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Biochemical Evaluation of Selected Renal Indices in Women with Metabolic Syndrome in Ilesa Metropolis South Western Nigeria

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aim: The aim of this study was to see the effects of metabolic syndrome on some selected renal indices for kidney biological functions.
Study Design: One-factor, one control - one test group quasi - experimental design.
Place and Duration of Study: Department of Chemical Pathology, Obafemi Awolowo University Teaching Hospitals Complex, Wesley Guild Hospital Unit, Ilesa, Osun State, Nigeria, between July 2015 and March 2016.
Methodology: A total of eighty (80) subjects were recruited for the study, and were grouped into normotensive women (n=40), and women with metabolic syndrome (n=40). Blood samples (10 mL venous blood) and 24 hour timed urine were collected, centrifuged and stored as plasma and aliquoted urine respectively before subjection to biochemical analysis. Blood plasma and urine samples were analyzed for renal indices using standard flame photometry and Spectrophotometric methods.
Results: Renal indices results revealed that Plasma (creatinine, urea, sodium and potassium)

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were significantly raised in women with metabolic syndrome when compared to normotensive women while significant decreases were observed in Urine (creatinine, potassium and urea) and creatinine clearance rates respectively in same comparison. However, a non-significant increase in Urine (volume and protein), as well as a non-significant decrease in urea clearance rates and urine sodium were seen in women with metabolic syndrome when compared to normotensive women.
Conclusion: This work revealed that metabolic syndrome has negative effects on the renal indices investigated which were attributed to the characterizing high blood pressure and vessel dysregulation to possibly result in kidney dysfunction in its sufferer. This work thus showed that renal indices can be employed with other investigations to diagnose metabolic syndrome, prevents its medical complications and monitor treatment progress.

Keywords: Renal indices; metabolic syndrome; ilesa metropolis; South Western Nigeria.

1. INTRODUCTION

Metabolic syndrome is defined as group of conditions that come together in a single individual for increased risk of heart disease, diabetes and stroke [1]. These group of conditions include insulin resistance for high blood glucose levels, hypertension (high blood pressure), cholesterol and triglyceride abnormalities, an increased risk for blood clotting and too much fat around the waist [1]. Metabolic syndrome also known as syndrome X, insulin resistance syndrome, or dysmetabolic syndrome is considered a multiplying risk factor for cardiovascular diseases, type 2 diabetes and stroke due to insulin resistance and an abnormal function and pattern of body fat [2].

Approximately 20 – 25 percent of the world's adult population has the cluster of risk factors of metabolic syndrome. They are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome [3]. In addition, people with metabolic syndrome have a fivefold greater risk of developing type 2 diabetes. They would add to the 230 million people worldwide who already have diabetes, one of the most common chronic diseases worldwide and the fourth or fifth leading cause of death in the developed world. The clustering of cardiovascular disease (CVD) risk factors found in metabolic syndrome is now considered to be the driving force for a new CVD epidemic [4]. This syndrome is a serious health condition that affects about 34 percent of adults in USA and places them at higher risk of cardiovascular disease, diabetes, stroke and diseases related to fatty build ups in artery walls [5]. The number of people with metabolic syndrome increases with age, affecting more than 40 percent of people in their 60s and 70s. According to World Health Organisation in 2004, more than one in five Americans has metabolic syndrome. Studies also show that 34% of overall African adults were affected with 43.3% in Ethiopia, 35.9% in Ghana, and 31.9% in Nigeria to mention a few [6].

The underlying cause of the metabolic syndrome continues to challenge the experts but both insulin resistance and central obesity are considered significant factors [7]. Abdominal obesity, hypertriglyceridemia, deranged cholesterol level, elevated blood pressure and blood sugar are prevalent signs and symptoms of metabolic syndrome [8]. Nevertheless, stress, overweight, sedentary lifestyle, aging, coronary heart disease, lipidystrophy, psychiatry illness, diet, genetics, physical inactivity, ageing, a pro-inflammatory state and hormonal changes may also have a potential risk factor effect, but the role of these may vary depending on ethnic group [9-11]. Possible complications of metabolic syndrome include mitochondrial dysfunction and insulin resistance, vascular and neurological disorders as well as diabetes mellitus type 2 to mention a few [12,13].

