Azhar Int J Pharm Med Sci 2025; Vol 5 (1):202 - 215
Research Article

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Genetic Factors in Preeclamptic Egyptian Women: Relation with Arginine Vasopressin; A Case-Control Study

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Article history: Received 2023-01-09 **Revised 2023-07-22** **Accepted 2024-06-04**

Abstract: Preeclampsia (PE) is one of the leading causes of prenatal and maternal deaths; therefore developing new biomarkers at the earliest stages of illness is essential to accelerating effectiveness. Using serum microRNA 149 and copeptin to diagnose preeclampsia will be investigated in this study. We conducted a matched case–control study in Egyptian *women*. Seventy women between 28 and 40 weeks of pregnancy participated in the study and divided into two groups: 20 pregnant women without PE and 50 with PE. Blood biochemical parameters were assessed for all subjects in addition to genetic analysis for microRNA 149 was detected by RT-PCR. Serum copeptin was detected by ELISA. The present study demonstrated that; preeclamptic patients had considerably lower serum levels of miR-149 than control cases at ($p \leq 0.01$). Meanwhile, preeclamptic patients had considerably higher serum levels of copeptin than controls at ($p \leq 0.01$). Serum copeptin levels may provide early indicators of preeclampsia.

Keywords: Preeclampsia; MicroRNA149; Copeptin; RT-PCR.

1. INTRODUCTION

Preeclampsia manifests as newly developed proteinuria (300mg/24 hours) in hypertensive women who do not have it before 20 weeks of pregnancy [1]. The Global Health Organization estimates that high blood pressure diseases contribute to 16% of all maternal mortalities in affluent nations, while their prevalence is nearly seven times higher in poor nations [2]. The inability to understand the mechanisms causing PE prevents early diagnosis and treatment. The discovery of novel biomarkers is essential for making an early diagnosis. Novel biomarkers may also help in the discovery of new treatment targets. Novel biomarkers may also make it possible to identify new therapeutic targets for this fatal condition. In the current study, we concentrated on the amounts of microRNA 149 and copeptin in the serum of Egyptian women with preeclampsia.

In preeclampsia, the placenta receives insufficient nutrition because of poor placental vascularization [3], Placental ischemia comes next, which creates a stressful environment that promotes inflammation and cell damage [4]. If the placenta is not operating at full capacity, the excess flow of blood that ought to be coming from the women may not pass via, resulting in high blood pressure, edema, and even renal complications. As a result of the mother's inability to remove waste products quickly enough, they accumulate in her blood as well as causing proteinuria due to the leakage of some essential proteins into her urine instead of staying in her bloodstream [5]. The generalized endothelial change in preeclampsia generates supplemental hypoxic injury of the endothelium by increasing vasoconstriction, promoting platelet adhesion and aggregation [6].

Animals, plants, and some viruses all have little non-coding RNAs called miRNAs. They are about 22 nucleotides in length. They attach to 3'-untranslated regions to control the post-transcriptional expression of the gene. By joining the 3' and 5' non-translated regions or coding sequences of targeted mRNAs, they control gene

Cite this article: Younis NF., Rashed LA., El-Mandoury AA., and Ellakwa DE. Genetic Factors in Preeclamptic Egyptian Women: Relation with Arginine Vasopressin; A Case-Control Stud. Azhar International Journal of Pharmaceutical and Medical Sciences, 2025; 5 (1): 202-215 doi: 10.21608/aijpms.2024.185070.1186

expression post-transcriptionally and cause RNA silence or mRNA destruction [7]. A few other genomic sites where miRNAs are encoded include introns/exons of non-coding RNA genes, introns of protein-coding genes, and intragenic regions [8].

MicroRNAs may influence gene expression and link with various diseases. Additionally, microRNAs aid in the conceiving and preserving of pregnancy [9] by controlling important processes such as immunological tolerance, inflammation, angiogenesis, and death. Several conditions connected to pregnancy disorders as pregnancy-induced hypertension, restricted intrauterine growth, and low birth weight, are characterized by aberrant expression of miRNAs [10].

