

ZONULIN AND SOME PARAMETERS AS EARLY BIOMARKERS OF PREGNANCY-INDUCED HYPERTENSION IN IRAQ

Hiba. H. Rasheed, Nazar. A. Abed, Ibrahim. F. Wahed

Department of Chemistry, College of Science, Tikrit University, Tikrit, Iraq

hibarasheed@tu.edu.iq

Abstract

Zonulin, Erythropoietin, Prolidase and increase in level ($P < 0.01$) but Renalase decrease in the levels ($P < 0.01$) are implicated in several metabolic disorders including hypertension, obesity and vascular weakness. The purpose of the current study was to estimate the serum erythropoietin, zonulin, Renalase content of pregnant women with preeclampsia caused by hypertension and obesity. Pregnancy induced hypertension which includes preeclampsia and gestational hypertension. There is a relationship linking collagen formation to the proportion of prolidase. The deficiency in this enzyme is a genetic condition that occurs due to mutations in the PEPD gene on the nineteenth chromosome and in some pathological cases. Preeclampsia is one of the social problems in the world in general and in Iraq in particular, as a result of the psychological, economic and environmental conditions lived by the family. This study was done to find the levels of these biochemical parameters of tight junction regulation among women with gestational hypertension. Keywords: preeclampsia (PE), (PIH), gestational hypertension (GH), Erythropoietin (EPO).

Introduction

Preeclampsia (Pre) is a multisystem vascular disease that complicates 8.5 million pregnancies each year and accounts for 40% of fetal mortality and 18% of maternal deaths ^[1]. Pre and fetal growth restriction often coexist and account for nearly 30% of premature deliveries, with an estimated ^[2]. Among the numerous factors known to increase the risk of Pre are preexisting vascular disease, primiparity, advanced maternal age, and hypoxia such as that experienced at high altitude residence raises the incidence of Pre threefold, highlighting the clinical relevance of persistent maternal hypoxia for hypertensive pregnancy disorders ^[3,4]. According to WHO estimates, the incidence of preeclampsia is seven times higher in developing countries compared to developed countries ^[5]. The prevalence of pre-eclampsia ranges from 1.8 to 16.7% in developing countries. Several studies have shown that preeclampsia is associated with failure of trophoblast invasion of maternal spiral arteries, resulting in increased vascular resistance of the uterine arteries and decreased uteroplacental flow ^[6,7]. If preeclampsia is not controlled, preeclampsia can prevent adequate blood flow and oxygen to the developing fetus. The fetus, causes liver and kidney damage in the mother and sometimes develops into pre-eclampsia, a serious condition that includes seizures ^[8,9]. There is no known cure for preeclampsia other than the birth of the child or the location. Successful pregnancy is accompanied by pronounced, systemic arterial and venous vasodilation, a 30% rise in cardiac output ^[10], and extensive remodeling of the maternal. Besides, the in which the high pressure in pregnant women increases with the increase in the production of

varieties Active oxygen is the result of exposure to many different agents such as chemicals, heavy metals, Smoking, radiation exposure, neuropsychological stress^[11] Serum zonulin concentrations are increased in T2D and obesity^[61] and strong correlations were observed with various metabolic markers, including fasting plasma glucose, IL-6, HDL-C, and triglyceride (TG) levels. However, there is insufficient information about the involvement of zonulin in pregnancy induced hypertension (PIH) which comprises preeclampsia (PE) and gestational hypertension (GH)^[12]. Erythropoietin is associated with preeclampsia, Pregnancy is a dynamic process associated with significant physiological changes in the cardiovascular system. Prolidase, also known as Peptidase D, has been found in various mammalian tissues and also in microorganisms. It is a cytosolic enzyme and it was purified from several sources, including human erythrocytes, fiber oblasts and different strains of bacteria^[13]. The enzyme activity is relatively high in kidneys, intestinal mucosa and erythrocytes, while it is low in plasma and liver. the serum levels of renalase levels in clinically normal gestations and correlations between serum levels of renalase and blood pressure levels, glomerular filtration rate and protein excretion in urine in gestations with mild and severe preeclampsia^[14].