Pathophysiologically, in metabolic syndrome there is development of visceral fat, after which the adipocytes (fat cells) of the visceral fat increase plasma levels of TNFα and alter levels of a number of other substances (e.g.adiponectin, resistin, and PAI-1). TNFα has been shown not only to cause the production of inflammatory cytokines, but also possibly to trigger cell signaling by interaction with a TNFα receptor that may lead to insulin resistance [14,15].

Several management methods can be applied to prevent and treat metabolic syndrome. Treating metabolic syndrome requires addressing several risk factors together for improving the quality and length of your life. This will improve the overall cardiovascular health and greatly improve the

individual risk factors that make up metabolic syndrome [16]. Lifestyle change had been considered to be potent way of preventing and treating this syndrome, as this has to do with eating a better and balanced diet, avoiding processed food, which often contains partially hydrogenated vegetable oils, and is high in salt and added sugar. Moreover, refraining from alcohol and smoking habits can do a lot to curb this syndrome. Since physical inactivity and excess weight are the few underlying contributors to the development of metabolic syndrome, getting more exercise and losing weight can help reduce or prevent the complications associated with this condition [17]. Health providers may also prescribe medications like antihypertensives and antidiabetics to manage some of the underlying problems [18].

Renal indices are substances (like chemicals, enzymes, molecules, indicators or signals) produced by kidney into body fluids [19]. These substances exhibit some characteristics such as specificity and sensitivity which makes them significant in monitoring the glomerular filtration rates and tubular functions of the kidneys and also detect alterations in renal functions [20].

Indices of use for kidney's specific functions include: Plasma creatinine, plasma urea, blood and urine electrolytes, clearance rates (of creatinine and urea), and 24 hour urine profile (volume, quantitative protein). However they aid in early diagnosis of renal dysfunction, assessment of nephrons, glomerular and tubular functions of the kidneys long before permanent damage will set-in to the kidneys [21].

This study investigated the biochemical evaluation of selected renal indices in women with metabolic syndrome in Ilesa Metropolis, South Western Nigeria. This was to see the effects of metabolic syndrome on some selected renal indices for kidney biological functions.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Reagents and chemicals

The reagents and chemicals used for this work were of analytical grade from sigma, and they include: urea acid reagent [$FeCl_3.6H_2O$ (5 g), phosphoric acid (85%), H_2SO_4 (200 mL)], urea colour reagent (diacetylmonoxime (20 g), thiosemicarbazide (5 g)], creatinine reagent [picric acid (9.3 g), NaOH (30 g), H_2SO_4 (18.8 ml), sodium tungstate (50 g)], urine protein reagent [trichloroacetic acid (30 g)] to mention a few.

2.2 Methods

2.2.1 Experimental design and grouping of subjects

Quasi-experimental design method was utilized in this study and subjects were divided into two (2) groups:

i. Group 1 was Normotensive Women (Control);
ii. Group 2 was Women with Metabolic Syndrome (Tests);

2.2.2 Sampling areas

The hospital selected for the purpose of this research in ilesa metropolis was Obafemi Awolowo University Teaching Hospitals Complex, Wesley Guild Hospital Unit, Ilesa, Osun State, Nigeria. This hospital is recognised as referral centres for metabolic syndrome cases in ilesa metropolis.

2.2.3 Recruitment of subjects

A total of eighty (80) subjects of both normotensive women (n=40), and women with metabolic syndrome (n=40) were recruited for this research and their samples were processed for analysis within 72 hours of collection employing standard diagnostic techniques.

2.2.4 Selection of subjects

The subjects for this project were selected according to the following criteria:

i. Obtaining brief clinical history from all subjects in outpatient department using questionnaire to take care of consent, age, body mass index, and other personal informations;
ii. Anthropometric measurements of the subjects blood pressure was done using sphygmomanometer, and ensuring test subject groups have readings greater or around 140 mmHg systolic and 90 mmHg diastolic measurement, while control subject groups have normal blood pressure readings;
iii. Ensuring test subject groups have central obesity and positive urine glucose as a sign

of diabetic state, while control subject groups have no central obesity with negative urine glucose readings;

iv. Notice of deranged lipid profile in test subject group while the control subject group have normal lipid profile analysis result.