Preeclampsia is governed by miRNAs. Several miRNAs (angiomas) change angiogenic pathways, which is crucial for the pathophysiology of PE. Human umbilical vein endothelial cells are prevented from migrating when the levels of the vascular endothelial growth factors (HUVECs) regulating miR-29 and miR-16 are decreased[11]. The cyclin-dependent kinase 1 (CDK1) and cyclin D1 (CCND1) are the targets of MiR-494, which prevents the transition between the growth and synthesis cycles [12].

According to estimates, microRNA-149's two isoforms target both tumor suppressors and oncogenes having a dual effect on cancer. While miR-149-5p5p participates in the insulin and chemokine signal transduction pathways, both are required for cancer progression, miR-149-3p includes participation in toll-like receptors up-regulating, T and B signal transduction pathway, cell adhesion pathway, smooth muscle contraction pathway and the lysosome pathway which are also connected to tumor progression and tumorigenesis [12]. However, its use for the pathogenesis of PE is still under study.

Pre-pro-AVP, a 164-amino-acid peptide encoded by the arginine vasopressin (AVP) gene, is made by neurons in the hypothalamic neurohypophysial system. AVP, which also contains a signal peptide, neurophysin II, and copeptin is derived from the mature pre-pro-AVP[13].Therapeutic significance of Arginine vasopressin (AVP) in preserving body fluid and vascular stiffness[14]. Unfortunately, due to its unstable nature and brief half-life, measurement is almost impossible [15].

Arginine vasopressin may play a role in the pathophysiology of preeclampsia, according to certain research. Pregnant mice given AVP showed signs of human PE, including placental oxidative stress, changed placental shape, hypertension, renal glomerular endothelins, intrauterine growth restriction, decreased placental growth factor (PGF), and altered placental gene expression[16].

Preeclampsia is distinguished from a normotensive pregnancy by a low-renin hypertensive condition. It is suggested that plasma arginine vasopressin (AVP) measures may be used to predict preeclampsia because other non-pregnant low-renin hypertensive diseases frequently reflect and often depend upon enhanced AVP secretion [17]**.**

Copeptin is an inactive prosegment of AVP that is released in a 1:1 molar ratio and has a significantly longer biological half-life than AVP, making it a potential biomarker of AVP secretion in clinical situations[18].

Contrasting to AVP, copeptin is a glycopeptide with 39 amino acids that is permanent in plasma and serum at cellar temperature and is simple to test as a sign of AVP[19]. Copeptin has recently gained attention as a promising biomarker in a number of acute illnesses, including sepsis, stroke, and acute pancreatitis[13]. Meanwhile, its application as a biomarker for PE is still being evaluated.

When exposed to hyperosmotic stimuli, the neurohypophysis co-secreted copeptin in an equimolar proportion with AVP. Pre-pro-AVP is thought to fold properly because of it. However the blood regulation of blood volume and electrolyte balance is a function of AVP, the exact function of copeptin's role is yet unknown[20].

The aim of this study is to investigate serum microRNA 149 level in PE and study the efficacy of dual usage of serum copeptin and microRNA 149 in early diagnosis of PE.

2. METHODS

2.1. Study Population

Seventy pregnant women participated in a case-control study of pregnant mothers in their second and third trimesters of gestation who visited Kasr Al-Ainy Hospital between March 2020 and March 2021.

Prior to sample collection, all participants underwent a clinical examination, and they were divided into two groups: 20 pregnant women (without any medical conditions) and 50 PE women (with proteinuria and hypertension >140/90 mm Hg). The Helsinki Agreement of 1975 and the hospital's ethical oversight committee both gave their approval to the study's protocol. The study's biochemical parameters were altered, so any patients with a history of cardiac, hepatic, renal, or other metabolic diseases were excluded.

2.1.1. Inclusion Criteria

The study involved pregnant women between 28 and 40 weeks gestation with preeclampsia (blood pressure ≥ 140/90 mm Hg and protein in urine ≥ 300 mg/24 hours).

2.1.2. Exclusion Criteria

- Pregnant women with any other medical disorders affecting blood Pressure such as chronic renal disease, thyroid disease and, long-standing diabetes were excluded.
- Pregnant women with chronic hypertension were excluded.