Materials and Methods

Subjects

Patients. One hundred preeclampsia patients and 80 healthy individuals were recruited Control group throughout the study from February 2019 to April 2020 in Tikrit Teaching Hospital, Tikrit, Iraq. The search was registered with Research Ethics Iraqi Ministry of Health Committee, Iraq. Ten milliliters of blood were collected in a test tube without anticoagulants. Blood samples were left for 20-30 minutes at 37 minutes Serum was obtained by centrifuging the blood at 4000 rpm ($1814 \times g$) for 10 min, Then serum was used for kinetic assays. Zonulin ng/ dl, prolidase U/L, Erythropoietin pg./ml and renalase ng/ dl were estimated from (Fine Test Biotech) (China) This kit was based on sandwich enzyme-linked immune-sorbent assay technology, Capture antibody was pre-coated onto 96- well plates.

RESULTS AND DISCUSSION:-

Biochemical Study

This study showed a significant increase in zonulin level to (49.300 ± 5.860) units for patients in the third stage of hypertension compared with the second stage (369.30 ± 26.22), The results shown in the Table (2) Were a significant ($P < 0.01$) in the levels of Zonulin in the blood serum of preeclampsia patients as compared with the control group in third and second stages These results consistent with previous studies (Ahmed Tijani Bawah^{1*}, Henry Tornyil, Mohammed Mustapha Seini²⁰²⁰), where there was also a strong correlation between Zonulin elevation and pregnancy-induced hypertension Elevated plasma zonulin affects the intestinal barrier and when the intestinal barrier is blocked, Infectious agents and nutritional antigens of mucosal immune elements reach the body, which may lead to increase immune reactions and induce inflammation leading to PIH. Elevated zonulin may also cause changes in gut proteins that lead to changes in permeability and dysbacteriosis. Gastrointestinal dysbacteriosis and Increased production of lactate bacteria^[15]. Neurotransmitters such as dopamine^[16] histamine^[17] and Serotonin^[18] which is potentially

involved in The regulation of blood pressure is also produced by these gut bacteria and so when there is dysbacteriosis An elevated zonulin level is usually associated with pregnancy, and hypertensive disorders may occur during pregnancy. That signs of permeable intestinal permeability, especially zonulin, predicted Systolic blood pressure and bowel barrier the composition of the gut microbiome is directly related High blood pressure in humans ^[19]. In another study, researchers reported that PE diagnosed during the third trimester of pregnancy was associated with a bowel disorder. Microorganism composition compared to those without PE Serum zonulin was higher in women with PE Compare with pregnancy, The association between zonulin levels and PIH is in accordance with previous studies which have shown that zonulin positively correlates with systolic blood pressure^[20] The mechanism by which zonulin can participate in PIH. It can lead to activation of nervous systems that include the renin-angiotensin-aldosterone system, Sympathetic nervous system and antidiuretic hormone. When these systems are activated, the plasma volume will increase resulting in high blood pressure ^[21].

Table 1. Patient characteristics by period and year

Parameter	Third Stage				Second Stage			
	Pre-E	Value	Control	Value	Pre-E	Value	Control	Value
Age, year	43.0± 8.7	P <0.01	29.8 ±3.8	NS	28.06 ±7.0	P <0.05	25.8 ± 2.8	NS
Height, cm	158 ± 2	P <0.05	151 ± 9	NS	150 ±1	NS	150 ± 4	NS
Weight, kg	114.0± 17.8	P <0.01	72.7± 10.5	NS	87.5± 15.4	P <0.01	63.7 ± 7.2	NS
BMI, kg/m ²	225.2±30.6	P <0.01	135±16.9	NS	38.7 ±3.8	P <0.05	26.3 ± 3.7	NS
Systolic Pressure(mm .Hg)	15.17±1.61	P <0.01	12.25±0.44	NS	14.53±1.13	P <0.05	12.61±1.35	NS
Diastolic pressure(mm .Hg)	9.42±0.85	NS	8.28±0.62	NS	9.13±0.91	P <0.05	8.50±0.96	NS
Cholesterol(mg /dl)	306.1± 4.15	P<0.01	171.0±2.13	P <0.05	223.46±11.5	P<0.01	176.13±8.09	NS