2.2.5 Collection of blood samples

2.2.5.1 Blood sample

About 10mL of venous blood was collected from each subject into lithium heparin bottle.

2.2.5.2 Preparation of blood plasma

The blood samples were separated as plasma into plain labeled bottles after spinning in a centrifuge at 4,000 rpm for 20 min.

2.2.5.3 24 hr urine sample

A preservative of 2M HCl (20mL) was added into a sterile 5 litre container for voided 24 hour timed urine by all subjects [28].

2.2.5.4 Preparation of 24 hr urine sample

Timed urine of 24 hour was collected from each of the subjects in each group and was measured using cylinder. About 10 mL of the measured urine was aliquoted into a plain labelled bottle before spinning in a centrifuge at 4,000 rpm for 20 min. The urine sample was used to confirm presence of protein in urine as well as clearance rates (of creatinine and urea).

2.2.6 Estimation of creatinine (in plasma and urine) and creatinine clearance

Plasma and urine concentration of creatinine was estimated spectrophotometrically according to Jaffe's reaction method [22].

2.2.6.1 Procedure

Distilled water (0.5 mL) was pipetted into test and standard tubes. 1 mL of plasma/urine and standard (1 mg/mL) was added into their tubes respectively. Thereafter, 0.5 mL of both sodium tungstate (5%) and sulphuric acid (0.33 mol/L) were added to all the tubes, and the content mixed thoroughly. The suspension was centrifuged for 10 min. at 5000 rpm. In the second stage, 1 mL of each tube supernatant was taken into other arranged tubes for test and standard. 1 ml of picric acid (0.04 mol/L) and sodium hydroxide (0.75 mol/L) were then added to all tubes, with the content mixed appropriately. It was incubate at room temperature for 15 min. before the absorbance was read at 520 nm against reagent blank.

Calculation:

Concentration of Plasma / urine (Creatinine (umol/L)) = Absorbance of test X Concentration of standard / Absorbance of standard

Creatinine Clearance Calculation:

Creatinine Clearance (mL/min) = (Urine creatinine concentration x 24 hr voided urine/ Plasma creatinine concentration x 1440 min)

2.2.7 Estimation of urea (in plasma and urine) and urea clearance

The concentration of plasma and urine urea was estimated spectrophotometrically using diacetylmonoxime method [22].

2.2.7.1 Procedure

Distilled water (1 mL) was pipetted into tubes of test and standard. 0.01 mL equal volume of plasma/urine and standard (200 mg / 100 mL) was added to their tubes respectively and the suspension mixed. The same volume of 1 mL of urea colour reagent [containing diacetylmonoxime (20 g/L) and thiosemicarbazide (5 g / L)] and urea acid reagent [containing $FeCl_3.6H_2O$ (20 g/L) and sulphuric acid (2.0 mol/L)] was added to all tubes. The mixture was boiled in water bath at 100℃ for 20 min., then removed and cooled in cold water. The absorbance of test and standard was read at 520 nm against reagent blank.

Calculation:

Concentration of Plasma / urine (Urea (mmol/L)) =Absorbance of test X Concentration of standard / Absorbance of standard

Urea Clearance Calculation:

Urea Clearance (mL/min) = (Urine urea concentration x 24 hr voided urine/Plasma urea concentration x 1440 min)

2.2.8 Determination of plasma and urine sodium and potassium concentrations

Plasma and urine concentrations of sodium and potassium were determined using the flame photometry method [23].

2.2.8.1 Procedure

Plasma/urine and standard [containing 58.5 g/L of NaCl and 7.46 g/L of KCl] of equal volume of 0.1 mL was taken with pipette into respective electrolyte bottle (of test and standard) and diluted with distilled water (10 mL). The flame photometer was turned on and blanked with distilled water. Required electrolyte filter was selected, after which the nebulizer was introduced into the diluted standard and test. The concentration of sodium and potassium in the plasma/urine samples and standard in mmol/L was visualised through the read-out device of the flame photometer.

2.2.9 Determination of urine glucose

Qualitative urine detection of glucose was determined using the clinistix strip method [23].

