Can FoxO1 and Sox2 TRANSCRIPTION FACTORS HELP IN PREDICTING PREECLAMPSIA!

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Preeclampsia (PE) is a complex and multifactorial disease of pregnancy, characterized by hypertension and proteinuria that typically occurs after 20 weeks of gestation. It is a significant cause of maternal and fetal morbidity and mortality worldwide. PE is estimated to affect 5-7% of all pregnancies globally and causes about 10% to 15% of maternal deaths (Rana *et al*., 2019). The incidence of PE is higher in developing countries, where access to adequate prenatal care may be limited. If left untreated, PE can lead to serious complications such as placental abruption, fetal distress, preterm delivery, and maternal organ failure (Phipps *et al*., 2019). Consequently, accurate and early diagnosis of PE is crucial for improving maternal and fetal outcomes (Huhn *et al*., 2020).

Many theories have been proposed to explain the pathogenesis of PE, including oxidative stress, endothelial dysfunction, inflammatory cytokines, genetic and dietary factors, and an imbalance between proangiogenic and antiangiogenic factors. These factors are thought to contribute to the development of the endothelial dysfunction that characterizes PE, leading to hypertension, proteinuria, and other clinical features of the disease (Opichka *et al*., 2021). Despite extensive research, the underlying causes of PE are not fully understood. Improving our understanding of the ultimate causes of PE is essential to develop better prevention and treatment strategies for PE (Johnson and Louis, 2022).

Current diagnosis of PE relies on the presence of hypertension and proteinuria, which are not always reliable indicators of the condition. Therefore, there has been a growing interest in identifying biomarkers that can be used for early diagnosis of PE (Kametas *et al*., 2022). Advances in genetics, epigenetics, and molecular biology have provided new insights into the mechanisms underlying PE (Amro *et al*., 2022). Forkhead transcription factor (TF) O1 (also called

FoxO1) and SRY (sex determining region Y)- box transcription factor 2 (also called Sox2) are products of genes that play important roles in various cellular processes, including differentiation, proliferation, and survival. Recent study has indicated that these transcription factors FoxO1 and Sox2 are linked to cell apoptosis and migration and may be associated with the behavior of trophoblast cells in PE (Sheridan *et al*., 2015; Weber *et al*., 2016).

Forkhead transcription factor subfamily O (FoxO) widely exists in various mammalian tissues and plays an important role in metabolism, cell proliferation, apoptosis, and stress resistance (Xu and Wang, 2021). The function of transcription factor FoxO1- a member of FoxO- is complex, which is mainly through the activation or inhibition of the transcription of its downstream target genes (Xing *et al*., 2018). FoxO1 in the endometrium has been shown to play an important role in the transformation of endometrium during menstruation, and in the protection of fetal mothers from oxidative damage during pregnancy (Kajihara *et al*., 2013). As a transcription factor, FoxO1 upregulation was found to promote adhesion and migration of trophoblast cells, thus inhibiting cell motility in PE (Chen *et al*., 2020). Furthermore, FoxO1 has been implicated in the pathogenesis of various diseases, such as diabetes, cancer, and neurodegenerative diseases (Liu *et al*., 2022). FoxO1 is also known to be involved in the regulation of the cell cycle, repair of DNA damage, and oxidative stress response (Lu and Huang, 2011).

On the other hand, Sox2 is the major regulator of the pluripotency of embryonic stem cells. It has a critical role in maintenance of embryonic and neural stem cells (Wang *et al*., 2012). Sox2 expression is found to be regulated by a negative feedback loop in embryonic stem cells that involves Protein kinase B (also called AKT) signaling and FoxO1 (Ormsbee-Golden *et al*., 2013). In this work, we examined the FoxO1 and Sox2 expression levels in sera of PE-Egyptian women and whether these can be checked during pregnancy to predict PE.

MATERIALS AND METHODS

In this study, 41 Egyptian pregnant women diagnosed with PE and 39 women with normal pregnancies- served as control group- were enrolled from outpatient and inpatient of the Obstetrics and Gynecology Department, Faculty of Medicine, Cairo University, Egypt. The study was performed with the approval of Faculty of Medicine, Cairo University local ethics committee and carried out in compliance with the Helsinki Declaration (2008) (Shaker *et al*., 2020). Informed consent was obtained from all the subjects enrolled in this study. The study excluded pregnant women with certain pre-existing conditions and risk factors, including a history of renal disease, diabetes, smoking, chromosomal abnormalities, alcoholism, and fetal congenital abnormalities. Characteristics of all enrolled subjects

including demographic, biochemical and haematological data were deducted from their clinical sheets.