Triglycerides(mg/dl)	270.5 ±16.1	P<0.01	147.00 ± 4.47	NS	229.61 ± 8.27	P <0.05	111.8±3.12	NS
HDL-C (mg/dl)	34.80±1.7	P<0.01	51.50±2.8	P <0.05	51.02 ± 2.30	P <0.05	61.80 ± 2.63	P <0.05
LDL-C(mg/dl)	100.52±18.99	NS	95.12±12.94	NS	148.5±7.35	P <0.05	177.7±7.73	P <0.05
VLDL-C(mg/dl)	56.7 ±2.8	P<0.01	23.4±1.2	P <0.05	40.72±2.97	P <0.05	30.53±1.91	NS

The risk of pre-eclampsia increases in the women with higher levels of oxidized low-density lipoprotein (LDL) and triglycerides (TG) and lower levels of circulating vitamin C as compared to normotensive pregnant women [22,23]. The oxidative conversion of LDL- cholesterol to oxidized LDL form is the key event for the in While the level of triglycerides, the difference was a significant increase (P<0.05) in patient compered of the control, This is in agreement with the studies conducted by both researchers (Thushari I. Alahakoon, Heather J. Medbury2020), Is being Elevation in maternal TG levels may have a role in the pathogenesis of PE. The implications of elevated maternal and fetal TG levels and elevated fetal Apolipoprotein B levels deserves further exploration of their role in long term cardiovascular risk in the mother as well as the off spring^[24] and development of atherosclerosis and hypertension ^[25].

high-density lipoprotein cholesterol in Note that the normal range for its concentration in the body is (less than 40 mg/dL). This decreased is due to endothelial dysfunction worsened by the physiological burden of pregnancy^[26]and insulin resistance and obesity, in the pathogenesis of preeclampsia, The concentration of low-density lipoprotein-cholesterol in the table shows that the concentration of very-low-density lipoprotein-cholesterol increased significantly (P<0.05) in the patients compared to the control, The results of the current study agreed with those of (Naorem et al., 2018). Which in turn leads to serious complications that lead to blockage of the arteries of the heart and blood vessels, and consequently the development of diseases that may be chronic after childbirth. Therefore, the goal was to study the effect of obesity on pre-eclampsia and heart diseases and to study variables that work on the early detection of pre-eclampsia and avoid its complications.

Table2. Biochemical Parameters Among Preeclampsia Patients as Compared with Control Group:

Third Stage mean ±SD					Second Stage mean ±SD			
Parameter	Pre-E	Value	Control	Value	Pre-E	Value	Control	Value

zonulin (ng/ dl)	49.300±5.8 60*	P<0. 01	23.290±1. 487	P>0. 05 NS	25.589±1. 188	P <0.0 5	19.085±1. 691	P>0. 05 NS
Erythropoi etin (pg./ml)	438.47 ±30.35*	P<0. 01	251.70 ±38.30	P>0. 05 NS	369.30 ±26.22	P <0.0 5	159.40 ±43.80	P>0. 05 NS
Renalase ng/dl	9.380 ±1.763	P<0. 01	21.491 ±3.453	NS	12.894 ±0.787	P<0. 05	20.746 ±3.354	NS
prolidase UI/ml	167.67± 27.08*	P<0. 01	80.42± 15.81	NS	115.00± 11.02	P <0.0 5	80.42±18. 64	NS