2.2. Blood Samples

In accordance with the commendations of the national committee of clinical laboratory standards, blood samples were collected. Following overnight fasting, ten ml of blood was punctured from each woman. Four sections of each blood sample were divided into using the automated hematology cell counter SYSMEX XT-2000i which operates on the idea of light deflection, two ml of blood were accumulated into test tubes containing (K3 EDTA) for a full blood picture, which includes hematocrit, hemoglobin, platelet count, RBCs count and total leukocytic count[21]. To obtain discrete plasma, two ml were drawn into citrate tubes and centrifuged. Using an automated coatron A4, prothrombin time and prothrombin concentration were measured on separate plasma samples[22], six ml of blood were drawn into vacutainers, allowed to sufficiently coagulate, and then the serum was isolated by centrifuging the blood at 3000 rpm for ten minutes. Dividing the collected serum into three aliquots; the first aliquot was used for determining liver function and kidney function. The second aliquot was stored at -80 ºC until the time of the microRNA149 analysis. The third aliquot was stored at -40 ºC until the date of copeptin measurement.

The concentration of serum microRNA 149 was measured using the RT-PCR method, using a microRNA149 from QIAGEN (USA) [23].

2.2.1. RNA Extraction

The 1,000 µl QIAzol lysis reagent, the miRNeasy extraction kit (Qiagen, Valencia, CA, and the USA), and room temperature incubation for 5 minutes were used to extract microRNAs from 200 µl of serum. 200µl of chloroform was added, vortexed for 15 s, and then allowed to rest for two or three minutes at room temperature. Centrifugation was then carried out at12, 000×g for 15 minutes at 4 °C. After becoming extracted, the upper aqueous phase was added to ethanol (100%) at a ratio of 1.5 times its volume. This mixture was put into a collecting tube with an RNeasy Mini spin column, and 700 µl of it was separated for 15 seconds at ambient temperature (at 8,000×g). Following a full pass of the mixture through each column, 700 µl of RWT buffer was poured into every column and they were individually centrifuged once more for 15 seconds (at 8,000×g, at RT). The column was filled with 500 µl of buffer RPE (centrifuged; 8,000×g; 15 s, RT). RNA was extracted from the column by centrifuging it for 1 minute at 8,000×g while introducing 50 µl of RNase-free water into a 1.5-ml collecting tube.

2.2.2. Reverse Transcription and Real-Time Quantitative PCR

Reverse transcription was carried on total RNA according to the manufacturer's recommendation using the miScript II RT package (Qiagen, USA) in a total volume of 20 µl RT processes which included 4µl of cDNA reaction buffer, 2 µl of 10x miScript Nucleics Mix, 5 µl of RNase-free water, 2 µl of miScript Reverse Transcriptase Mix and 7 µl of Template RNA. 20 µl of RT processes (cultured for 60 min at 37°C and 5 min at 95°C), a kit with miScript SYBR Green PCR (Qiagen, USA) in a total volume of 25 µl PCR processes which included12.5 µl of 2x QuantiTect SYBR Green PCR Master Mix, 2 µl of 10x of miscript Primer Assay, 2 µl of 10x miscript Universal Primer, 3.5 µl of RNase-free water and 5 µl of template cDNA, as well as primers for miR-149 and mir U6 as a housekeeping gene, with the sequences listed below. For real-time qPCR, the forward primers 5-AGGGAGGGACGGGGGCTGTGC-3, 5-CTCGCTTCGGCAGCACA-3 and reverse 5-CTCAACTGGTGTCGTGGA-3, 5-CTCGCTTCACGAATTTGCGT-3 were used (Qiagen, USA). Fluorescence was detected and recorded using the Rotor-Gene Q Real-Time PCR System for 40 cycles of 94 °C for 15 seconds, 55 °C for 30 seconds, and 70 °C for 34 seconds. ROX was used as the reference dye. Twenty nanograms of cDNA were utilized as a guide in reaction with a total amount of twenty microliters (Qiagen, USA). The Ct method was used to find the miR-149 expression levels. The cycle cut-off point (Ct) value is the number of qPCR cycles required for the fluorescent signal to pass a specified level. Ct qualities were obtained by deducting target microRNA Ct qualities from U6 Ct calculated values. The value of ΔΔCt was estimated by deducting the ΔCt of the control specimen from the ΔCt of the infected specimen by using equation $2^{-\Delta\Delta Ct}$; the percent change in miR-149 expression was measured.