The used technique- for assessment of FoxO1 and Sox2 expression level- is quantitative PCR (qPCR) technique that allows for the quantification of mRNA expression levels of both FoxO1 and Sox2 in a sample. It involves reverse transcription of RNA to cDNA followed by amplification of the cDNA using specific primers for FoxO1, Sox2 and a housekeeping gene for normalization of both.

RNA extraction

miRNeasy extraction kit from Qiagen (Valencia, CA) was used for total RNA extraction from serum, according to the manufacturer's instructions. This kit is designed for the extraction of both small and large RNA molecules, including miRNA, from a variety of sample types, including plasma, serum, and other biofluids. The kit utilizes QIAzol lysis reagent for cell lysis and RNA stabilization, followed by purification of total RNA using spin columns. Then, RNA concentration and purification were measured using a NanoDrop-2000 spectrophotometer (Thermo Scientific-USA).

Reverse transcription reactions

In this study, reverse transcription (RT) was performed on 60 ng of total RNA using the RT2 strand kit from Qiagen (Valencia, CA) following the manufacturer's guidelines. The RT2 strand kit is designed to convert RNA to cDNA in a two-step reaction, involving the reverse transcription of RNA to complementary DNA (cDNA) using a mix of oligo-dT and random hexamer primers, followed by a reaction that converts RNA-cDNA hybrids to double-stranded cDNA. The RT reaction was performed in a final volume of 20 µl, which contained the RNA template, RT2 First Strand Master Mix, and RT2 First Strand Enzyme Mix, according to the guidelines of the manufacturer [https://www.qiagen.com/us/products/discovery-and-translation-research/pcr-qpcr-dpcr/qpcr].

Quantitative PCR (qPCR)

Expression levels FoxO1 and Sox2 in serum determined using glyceraldehyde-3-phosphate dehydro-genase (GAPDH) gene as the internal control (Shaker *et al*., 2020). The expression levels were measured using quantitative real-time PCR (qPCR) and the Maxima SYBR Green PCR kit from ThermoScientific (USA) according to the manufacturer's instructions; qPCR is a sensitive and accurate technique that enables the quantification of nucleic acid molecules in real-time during the amplification process. The primer sequences used for GAPDH were: 5′-CCCTTCATTGACCTCAACTA-3′ for the GAPDH-forward and 5′-TGGAAGATGGTGATGGGATT-3′ for the GAPDH-reverse. These primers were designed to specifically amplify the target sequences, and GAPDH was used as an

internal control to normalize the data for variations in RNA input and reverse transcription efficiency. The qPCR reactions were performed using a thermal cycler with the following conditions: 95ºC for 10 min, followed by 45 cycles at 95ºC for 15s, 60ºC for 60s, and 70ºC for 30s. Afterwards, melting curve analysis was performed to validate the specific generation of the expected PCR product: [95ºC for 15s, 60ºC for 1 min. and 95ºC for 15s]. The relative expression level of FoxO1 and Sox2 were calculated using the comparative Ct (ΔΔCt) method, which involves normalizing the Ct values of FoxO1 or Sox2 to the housekeeping gene and comparing the normalized FoxO1 Ct (or Sox2) value to a control sample, *i.e.*, all the relative fold change (RFC) were calculated by using the $2^{-\Delta\Delta Ct}$ equation (Abd-Elkader *et al*., 2020).

Statistical analysis

Data were collected and coded to facilitate data manipulation and data analysis was performed using SPSS software *version* 22 (SPSS Inc., Chicago, IL, USA). Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data. For quantitative parametric data, an independent student t-test was used to compare measures of two independent groups of quantitative data. For quantitative nonparametric data, Kruskal–Wallis and Mann–Whitney tests were used in comparing more than two independent groups (Chan, 2003a). Receiver Operating Characteristic (ROC) curve was used to determine the sensitivity and specificity for the FoxO1 and Sox2 in predicting PE. *P* was considered statistically significant at values <0.05 (Chan, 2003b; Florkowski, 2008).