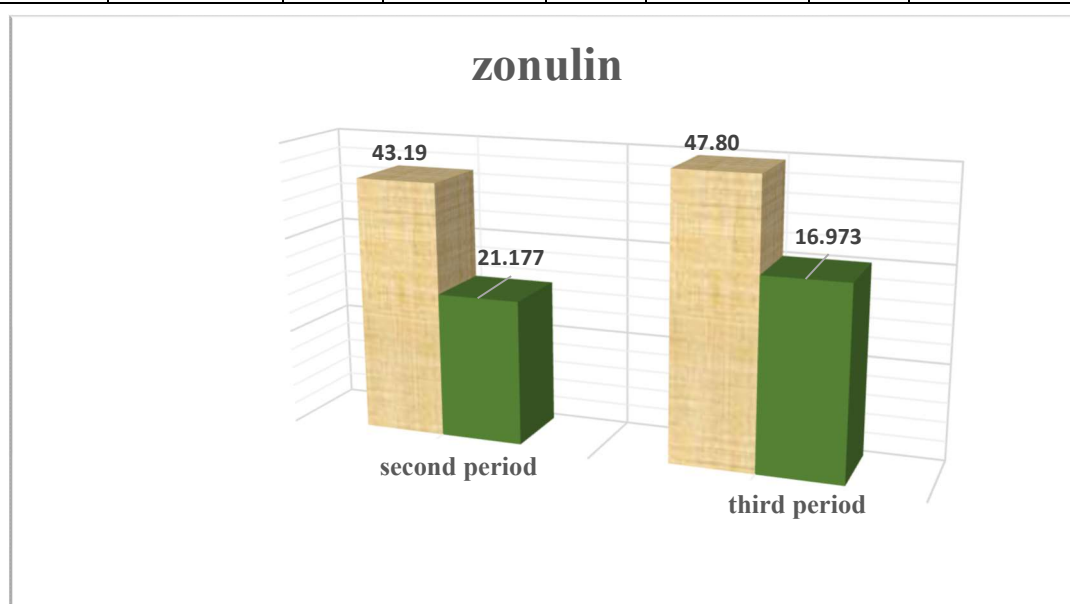


Figure (1) zonulin concentration in patients compared of control.

Our study showed a significant increase in the level of Erythropoietin (438.47 ± 30.35) for patients in the third stage of pregnancy hypertension compared with the second stage (369.30 ± 26.22). The results shown a significant ($P < 0.01$) increase in stage as compared with the second stage with the control. The results we obtained were similar to those of previous studies (Dmitriy Vazenmiller, Olga Ponamaryova, 2018). EPO synthesis is inversely related to tissue oxygen availability, with the main determinant being the transcriptional activity of its gene by hypoxia-inducible factor-1 (HIF-1) [27]. During fetal development, EPO synthesis gradually changes from hepatocytes that stimulate erythropoiesis in the liver to EPO-producing renal cells in the cerebral cortex and outer marrow that stimulate erythropoiesis in the bone marrow, with the timing of this transition dependent on species. However, secretion of small amounts of EPO has been identified in other fetal tissues, and for the placenta this can become significant in response to fetal hypoxemia [28]. Our findings demonstrate that environmental hypoxia increased the pregnancy-associated rise in EPO level.

despite a similar reduction in Hb (Gabriel H. Wolfson, Enrique Vargas, Vaughn A. Browne, Lorna G. Moore, and Colleen G. Julian2022).

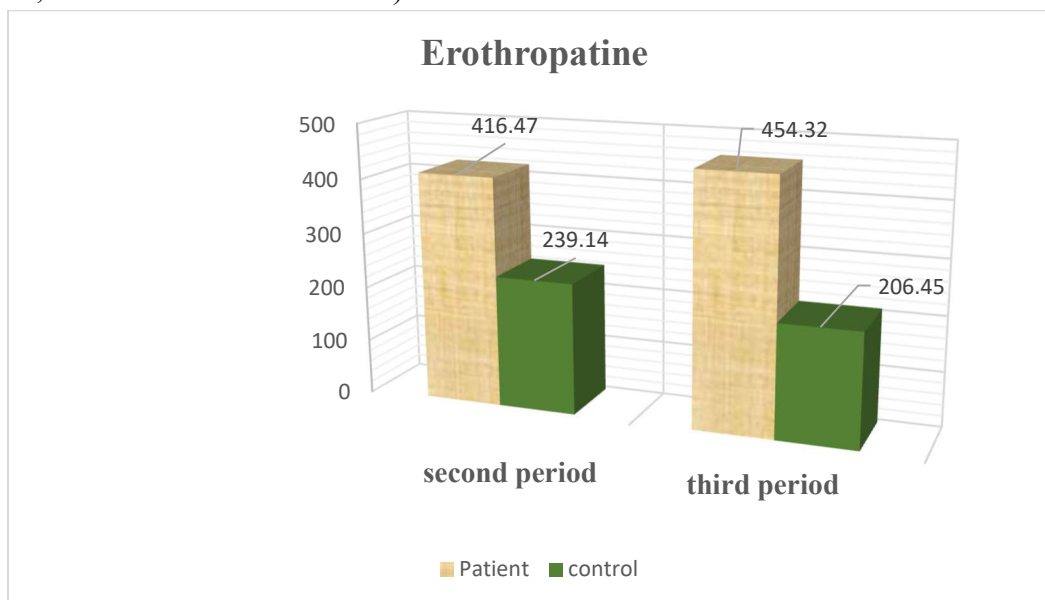


Figure (2) Erothropatine concentration in patients compared of control.