An ELISA approach was used to determine serum copeptin using a copeptin kit bought from WKEA med supplies (China) [24].

The kit "Human copeptin level in the serum sample" coated microtiter plate wells with pure human copeptin antibody, creating a solid-phase antibody, and then copeptin was introduced to wells. Copeptin antibody was mixed with enzyme-labeled, horse radish peroxidase-catalyzed became antibody-antigen - enzyme-antibody compound was formed after washing completely; organic matter was added that gave blue color. The process was stopped by adding a sulphuric acid solution (stop solution) and the color turned to yellow, which was detected spectrophotometrically at 450 nm. The optical density of the samples was compared to calculate the amount of copeptin in the samples.

2.3. Urine Samples

Protein in urine was evaluated using the dipstick procedure after collecting urine specimens in sterile, clean containers and the color obtained was matched to a chromatic scale. Color progression was detected by various degrees of green to blue. Observations were recorded in the form of negative, trace, 1+, 2+, 3+, and 4+ corresponding to each alteration. Preeclampsia must be evaluated with proteinuria (300mg/24h or 1+ by dipstick) at least two times discovered at least 4 hours apart. Preeclampsia is categorized as mild if there is albumin in the urine of 1+, no sign of organ damage, and albumin in the urine of more than 5 g in 24 hours, or if there is ≥3+ on two random urine samples that are categorized as severe [25].

2.4. Statistical Data Analysis

IBM SPSS statistics (V. 25.0, IBM Corp., USA, 2021) were employed for the analysis of data. For quantitative parametric measures, data were displayed as Mean±SD in addition to number and percentage for category data.

The corresponding tests were conducted:

1. To check the data distribution between two groups, use Kolmogorov-Smirnov and Shapiro-Wilk tests.
2. Using an independent t-test to compare the two groups for parametric data.
3. Using chi-square test to analyze frequencies and percentages of data

3. RESULTS

3.1 Clinical, hematological and biochemical characteristics of the study subjects

The clinical and demographic data of the enrolled subjects are shown in **Table 1**. The average age of the participants was 31.45 years for the control group and 32.68 years for the preeclampsia group. Statistical analysis revealed no significant difference in age between the two groups (P = 0.1). Similarly, there was no significant difference in gestational age (P = 0.3) between groups. However, significant differences were observed in systolic blood pressure (P < 0.001) and diastolic blood pressure (P < 0.001) between the groups **(Table 1 & Figure 1)**. The preeclampsia group exhibited significantly higher blood pressure levels compared to the control group. Furthermore, the magnitude of body mass index (BMI) in the preeclampsia group was found to be significantly higher than that of the control group (P < 0.001). This indicates a greater prevalence of obesity among individuals with preeclampsia compared to those without any medical conditions.

Table1. Clinical and demographic parameters in control, and preeclamptic groups.

Variables	Control (n=20)	Preeclampsia (n=50)	P Value	Significance
	Mean±SD	**Mean±SD**		
Age (years)	31.45±2.65	32.68±2.92	0.1	NS
Gestational age (weeks)	36.8±1.77	36.08±2.88	0.3	NS
Body Mass Index (Kg/ m^2)	32.08±2.15	36.35±4.07	<0.001	HS
Diastolic BP (mmHg)	77.25±6.58	104±10.1	<0.001	HS
Systolic BP (mmHg)	122.75±11.53	166.5±17.68	<0.001	HS

NS: No significant difference was observed (P >0.05), HS: High significant difference was observed (P≤0.01)