RESULTS AND DISCUSSION

Demographic data

These were obtained from the two studied groups (41 pregnant women who had PE and 39 apparently healthy pregnant females) and compared as illustrated in Table (1). The data were classified into maternal and neonatal data. Nonsignificant differences appeared between the two groups in respect to: age, body mass index (BMI), gravity, and parity. Blood pressure showed a highly significant difference between the two groups as all our PE patients were hypertensive (100%); as this is a characteristic of PE (Zhang *et al*., 2016). Intrauterine growth retardation (IUGR) showed highly significant (P<0.001) difference between the two groups. This is in line with another study made by Roberts and Escudero (2012), which referred this condition to problems with the blood supply to the placenta. As expected, fetal birth weight showed a highly significant difference to the sake of the control group (P<0.001), which is in harmony with preceding work too (Mansour *et al*., 2002).

Analysis of the clinical data obtained is illustrated in Table (2). Increased serum uric acid level in PE group significantly than control group was logical, as it

was reflected after deliver to the low feal birth weight. This result is in line with other study reported that in women with PE, maternal serum uric acid level is an important parameter for predicting low birth weight (Aelie *et al*., 2019). Increase in c-reactive protein (CRP) level to sake of the PE group (P<0.001) confirming the inflammation status of our PE patients. This result was in harmony with another study which attributed the elevation of CRP in PE to the systemic inflammation and endothelial and placental dysfunction that are characteristic of this condition (Sánchez-Aranguren *et al*., 2014). Haematological parameters and blood indices: mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) did not show significant difference between the two groups, this may be attributed to the small sample size. A qualitative evaluation for proteinuria was done and all our PE patients had proteinuria ranging from +1 to +4, as PE is considered a common reason for proteinuria (Müller-Deile and Schiffer, 2014).

Expression level of FoxO1 and Sox2

Our study revealed that FoxO1 transcription factor decreased significantly in the PE group than the control group as shown in Table (3), which is in line with a study by Hosaka *et al*., (2004) who had shown that FoxO1 knockout leads to embryo death due to vascular dysplasia. Another study by Lu *et al*., (2021) suggested that FoxO1 is not only involved in pregnancy but also related to restraint stress. This may be referred to indirect effect of FoxO1, that is accompanied by changes in the β2-adrenergic receptor (β2-AR) pathway- which is a signaling pathway that is involved in the regulation of many physiological processes- including the stress response which may be generated by mechanical stimuli on the vascular wall, that increase with hypertension. This explanation may fit with our study as all our PE patients were suffering from high blood pressure. However, FoxO1 has been shown to play a critical role in the regulation of glucose metabolism, insulin sensitivity, and cell survival as well (Rodrigo *et al*., 2011 and Zhang *et al*., 2016).

The FoxO1 pathway has been shown to play a role in the acute inflammation process too (Lu *et al*., 2021). When activated, this pathway can lead to an increase in glucocorticoid production, which in turn can have various effects on the body, including reducing insulin growth factor-1 (IGF-1) production and increasing tumour necrosis factor (TNF) alpha/NF-kB signaling, which is associated with protein hydrolysis (Xu and Wang, 2021), this explanation is in concordance with our results, where all subjects of the PE group were suffering from proteinuria. This may also explain the highly significant difference in CRP between the two studied groups.

Our results revealed also that Sox2 decreased in the PE group than the control group (Table 3), which is in harmony with several studies have shown that the gene expression level of Sox2 is altered in

preeclampsia. One study found that the expression of Sox2 decreased significantly in the placental tissue of women with PE compared to normal pregnancy controls (Vishnyakova *et al*., 2016). Another study concluded that it decreased in maternal serum samples from women with PE compared to normal pregnancy controls (Huhn *et al*., 2020). A third study analyzed the gene expression profiles of placental tissues from women with PE and found that *Sox2* was one of several genes that were significantly downregulated in the placental tissue of women with PE compared to normal pregnancy control group (Cui *et al*., 2021). Together, these studies suggest that changes in expression level of Sox2 may play a role in the pathogenesis of this condition. This may be explained as Sox2 interacts with other transcription factors in multiple signaling pathways to control growth and survival (Liu *et al*., 2013). Świstowska *et al*., (2019) reported its potential inhibitory effects on the expression of cyclin D1 and CDK4 kinase, where they analyzed the gene expression of Sox2 in the stem cells of Wharton's jelly isolated from twenty umbilical cords collected during childbirth.