2.2.9.1 Procedure

The clinistix band area was completely immersed in urine specimen collected into a sterile container. The strip was removed and tapped against the side of urine container to remove excess urine. The strip was held close to colour chart on the clinistix bottle, and the investigation was carefully recorded by comparing glucose band colour area with corresponding colour chart on the bottle after 2 min.

2.2.10 Estimation of 24 hour urine protein concentration

Concentration of 24 hour urine protein was estimated spectrophotometrically according to turbidimetric method [24].

2.2.10.1 Procedure

24 hour urine sample and standard (100 mg/dL) of equal volume of 0.5 mL were pipetted to their test tubes and 3% trichloroacetic acid (1.5 mL) was added to each tubes. The reaction mixture were mixed and incubated for 5 min. at room temperature, after which the absorbance of the urine and standard were read against reagent blank at 470 nm.

Calculation:

Concentration of 24 hours (Urine Protein (mg/24 hr)) = Absorbance of test in mg/dL x 10 x 24 hr urine volume/1000 x 1000

2.3 Statistical Analysis

Results are expressed as mean ± SEM. Statistical difference was determined by one-way analysis of variance (ANOVA) followed by a post hoc test (Student Newman-Keuls Test (SNK)). Difference was considered statistically significant with $p < 0.05$. Computer software Graph pad PRISM® version 3.00 was used for the analysis.

3. RESULTS

The results of this study showed that in women with metabolic syndrome plasma concentrations of creatinine and potassium were significantly raised at ($P<0.05$) while the levels of urea and sodium were significantly raised at ($P<0.01$) in the investigated renal indices when compared with normotensive women (see Table 1).

Women with metabolic syndrome however revealed non-significant increase at ($P>0.05$) in urine volume and urine protein, as well as a non-significant decrease ($P>0.05$) in urea clearance rates and urine sodium in women with metabolic syndrome when compared to normotensive women. Moreover, in the same comparison, significant decreases were observed in urine creatinine, creatinine clearance rates and urine potassium respectively at ($P<0.001$) but at ($P<0.05$) in urine urea (see Table 2).

4. DISCUSSION

Results obtained from this study showed that plasma creatinine and urea concentrations were significantly raised in women with metabolic syndrome than the normotensive groups. This observation could be due to endogenous degradation/destruction of muscle and tissues from raised blood pressure vasoconstriction (kidney hypertension) in metabolic syndrome individual as well as vascular dysregulation/ endotheliosis of arteries to facilitate kidney necrosis that elevates synthesis of creatinine and urea. This result is similar to previous reports [4,25].

Table 1. Renal indices in plasma of normotensive women [A] and women with metabolic syndrome [B]

Group/Indices	CR (umol/L)	UR (mmol/L)	Na^+ (mmol/L)	K^+ (mmol/L)
A (n=40)	81.2±7.12	3.5±0.28	131.2±1.36	3.5±0.10
B (n=40)	102.8±5.34	4.6±0.24	136.5±1.39	3.8±0.09
Differences between means	B>A	B>A	B>A	B>A
Levels of significance	P<0.05	P<0.01	P<0.01	P<0.05

Table showed Means ± Standard error of mean (SEM), Differences between means and the Levels of significance (P<0.01, and P<0.05). CR = Plasma creatinine, UR = Plasma urea, Na^+ = Plasma sodium, K^+ = Plasma potassium

Table 2. Renal indices in urine of normotensive women [C] and women with metabolic syndrome [D]

Group/Indices	UV (mL)	UCR (umol/L)	UUR (mmol/L)	CRCL (mL/min)	URCL (mL/min)	QUP (g/24hr)	UNa^+ (mmol/L)	Uk^+ (mmol/L)
C (n=40)	1,894.2 ± 85.04	3,178.0 ± 107.45	127.4 ± 8.25	74.1 ± 8.35	59.2 ± 3.07	0.3 ± 0.06	67.6 ± 3.98	15.2 ± 0.85
D (n=40)	2,019.6 ± 87.25	2,095.7 ± 86.84	103.1 ± 3.57	38.5 ± 4.08	56.4 ± 5.01	0.5 ± 0.06	65.4 ± 4.71	9.5 ± 0.51
Differences between means	D>C	D<C	D<C	D<C	D<C	D>C	D<C	D<C
Levels of significance	P>0.05	P<0.001	P<0.05	P<0.001	P>0.05	P>0.05	P>0.05	P<0.001