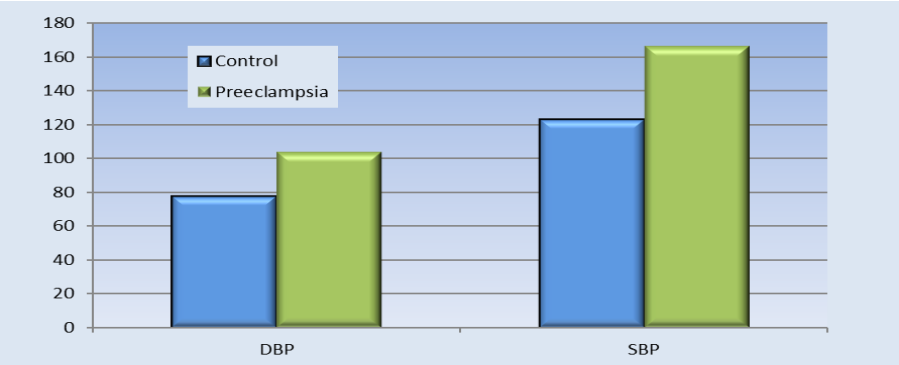

Figure1. Mean ± SD of diastolic and systolic blood pressure in control and preeclamptic groups (SBP, systolic blood pressure; DBP, diastolic blood pressure).

The analysis of various hematological parameters between the preeclampsia (PE) and control groups revealed no significant differences in several key measures. Hemoglobin (Hb) levels were similar between the groups, with a mean of 11.24 mg/dL in the PE group and 11.21 mg/dL in the control group ($P > 0.05$). Similarly, hematocrit (Hct) levels indicated no significant variation, with the PE group having a mean Hct level of 40.86% compared to 42% in the control group ($P > 0.05$). For the red blood cell (RBC) count, the mean value in the PE group was 5.32 cells/mcL, while the control group had a mean of 4.18 cells/mcL; however, this difference was not statistically significant ($P > 0.05$). The total leukocyte count (TLC) also revealed no significant difference, with the PE group having a mean TLC of 8.52 cells/mcL compared to 8.93 cells/mcL in the control group ($P > 0.05$) shown in **Table 2**

A highly significant difference was observed in platelet (PLT) counts between the two groups ($P < 0.001$). The mean platelet count was significantly lower in the control group ($31 \times 10^3/mm^3$) compared to the PE group ($200.38 \times 10^3/mm^3$) **(Figure 2).** However, other coagulation parameters, such as prothrombin time (PT) and prothrombin concentration, did not differ significantly. The mean PT was 13.43 seconds in the PE group and 13.39 seconds in the control group ($P > 0.05$), while the mean prothrombin concentration was 99.52 mg/dL in the PE group and 99.1 mg/dL in the control group ($P > 0.05$). Finally, the International Normalized Ratio (INR) was comparable between the groups, with a mean of 1.00 in the PE group and 0.99 in the control group, showing no significant difference ($P > 0.05$) shown in **Table 2.**

Table2. Mean ± SD of hematological parameters in control, and preeclamptic groups.

Variables	Control (n=20)	PE (n=50)	P Value	Significance
	Mean±SD	Mean±SD		
Hemoglobin (mg/dL)	11.21±0.59	11.24±1.06	0.9	NS
Hematocrit (percent)	42±1.52	40.86±2.51	0.06	NS
Red Blood Cell (cell/mcL)	4.18±0.34	5.32±6.19	0.4	NS
Total Leukocyte Count (cell/mcL)	8.93±1.82	8.52±1.97	0.4	NS
Platelets ($10^3/mm^3$)	313.15±28.79	200.38±36.5	<0.001	HS
Prothrombin time (seconds)	13.39±0.26	13.43±0.45	0.6	NS
Prothrombin concentration (mg/dl)	99.1±2.15	99.52±3.35	0.6	NS
International normalized ratio (INR)	0.99±0.02	1±0.03	0.6	NS

NS: No significant difference was observed (P >0.05), HS: High significant difference was observed (P≤0.01)