Preceding number of abortions had reciprocal relationship with both FoxO1 and Sox2 transcription factors. Such association can be explained as both can lead to increase in ROS in endometriosis patients, which can lead to adverse effects on embryos, such as IUGR, spontaneous abortion, or fetal dysmorphogenesis (Lu *et al*., 2018). This may encourage for more research on this topic as both of FoxO1 and Sox2 expression can be used to predict PE in pregnant women at risk.

Correlations between FoxO1 and Sox2 with the investigated parameters

Pearson's and Spearman's correlations between each of FoxO1 and Sox2 and the investigated parameters are illustrated in Table (4). Both showed inverse significant correlation with qualitative alb. concentration in urine, and AF volume. FoxO1 did not show significant correlation with either IUGR or fetal BW, in contrast to Sox2. These results are augmented by preceding studies by Świstowska *et al*., (2019) in Wharton's jelly-derived stem cells, by Lien *et al*. (2020) in rats and finally by Brown *et al*., (2021) in mice. Surprisingly, none of FoxO1 or Sox2 showed significant correlation with CRP, this may be referred to the small sample size of the present work.

Performance of FoxO1 and Sox2 to predict PE

Efficacy of FoxO1 and Sox2 to predict PE- during pregnancy- was tested using ROC curve analyses (Fig. 1). Fig. (1a) shows potentiality of both in predicting PE due the AF volume, while Fig. (1b) shows the potentiality of both in predicting PE due the IUGR. Sensitivity and specificity with the cut off values are illustrated in Tables (5 and 6). FoxO1 was more sensitive (57.7%) and more specific (22.2%), than Sox2 (sensitivity was 42.3% and specificity was 11.1%), with $P< 0.01$ for both in predicting PE due to

the AF volume. While diagnosing PE depending on the IUGR showed more efficacy for FoxO1; it showed 69.6% sensitivity and 29.8% specificity, at cut off value <0.636 RFC. On the other hand, Sox2 showed 30.4% sensitivity and 12.3% specificity, at cut off value <0.685 RFC in the same context. This implies that FoxO1 is more efficient in predicting PE depending on either AF volume or IUGR. The relatively low performance of each of them separately may be attributed to the small sample size. Further research on larger PE cohort is needed to fully assure the role of both biomarkers in predicting or diagnosing PE.

CONCLUSION

- Our results concluded that FoxO1 and Sox2 transcription factors may act as molecular biomarkers for predicting PE.
- Further studies are needed- on large cohort- to confirm their diagnostic and therapeutic potentiality in PE, which will be a promising step towards improving the early diagnosis and treatment of PE, which could ultimately lead to better maternal and fetal outcomes.
- Studying polymorphism in both of FoxO1 and Sox2 transcription factors will be of great importance- as research point- among Egyptian women with repeated PE.

SUMMARY

Preeclampsia (PE) is a multisystem pregnancy disorder that affects about 10 million women worldwide. It is significantly associated with pregnancy-related fetal and maternal morbidity and mortality. PE has been linked to an increased risk of cardiovascular disease in women later in life too. Determining the exact etiology of PE has proved to be a hard task. So, it is critical to demonstrate how the gene regulatory mechanisms may help as therapeutic targets and diagnosis of PE. We assessed the expression level of FoxO1 and Sox2 transcription factors in sera of PE cases compared to control group. FoxO1 and Sox2 were estimated in all subjects using real time PCR technique, acting on mRNA of FoxO1 and Sox2 as a starting material in serum. Both FoxO1 and Sox2 showed decreased expression level in PE group than the control group. Each of them showed an inverse association with the increased preceding abortion numbers among the PE patients. Sensitivity and specificity of both FoxO1 and Sox2 for predicting PE were assessed by applying receiver operating characteristic (ROC) curve analysis; FoxO2 showed more sensitivity and specificity than Sox2 in predicting PE in women at risk. FoxO1 and Sox2 seem to play a critical role in PE pathogenesis. Our results suggest that FoxO1 and Sox2 might be promising predicting and/ or diagnostic molecular biomarkers for PE.

REFERENCES

Abd-Elkader A., Ismail H., Shaker O., Lotfy M., Abdel-Mageed W. and Keshk M., (2020). Alterations in

microRNA-15a and "γ-synuclein" expression levels among HCV-related hepatocellular carcinoma patients in Egypt. Research Journal of Applied Biotechnology, 6: 1-10. DOI: 10.21608/RJAB. 2020. 219593.