Table showed Means ± Standard error of mean (SEM), Differences between means and the Levels of significance (P<0.001, P<0.05 and P>0.05). : UV = Urine volume, UCR = Urine creatinine, UUR = Urine urea, CRCL = Creatinine clearance, URCL = Urea clearance, QUP = Urine protein, UNa^+ = Urine sodium, UK^+ = Urine potassium

However, the urine creatinine and urea concentrations, as well as creatinine clearance rates were decreased significantly while urea clearance rates were non-significantly decreased in women with metabolic syndrome than the normotensive groups. The observations are similar to the previous findings [4,25,26]. This result could be attributed to reflections of endogenous degradation / destruction of muscle and tissues as well as vascular dysregulation/ endotheliosis of arteries to facilitate kidney necrosis that elevates synthesis of plasma creatinine and urea with reduced clearance rates of creatinine and urea in urine.

Plasma concentrations of sodium and potassium were observed to be significantly raised in women with metabolic syndrome than the normotensive groups, while in urine, sodium was non-significantly decreased and potassium concentration was significantly decreased in same comparison. The observed hypernataemia and hyperkalaemia might be due to increased kidney vasoconstriction in metabolic syndrome with resultant changes in the kidneys' ability to remove salt for increased retention of sodium and potassium in blood with simultaneous reduction of sodium and potassium in urine, thus enhancing high blood pressure, heart disease and stroke. This result is as well similar to previous reports [27,28].

The 24 hour urine volume and urine protein concentration were non-significantly raised in women with metabolic syndrome when compared with the normotensive groups. Similar previous reports have been made [29,28,30-32]. The result might be due to increased blood pressure (kidney hypertension) vasoconstriction in metabolic syndrome that initiates resultant changes in the kidneys' ability for increased thirst and increased urination as well as hyperproteinuria due to increase kidney intratubular pressure with ureteral obstruction, and efferent glomerular arteriole constriction to filter out protein with failure of tubular reabsorption.

5. CONCLUSION

Metabolic syndrome as expressed in this study has negative effects on the renal indices investigated which were attributed to the characterizing high blood pressure and vessel dysregulation to possibly result in kidney dysfunction in its sufferer. This work thus showed that renal indices can be employed with other investigations to diagnose metabolic syndrome, prevents its medical complications and monitor treatment progress.

6. LIMITATIONS OF THE STUDY

The limitation attached to this study is based on the rare occurrence rate of metabolic syndrome in ilesa metropolis that has warranted using smaller subjects number for the study.

CONSENT

Author declares that written informed consent was obtained from all the subjects studied in this work.

ETHICAL APPROVAL

Author hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Stöppler MC, Shiel WC. Metabolic syndrome. Medicine Net. 2012;1:1-5.
2. Carr DB, Utzschneider KM, Hull RL, Kodama K, Retzlaff BM, Brunzell JD, Shofer JB, Fish BE, Knopp RH, Kahn SE. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III Criteria for the Metabolic Syndrome. Diabetes. 2004;53(8):2087-94.
3. Carson C. How to prevent or reverse metabolic syndrome. Dailystrenght. 2011; 2:1-3.
4. International Diabetes Federation. The IDF consensus worldwide definition of the metabolic syndrome. IDF. 2006;5:1-24.