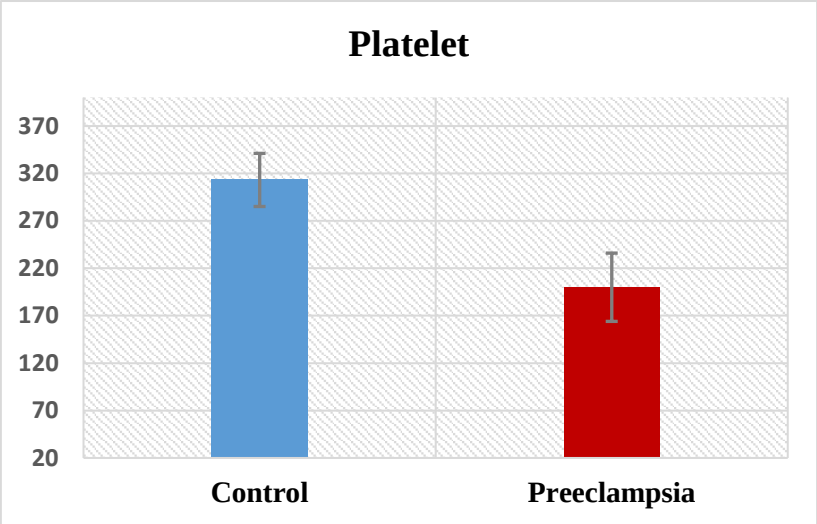

Figure2. Mean ± SD of platelet count in control and preeclamptic groups.

A comparison of liver and kidney function data between the control and preeclampsia (PE) groups, as provided in **Table 3**, reveals significant differences across several variables. The variables examined include alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin, urea, and creatinine.

For ALT, the mean level was 33.48 IU/L in the PE group compared to 16.8 IU/L in the control group, indicating a highly significant difference ($P < 0.001$). Similarly, AST levels were significantly higher in the PE group, with a mean of 45.28 IU/L compared to 19 IU/L in the control group ($P < 0.001$). Serum albumin levels were also markedly different, with the PE group having a mean of 2.8 g/dL and the control group having a mean of 3.5 g/dL ($P < 0.001$).

Regarding kidney function, the mean urea level in the PE group was 33.04 mg/dL, significantly higher than the control group's mean of 21.6 mg/dL ($P < 0.001$). Creatinine levels also showed a significant difference, with the PE group having a mean level of 0.76 mg/dL compared to 0.59 mg/dL in the control group ($P = 0.002$). These results highlight substantial alterations in liver and kidney function associated with preeclampsia shown **in Table 3&** Figure 3**, 4.**

The distribution of urine albumin levels in the preeclamptic group is shown in **Figure 5**, indicating varying degrees of severity among the cases. Specifically, 32% of cases exhibited a 1+ level of urine albumin. Another 32% of cases had a 2+ level, while 24% showed a 3+ level. The remaining 12% of cases had a 4+ level of urine albumin.

Table 3. Mean ± SD of liver and kidney function data in control and preeclamptic groups.

Variables	Control (n=20)	Preeclampsia (n=50)	P Value	Significance
	Mean±SD	Mean±SD		
ALT (IU/L)	16.8±2.57	33.48±16.07	<0.001	HS
AST (IU/L)	19±2.73	45.28±17.62	<0.001	HS
Serum Albumin (g/dl)	3.5±0.06	2.8±0.43	<0.001	HS
Urea (mg/dl)	21.6±3.4	33.04±13.33	<0.001	HS
Creatinin (mg/dl)	0.59±0.15	0.76±0.22	0.002	HS

HS: High significant difference was observed (P≤0.01), ALT, alanine aminotransferase; AST, aspartate aminotransferase..

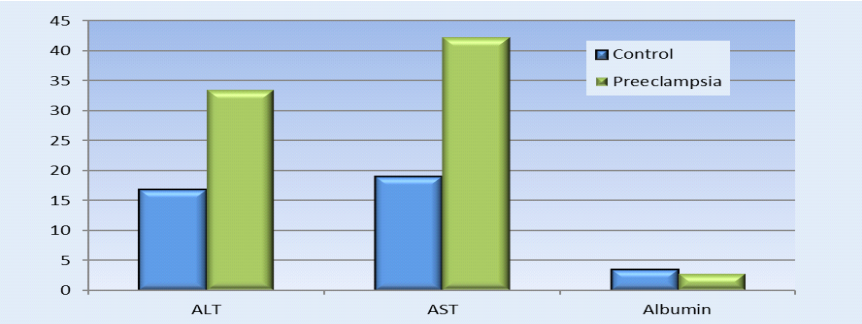

Figure 3. Mean ± SD of alanine aminotransferase, aspartate aminotransferase, and serum albumin in control and preeclamptic groups.