Aelie R., Cho N., Kim Y. and Lee E., (2019). Predictive value of serum uric acid levels for adverse perinatal outcomes in preeclampsia. Medicine, 98: p e15462. DOI: 10.1097/MD.0000000000015462.

Amro B., Aristondo M., Alsuwaidi Sh., Almaamari B., Hakim Z., Tahlak M., Wattiez A. and Koninckx P., (2022). New understanding of diagnosis, treatment and prevention of endometriosis. International Journal of Environmental Research and Public Health, 19: 6725. https://doi.org/10.3390/ijerph19116725.

Brown A., Wieben M., Murdock S., Chang J., Dizon M., St Pierre M., Chavez-Valdez R., Dorsky R. and Fung C., (2021). Intrauterine growth restriction causes abnormal embryonic dentate gyrus neurogenesis in mouse offspring that leads to adult learning and memory deficits. eNeuro, 8. DOI:10.1523/ENEURO.0062-21.2021.

Chan Y., (2003a). Biostatistics102: Quantitative Data – Parametric & Non-parametric Tests. Singapore Med J.; 44: 391-396. https://pubmed.ncbi.nlm.nih.gov/14700417/#.

Chan Y., (2003b). Biostatistics 103: Qualitative Data –Tests of Independence. Singapore Med J.; 44: 498-503. https://pubmed.ncbi.nlm.nih.gov/15024452/#.

Chen Y., Ding H., Wei M., Zha W., Guan S., Liu N., Li Y., Tan Y., Wang Y. and Wu F., (2020). MSC-Secreted Exosomal H19 Promotes Trophoblast Cell Invasion and Migration by Downregulating let-7b and Upregulating FoxO1. Molecular Therapy Nucleic Acids, 19: 1237-1249. Doi: 10.1016/j.omtn.2019.11.031.

Cui K., Zhu Y., Shi Y., Chen T., Wang H., Guo Y., Deng P., Liu H., Shao X. and Qin J., (2021). Establishment of trophoblast-like tissue model from human pluripotent stem cells in three-dimensional culture system. Advanced Science, 9: 2100031. https://doi.org/10.1002/advs.202100031.

Florkowski C., (2008). Sensitivity, specificity, receiver-operating characteristic (ROC) curves and likelihood ratios: communicating the performance of diagnostic tests. Clinical Biochemist Reviews, (Suppl 1): S83-87.

Hosaka T., Biggs W., Tieu D., Boyer A., Varki N., Cavenee W. and Arden K., (2004). Disruption of Forkhead transcription factor (FoxO) family members in mice reveals their functional diversification. Proceedings of the National Academy of Sciences U S A, 101: 2975-2980. DOI: 10.1073/pnas. 0400093101.

Huhn E., Hoffmann I., De Tejada B., Lange S., Sage K., Roberts Ch., Gravett M., Nagalla S. and Lapaire O., (2020). Maternal serum glycosylated fibronectin as a short-term predictor of preeclampsia: a prospective cohort study. BMC Pregnancy and Childbirth, 20: 128. https://doi.org/10.1186/s12884-020-2809-2.

Johnson J. and Louis J., (2022). Does race or ethnicity play a role in the origin, pathophysiology, and outcomes of preeclampsia? An expert review of the literature. The American Journal of Obstetrics and Gynecology, 226: S876-S85. DOI: 10.1016/j.ajog .2020.07.038.

Kajihara T., Brosens J. and Ishihara O., (2013). The role of FoxO1 in the decidual transformation of the endometrium and early pregnancy. Medical Molecular Morphology, 46: 61– 68. DOI: 10.1007/s00795-013-0018-z.

Kametas N., Nzelu D. and Nicolaides K., (2022). Chronic hypertension and superimposed preeclampsia: screening and diagnosis, American Journal of Obstetrics and Gynecology, 226: S1182-S1195. https://doi.org/10.1016/j.ajog.2020.11.029.

Lien Y., Wang P., Lu X. and Simmons R., (2020). Altered transcription factor binding and gene bivalency in islets of intrauterine growth retarded rats. Cells, 9: 1435. https://doi.org/10.3390/cells9061435.

