. Kayacan, G. Süleymanlar and F. Ersoy, 2015. Hemodiyaliz uygulanan kronik böbrek yetmezlikli hastalarda ağrı değerlendirmesi. Pain, 27(4): 197-204.