While The results shown a significant decrease in the levels of renalase in the blood serum of preeclampsia patients in third stage and second stage decrease ($P < 0.05$) compared with the control, The results we obtained were similar to those of previous(Heba Abdel basset Allam, Lecturer in department of Obstetrics and Gynecology2018), The results displayed that the pathophysiological development of preeclampsia in gestations is linked with alteration in levels of serum Renalase. Elevated blood pressure levels and renal damage that feature this pathological disorder is impacted by low serum. Renalase is a variety of monoamine oxidase enzyme that works Directly to the breakdown of catecholamines (noradrenaline, adrenaline and dopamine). Reduces in vivo blood pressure levels By inhibiting the systolic property, heart rate and Obstruction of the compensatory mechanism rises in the peripheral levels Vascular tone ^[29].

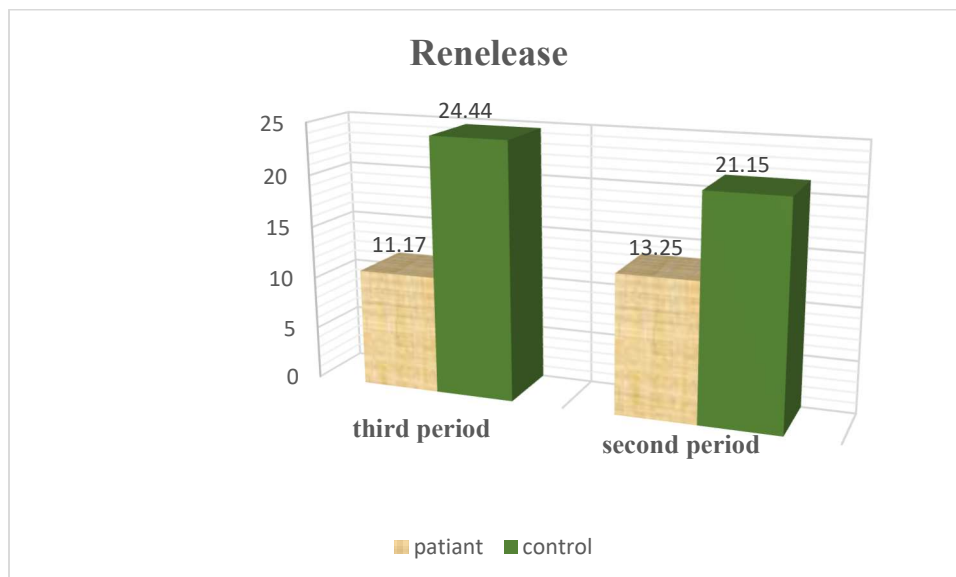


Figure (3) Renalase activity in patients compared of control.

Table(1) shows that the activity of prolidase enzyme increased significantly ($P \leq 0.01$) in patients compared with the control. This may indicate the association of increased enzyme activity with hypertension at week 26 and week 40 of pregnancy. The results of the current study agreed with the results of the study (Pehilvanet al., 2017), which indicated that there is a significant correlation between the activity of the enzyme prolidase and preeclampsia. Where it decreased significantly in the 13th week and after birth, this is confirmed by studies as indicated by Güven and his group (2014) to the low activity of the enzyme in the serums of pregnant women patients, as the enzyme plays an important role in the disease, The enzyme activity in the placenta is also increased.