Liu K., Lin B., Zhao M., Yang X., Chen M., Gao A., Que J. and Lan X., (2013). The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. Cell Signal, 25: 1264- 1271. DOI:10.1016/j.cellsig.2013.02.013.

Liu L., Bai J., Liu F., Xu Y., Zhao M., Zhao Ch. and Zhou Z., (2022). Cross-talking pathways of forkhead box O1 (FoxO1) are involved in the pathogenesis of alzheimer's disease and huntington's disease. Oxidative Medicine and Cellular Longevity, 7619255. DOI:10.1155/2022 /7619255.

Lu H. and Huang H., (2011). FoxO1: a potential target for human diseases. Current Drug Targets,12: 1235-1244.DOI:10.2174/138945011796150280.

Lu J., Wang Z., Cao J., Chen Y. and Dong Y., (2018). A novel and compact review on the role of oxidative stress in female reproduction. Reproductive Biology Endocrinolo-

gy, 16: 80. https://doi.org/10.1186/s12958- 018-0391-5.

Lu J., Chen Y., Wang Z., Cao J. and Dong Y., (2021). The role of the FoxO1/β_2-AR/p-NF-κB p65 pathway in the development of endometrial stromal cells in pregnant mice under restraint stress. International Journal of Molecular Sciences, 22: 1478. DOI: 10.3390/ijms22031478.

Mansour E., Eissa A., Nofal L., Kharboush I., Anwar W. and Sallam I., (2002). Incidence and factors leading to low birth weight in Egypt. International Pediatrics, 17: 223-230.

Müller-Deile J. and Schiffer M., (2014). Preeclampsia from a renal point of view: Insides into disease models, biomarkers and therapy. World Journal of Nephrology, 6:169-81. DOI: 10.5527/wjn.v3.i4.169.

Opichka M., Rappelt M., Gutterman D., Grobe J. and McIntosh J., (2021). Vascular dysfunction in preeclampsia. Cells, 10: 3055. https://doi.org/10.3390/cells10113055.

Ormsbee-Golden B., Wuebben E. and Rizzino A., (2013). Sox2 expression is regulated by a negative feedback loop in embryonic stem cells that involves AKT signaling and FoxO1. PLoS ONE, 8: e76345. DOI: 10.1371/journal.pone.0076345.

Phipps E., Thadhani R., Benzing T. and Karumanchi S., (2019). Pre-eclampsia: pathogenesis, novel diagnostics and therapies [published correction appears in Nat. Rev. Nephrol. 2019 Jun; 15 (6):386]. Nature Reviews Nephrology, 15: 275-289. DOI:10.1038/s41581-019-0119-6.

Rana S., Lemoine E., Granger J. and Karumanchi S., (2019). Preeclampsia: pathophysiology, challenges, and perspectives. Circulation Research, 124: 1094-1112. https://doi.org/10.1161/CIRCRESAHA.118.313276.

Roberts J. and Escudero C., (2012). The placenta in preeclampsia. Pregnancy Hypertension, 2: 72-83. DOI: 10.1016/j.preghy.2012.01.001.

Rodrigo R., González J. and Paoletto F., (2011). The role of oxidative stress in the pathophysiology of hypertension. Hypertension Research, 34: 431-440. https://doi.org/10.1038 /hr.2010.264.

Sánchez-Aranguren L., Prada C., Riaño-Medina C. and Lopez M., (2014). Endothelial dysfunction and preeclampsia: role of oxidative stress. Frontiers in Physiology, 5:

372. DOI:10.3389/fphys.2014.00372.

Shaker O., Mahfouz H., Salama A. and Medhat E., (2020). Long non-coding HULC and miRNA-372 as diagnostic biomarkers in hepatocellular carcinoma. Reports of Biochemistry & Molecular Biology, 9: 230-240. Doi:10.29252/rbmb.9.2.230.

Sheridan R., Belludi C., Khoury J., Stanek J. and Handwerger S., (2015). FOXO1 expression in villous trophoblast of preeclampsia and fetal growth restriction placentas. Histology and Histopathology, 30: 213-222. Doi:10.14670/HH-0.3213.

Świstowska M., Gil-Kulik P., Krzyżanowski A., Bielecki T., Czop M., Kwaśniewska A. and Kocki J., (2019). Potential effect of Sox2 on the cell cycle of Wharton's Jelly stem cells (WJSCs). Oxidative medicine and cellular longevity, 5084689. DOI: 10.1155/2019/5084689.