5. American Heart Association. About metabolic syndrome. American Medical Network. 2016;3:92-6.
6. World Health Organisation. Global Burden of Disease for the Year 2001 by World Bank Region, for use in Disease Control Priorities in Developing Countries. Disease Control Priorities Project. 2004;52:6-500.
7. National Heart Lung and Blood Institute. What is metabolic syndrome? NHLBI. 2016;7:1-9.
8. Rosmond R, Björntorp P. The hypothalamic–pituitary–adrenal axis activity as a predictor of cardiovascular disease, type 2 diabetes and stroke. J Intern Med. 2000;247:188-97.
9. Brunner EJ, Hemingway H, Walker BR, Page M, Clarke P, Juneja M, Shipley MJ, Kumari M, Andrew R, Seckl JR, Papadopoulos A, Checkley S, Rumley A, Lowe GD, Stansfeld SA, Marmot MG. Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: Nested case-control study. Circulation. 2002;106(21):2659-65.
10. Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J. Harrison's Principles of Internal Medicine, 17th Edition (Single Vol.). McGraw-Hill Medical. 2008;8:5-14.
11. Goldberg RB, Mather K. Targeting the consequences of the metabolic syndrome in the diabetes prevention program. Arterioscler Thromb Vasc Biol. 2012;32: 2077-90.
12. John AP, Koloth R, Dragovic M, Stephen CB. Prevalence of metabolic syndrome among Australians with severe mental illness. Med J Aust. 2009;190:176-9.
13. Renaldi O, Pramono B, Sinorita H, Purnomo LB, Asdie RH, Asdie AH. Hypoadiponectinemia: A risk factor for metabolic syndrome. Acta Med Indones. 2009;41:20-4.
14. Hallfrisch J. Metabolic effects of dietary fructose. The FASEB J. 1990;4:2652-60.
15. Hotamisligil GS. The role of TNF-alpha and TNF receptors in obesity and insulin resistance. J Intern Med. 1999;245:621-5.
16. Awofadeju SO, Imoru JO, Asaolu MF. Effect of magnesium sulphate on cardiac biomarkers in preeclamptic patients in selected tertiary hospitals in Osun State South Western Nigeria. CA J. 2016;5:1-11.
17. Farooqui AA. Farooqui T, Panza F, Frisardi V. Metabolic syndrome as a risk factor for neurological disorders. Cell Mol Life Sci. 2012;69:741-62.
18. Fukuchi S, Hamaguchi K, Seike M, Himeno K, Sakata T, Yoshimatsu H. Role of fatty acid composition in the development of metabolic disorders in sucrose-induced obese rats. Exp Biol Med (Maywood). 2004;229:486-93.
19. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr. Harmonizing the metabolic syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
20. Harper HA, Rodwell VW, Mayer PA. Biochemistry. 5th ed. Churchill Livingstone. 1979;15:124-37.
21. Waikar SS, Betensky RA, Bonventre JV. Creatinine as the gold standard for kidney injury biomarker studies? Nephrol Dial Transplant. 2009;24:3263-5.
22. Cheesbrough M. District Laboratory Practice in Tropical Countries (Part 1). Cambridge University Press, Cambridge. 1999;6:340-72.
23. Burtis CA, Edward RA. Tietz Textbook of Clinical Chemistry. 7th ed. W.B Saunders Company, United States of America. 1999; 23:432-57.
24. Tietz NW, Fiereek EA. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia Publishers. 1990;12:301-43.
25. Zhou H, Hewitt SM, Yuen PS, Star RA. Acute kidney injury biomarkers - needs, present status, and future promise. Nephrol Self Assess Program. 2006;5:63-71.
26. Ziaei S, Ranjkesh F, Faghihzadeh S. Evaluation of 24 hour urine copper in preeclamptic versus normotensive pregnant and non-pregnant women. Royan Institue International Journal of Fertility and Sterility. 2008;2:9-12.
27. Moran P, Lindheimer MD, Davison JM. The renal response to preeclampsia. Semin Nephrol. 2004;24:588-95. Burtis CA, Edward RA. Tietz Textbook of Clinical Chemistry. 7th ed. W.B Saunders Company, United States of America. 1999; 23:432-57.

28. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308:1592-4.
29. Witlin AG, Friedman SA, Sibai BM. The effect of magnesium sulfate therapy on the duration of labor in women with mild preeclampsia at term: A randomized, double-blind, placebo-controlled trial. Am J Obstet Gynecol. 1997;176:623-7.
30. Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006; 444:881-7.
31. Edgar VL. Novel biomarkers of renal function. Medscape. 1994;69:89-95.
32. de Geus HR, Betjes MG, Bakker J. Biomarkers for the prediction of acute kidney injury: A narrative review on current status and future challenges. Clin Kidney J. 2012;5:102-8.

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