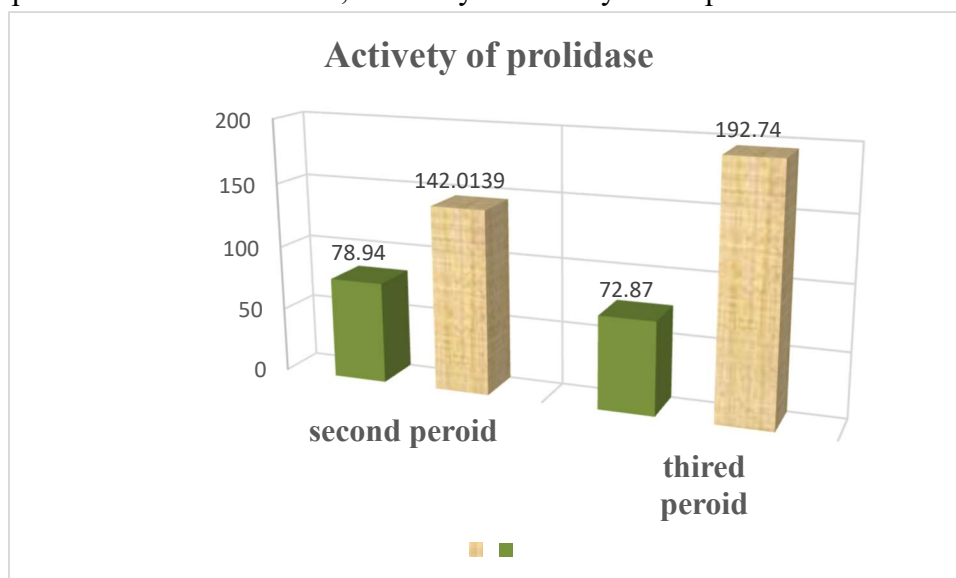


Figure (4) Activity of prolidase in patients ■ Second and third period compared of control ■.

Conclusion

For clinical examinations:

- 1-(high of zonulin protein prolidase enzyme, and erythropoietin hormon for women with preeclampsia in the second and third period of pregnancy compared to the control groups.
- 2- The level of Renalase decreased in the second and third period of pregnancy compared with the control group.
- 3- An increase in the level of triglycerides, which is directly related to obesity, and thus leads to high pregnancy pressure.

References

- 1- Say L, Chou D, Gemmill A, et al. Global causes of maternal death: a WHO systematic analysis. *Lancet Glob Health* ;2(6), 323–33(2014).
- 2-Grand'Maison, S. Pilote, L.; Okano, M.; Landry, T.; Dayan, N. Markers of vascular dysfunction after hypertensive disorders of pregnancy: A systematic review and meta-analysis. *Hypertension* 68,1447–1458 (2016).
- 3- Behrman RE, Butler AS, eds. *Preterm Birth: Causes. Washington, DC: Consequences, and Prevention*; 2007.
- 4-Keyes LE, Armaza JF, Niermeyer S, Vargas E, Young DA, Moore LG. Intrauterine growth restriction, preeclampsia, and intrauterine mortality at high altitude in Bolivia. *Pediatr Res.* 54(1), 20–25 (2003).
- 5-Osungbade, K. O. & Ige, O. K. Public health perspectives of preeclampsia in developing countries: Implication for health system strengthening. *J. Pregnancy*, 48(10),95 (2011).
- 6- Belay, A. S. & Wudad, T. Prevalence and associated factors of pre-eclampsia among pregnant women attending anti-natal care at Mettu Karl referral hospital, Ethiopia: Cross-sectional study. *Clin. Hypertens.* 25, 1–8 (2019).
- 7-Phupong, V. et al. Predicting the risk of preeclampsia and small for gestational age infants by uterine artery Doppler in low-risk women. *Arch. Gynecol. Obstet.* 268, 158–161 (2003).
- 8-Khanum, H. Prevalence of pre-eclampsia and factors responsible among third trimester pregnant women in hospital of dhaka. *Biomed. J. Sci. Tech. Res.* 33, 26089–26097 (2021).
- 9-Wallis, A. B., Saflas, A. F., Hsia, J. & Atrash, H. K. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987–2004. *Am. J. Hypertens.* 21, 521–526 (2008).
- 10- Vest, A. R. & Cho, L. S. Hypertension in pregnancy. *Curr. Atheroscler. Rep.* 16, 395 (2014).
- 11- Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. *Hypertension.*62(6),1046–1054(2013).
- 12- Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schomig A, et al. Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. *Clin Chem*48,1377–82 (2002).