Vishnyakova P., Volodina M., Tarasova N., Marey M., Tsvirkun D., Vavina O., Khodzhaeva Z., Kan N., Menon R., Vysokikh M. and Sukhikh G., (2016). Mitochondrial role in adaptive response to stress conditions in preeclampsia. Scientific Reports, 6: 32410. DOI: 10.1038/srep32410.

Wang Z., Oron E., Nelson B., Razis S. and Ivanova N., (2012). Distinct Lineage Specification Roles for NANOG, OCT4, and SoX2 in Human Embryonic Stem Cells. Cell Stem Cell, 10, Issue 4: 440-54. DOI 10.1016/j.stem.2012.02.016.

Weber M., Göhner C., San Martin S., Vattai A., Hutter S., Parraga M., Jeschke U., Schleussner E., Markert U. and Fitzgerald J., (2016). Unique trophoblast stem cell- and pluripotency marker staining patterns depending on gestational age and placenta-associated pregnancy complications. Cell Adhesion & Migration, 10: 56- 65. Doi: 10.1080/19336918.2016.1142035.

Xing Y., Li A., Yang Y., Li X., Zhang L. and Guo H., (2018). The regulation of FoxO1 and its role in disease progression. Life Sciences, 193: 124-131. DOI: 10.1016/j.lfs.2017.11. 030.

Xu R. and Wang Z., (2021). Involvement of transcription factor FoxO1 in the pathogenesis of polycystic ovary syndrome. Frontiers in Physiology, 12: 649295. DOI: 10.3389/fphys.2021.649295.

Zhang T., Kim D., Xiao X., Lee S., Gong Z., Muzumdar R., Calabuig-Navarro V., Yamauchi J., Harashima H., Wang R., Bottino R., Alvarez-Perez J., Garcia-Ocaña A., Gittes G. and Dong H., (2016).

FoxO1 plays an important role in regulating β-cell compensation for insulin resistance in male mice. Endocrinology, 157:1055-1070. DOI: 10.1210/en.2015-1853

Electronic References:
https://www.qiagen.com/us/products/discovery-and-translation-research/pcr-qpcr-dpcr/qpcr.

Table (1): Demographic data of the studied groups.

Variable	Control group (n=39)	PE group (n=41)	*P*
Maternal data:			
Age: Years	31.00±6.60	29.73±6.87	N.S.
(Range)	(17-41)	(18-42)	
BMI	32.36±4.73	31.20±5.29	N.S.
BP:			
Systolic	116.79±12.95	164.39±17.57	<0.001
Diastolic	72.31±7.05	108.29±11.05	<0.001
IUGR	0.08±0.27	0.49±0.51	<0.001
Gravity	2.15±2.18	2.10±2.11	N.S.
Parity	1.64±1.80	1.71±1.60	N.S.
Neonatal data: BW	3.28±.39	2.84±.49	<0.001

Values are presented as mean± standard deviation or frequency as numbers (%). BMI: Body Mass Index; Bp: blood pressure; IUGR: intrauterine growth retardation; BW: birth weight, N.S.: non-significant; *P*< 0.01: highly significant; *P*< 0.001: very highly significant.

able (2): Biochemical and haematological parameters of the studied groups.

Variable	Control group (n=39)	PE group (n=41)	*P*
Biochemical parameters			
ALT	18.36±10.16	33.83±50.05	N.S.
AST	18.46±9.01	36.37±44.05	<0.05
Total Bil.	0.59±0.25	0.53±0.26	N.S.
Direct Bil	0.13±0.09	0.09±0.08	N.S.
ALP	73.82±2.13	74.85±5.98	N.S.
Urea	26.23±10.20	25.24±9.96	N.S.
Uric acid	3.46±0.70	3.89±0.70	<0.01
Albumin	3.19±0.27	3.12±0.39	N.S.
CRP	15.15±15.38	37.24±39.83	<0.01
Haematological parameters:			
Hgb	10.74±1.25	10.74±1.19	N.S.
Hct	33.34±2.71	33.41±2.45	N.S.
RBCs ($x10^6$)	4.15±0.28	4.09±0.34	N.S.
MCH	27.08±3.52	26.73±3.24	N.S.
MCHC($x10^3$)	32.24±2.85	33.33±8.74	N.S.
WBCs ($x10^3$)	8.12±2.61	8.23±3.027	N.S.
Platelets 10^3)	304.26±80.79	286.78±95.59	N.S.