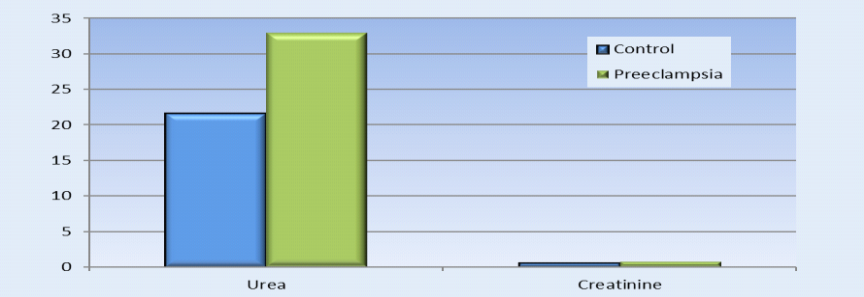

Figure 4: Mean ± SD of urea and creatinine in the healthy pregnant control and preeclamptic groups.

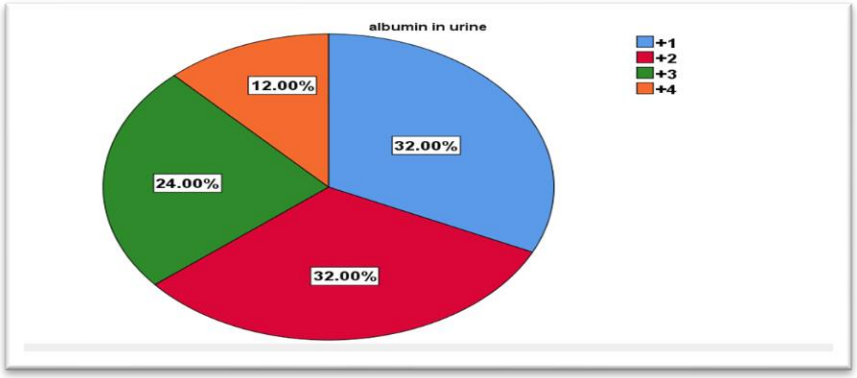

Figure5. Urine albumin percentage in the preeclamptic group.

3.2 Comparison statistics of miRNA149 and copeptin between patients and controls

Comparing MIR149 and Copeptin levels between the preeclampsia (PE) and the control groups, revealed highly significant differences. The mean value of MIR149 was significantly lower in the PE group, with a mean of 0.37 compared to 1.01 in the control group ($P < 0.001$). Conversely, Copeptin levels were markedly higher in the PE group, with a mean of 1738.8 pg/mL, compared to 243 pg/mL in the control group ($P < 0.001$). These findings indicate that the PE group had significantly lower levels of MIR149 and significantly higher levels of Copeptin, highlighting the potential roles of these biomarkers in the pathophysiology of preeclampsia shown in **Table 4 & Figure 6.**

Table 4. Mean ± SD of miRNA 149 and copeptin in control and preeclamptic groups.

Variables	Control (n=20)	Preeclampsia (n=50)	P value	Significance
	Mean±SD	Mean±SD		
MIR149	1.01±0.02	0.37±0.17	<0.001	HS
Copeptin (pg/ml)	243 ± 62.66	1738.8±190.89	<0.001	HS

HS: High significant difference was observed (P≤0.01), pg/ml, picograms per milliliter