- 13- Surazynski A, Miltyk W, Palka J, Phang JM. Prolidase-dependent regulation of collagen biosynthesis. *Amino Acids* 35: 731–738(2008).
- 14- Tkachenko O, Shchekochikhin D, Schrier RW. Hormones and Hemodynamics in Pregnancy. *Int J Endocrinol Metab* 12(2): e14098(2014).
- 15- Yeboah FA, Ngala RA, Bawah AT, Asare-Anane H, Alidu H, Hamid AM, Wumbee JDK. Adiposity and hyperleptinemia during the first trimester among pregnant women with preeclampsia. *Int J Womens Health*. 9, 449–54(2017).
- 16- Makharia GK. Intestinal permeability in portal hypertension: Still a dilemma. *Trop Gastro Enterol*. 34,119–22(2014).
- 17- Kim S, Goel R, Kumar A, Qi Y, Lobaton G, Hosaka K, Mohammed M, Handberg EM, Richards EM, Pepine CJ, Raizada MK. Plasma Zonulin, Along With a Unique Gut Microbiome Profile, Are Potential Predictors of Systolic Blood Pressure in Humans. *Hypertension*. 70,(suppl_1)(2017).
- 18- Moreno-Navarrete JM, Sabater M, Ortega F, Ricart W, Fernández-Real JM. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. *PloS One*. 7,37160(2012).
- 19- Lv LJ, Li SH, Li SC, Zhong ZC, Duan HL, Tian C, Li H, He W, Chen MC, He TW, Wang YN, Zhou X, Yao L, Yin AH. Early-onset preeclampsia is associated with gut microbial alterations in antepartum and postpartum women. *Front Cell Infect Microbiol*. 9-224 (2019).
- 20- Ohlsson B, Orho-Melander M, Nilsson PM. Higher levels of serum zonulin may rather be associated with increased risk of obesity and hyperlipidemia, than with gastrointestinal symptoms or disease manifestations. *Int J Mol Sci*. 18(3),582(2017).
- 21- Bah A, Pasricha SR, Jallow MW, Sise EA, Wegmuller R, Armitage AE, Drakesmith H, Moore SE, Prentice AM. Serum Hepcidin Concentrations Decline during Pregnancy and May Identify Iron Deficiency: Analysis of a Longitudinal Pregnancy Cohort in The Gambia–3. *The Journal of nutrition*.; 147(6):1131-7. (2017).
- 22- Qiu C, Phung TTT, Vadachkoria S, Muy-Rivera M, Sanchez SE, Williams MA: Oxidized Low-Density Lipoprotein (Oxidized LDL) and the Risk of Preeclampsia. *Physiol Res* 55:491–500(2006).
- 23- Timalsina S, Gyawali P, Bhattarai A: Comparison of lipid profile parameters and oxidized low-density lipoprotein between normal and preeclamptic pregnancies in a tertiary care hospital in Nepal. *Int J Womens Health* 8:627–631(2016).
- 24- YANG, Ying; LI, Jian-xin; CHEN, Ji-chun; CAO, Jie; LU, Xiang-feng; CHEN, Shu-feng; WU, Xi-gui; DUAN, Xiu-fang; MO, Xing-bo; GU, Dong-feng. Effect of elevated total cholesterol level and hypertension on the risk of fatal cardiovascular disease: a cohort study of Chinese steelworkers, *Chinese Medical Journal*: Nov- Volume 124 - Issue 22 - p 3702-3706(2017).
- 25- Lain KY, Catalano PM: Metabolic Changes in Pregnancy. *CLINICAL OBSTETRICS AND GYNECOLOGY*, 50(4):938–948(2017).

- 26- Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. *Circ Res.* 124(7):1094–112(2019).
- 27-Hershkovitz R, Ohel I, Sheizaf B, Nathan I, Erez O, Sheiner E, Pinku A, Mazor M. Erythropoietin concentration among patients with and without preeclampsia. *Arch Gynecol Obstet.* 273(3): 140–143(2005).
- 28- Osol G, Moore LG. Maternal uterine vascular remodeling during pregnancy. *Microcirculation.* 21(1):38–47(2014).
- 29- Hennebry SC, Eikelis N, Socratous F, Desir G, Lambert G, et al. Renalase, a novel soluble FAD-dependent protein, is synthesized in the brain and peripheral nerves. *Mol Psychiatry* 15(3): 234-236(2010).