Values are presented as M±SD. ALT: alanine transaminase; AST: aspartate transaminase; Bil.: bilirubin; ALP: alkaline phosphatase; CRP: C-reactive protein; Hgb: haemoglobin; Hct: haematocrite; RBCs: Red blood cells; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBCs: White blood cells; N.S.: non-significant; *P*-value< 0.05: significant; *P*-value< 0.01: highly significant.

Table (3): Expression level of FoxO1 and Sox2 in both groups.

Variable		Control group (n=39)	PE group (n=41)	*P*
FoxO1	(RFC)	1.0060±0.15	0.50±0.42	<0.001
Sox2	(RFC)	1.0005±0.05	0.83±0.89	N.S.

Values are presented as mean± standard deviation; RFC: relative fold change; N.S.: non-Significant; *P*-value< 0.001: very highly significant.

Table (4): Correlations between each of FoxO1, Sox2 with different estimated parameters.

Biomarker	FoxO1		Sox2	
	r	*P*	*r*	*P*
AST	0.294	<0.01	-0.041	N.S.
MAP	-0.470	<0.01	-0.262	<0.05
Uric acid	-0.211	N.S.	-0.275	<0.05
CRP	-0.163	N.S.	-0.085	N.S.
IUGR	-0.097	N.S.	-0.363	<0.01
BW	0.214	N.S.	-0.295	<0.01
Severity	-0.633	<0.01	-0.480	<0.01
Alb. in urine (qualitative)	-0.598	<0.01	-0.519	<0.01
AF	-0.361	<0.01	-0.323	<0.01

MAP: mean arterial pressure (mmHg); CRP: c-reactive protein; IUGR: intra uterine growth retardation; BW: birth weight; AF: amniotic fluid, Alb.: albumin.

Table (5): Performance of FoxO1 and Sox2 molecular biomarkers to predict PE due to AF volume.

Biomarker	AUC	Cut off value	Sensitivity	Specificity	95% CI	*P*
FoxO1 (RFC)	0.278	<0.592	57.7%	22.2%	0.162-0.394	<0.01
Sox2 (RFC)	0.301	<0.567	42.3%	11.1%	0.153-0.450	<0.01

RFC: relative fold change; AUC: area under the curve; CI: confidence interval; *P*< 0.01: highly significant.

Table (6): Performance of FoxO1 and Sox2 molecular biomarkers to predict PE due to IUGR.

Biomarker	AUC	Cut off value	Sensitivity	Specificity	95% CI	*P*
FoxO1 (RFC)	0.361	<0.636	69.6%	29.8%	0.236-0.486	N.S.
Sox2 (RFC)	0.227	<0.685	30.4%	12.3%	0.091-0.362	<0.001

RFC: relative fold change; AUC: area under the curve; CI: confidence interval; N.S.: non-significant; *P*<0.001: very highly significant.

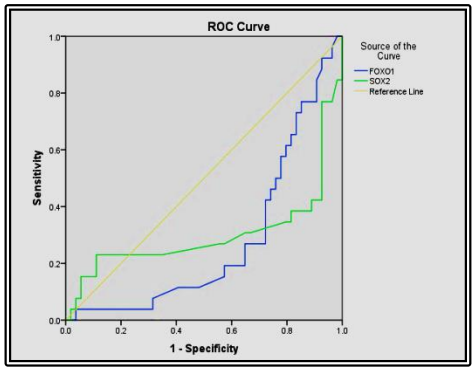

Figure (1a)

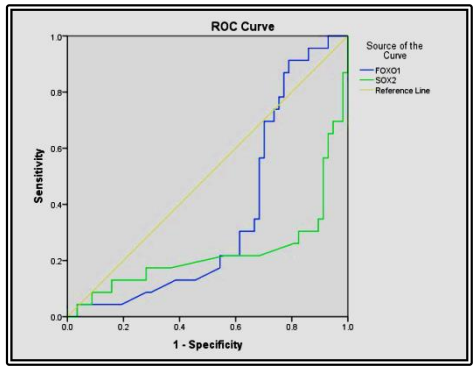

Figure (1b)

Fig. (1): ROC curve analysis for FoxO1 and Sox2 to predict PE cases (a) due to AF volume, and (b) due to IUGR.