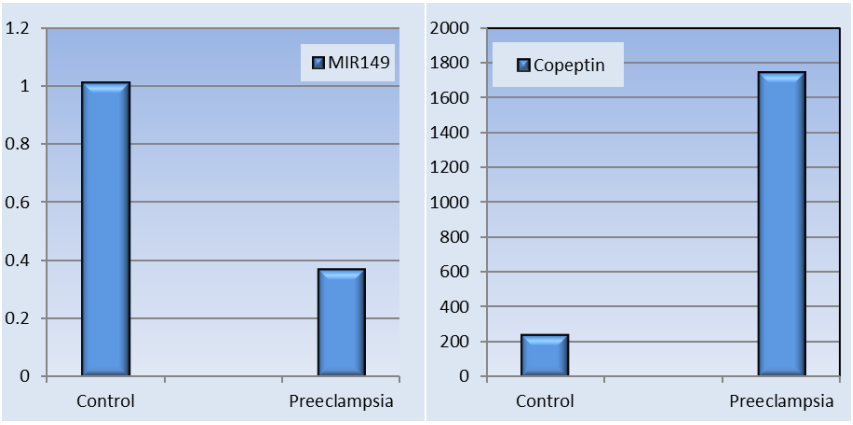

Figure 6. Mean ± SD of miRNA149 and copeptin in control and preeclamptic groups.

4. DISCUSSION

Pregnancy complications like hypertension are extremely serious. Preeclampsia and eclampsia are both severe types and are accompanied by problems in coagulation and extensive endothelium damage, which cause vasoconstriction and platelet aggregation, as well as further hypoxic endothelium injury[26].

In the present research, we have concentrated on the promising involvement of serum microRNA 149 and copeptin in the pathogenesis of preeclampsia. The age span of the study covered nearly entire of the females' sexual activity years (20 to 40) and the PE group′s body mass index was considerably higher than the control group′s (Table 1).

According to *Ramsey et al., Yeung et al., Alkinade et al., and Bartsch et al., al.,* preeclamptic mothers were considerably more probable to have a greater BMI and produce offspring with infants with lower birth weights. These findings were similar to those findings[27,28,25,29]. Also, *Vince et al.* discovered that an increased incidence of preeclampsia during pregnancy is maternal obesity[30]. *Zulfikaroglu et al.* contradicted these results and found no clinically significant between the gestational ages, body mass index, or mean ages of the preeclampsia group and control group at sampling[31].

In the current study, preeclampsia groups had Markey higher diastolic and systolic blood pressures than control groups (Table 1 and Figure 1).
Stepan et al. defined preeclampsia as a new development of albumin in the urine in hypertensive women after 20 weeks of pregnancy, whereas pregnancy-induced hypertension is defined as blood pressure ≥140/90M Hg without proteinuria and identified beyond that time[32]. Furthermore, *Al-Amin et al*. discovered that blood pressure increased 5.2% in the normal blood pressure group, but 13.3% in the group with preeclampsia late-onset[33].

There were no significant changes between the control and the PE groups in Hb, HCT, TLC, and RBCs (Table 2). These conclusions were in line with those made by *Elgar et al* [34]. In contrast hand, *Han et al*. proposed a significant hematocrit value might be an indicator of preeclampsia and supported their remark with the hypothesis that preeclampsia typically results in the body's tissues accumulating blood plasma. An elevated hematocrit value is a result of the blood becoming more concentrated[35].

In the present work, compared to the healthy group, there was an extremely reduced platelet count in the preeclampsia group (Table 2 and Figure 2). *Cines and Levine* claimed that preeclampsia could result in thrombocytopenia[36]. According to *Juan et al* low-grade intravascular coagulation includes platelet consumption. Additionally, platelets seem to move more actively and have a tendency to bind to the injured endothelium cells[37]. Meanwhile, *Alkholy et al.* clarified that preeclampsia causes accelerated platelets turnover & degradation[38].

According to *Han et al.,* a physiological hypercoagulable state in the mother develops during normal pregnancy in the third trimester. Preeclampsia can cause complex disturbances in the natural coagulation mechanisms. It attacks fibrin and platelets, thus causing fibrinolysis and thrombopoiesis[35]. Nevertheless, these findings contradicted with *El-Garhey et al.,* who discovered that mean platelet volume values do not influence the presence of preeclampsia[39].

There were no significant changes in PC, PT, and INR between the preeclamptic and control groups (Table 2).

The present result agreed with the results of *Chandra et al.,* who discovered no significant alterations in TT or PT in women with PIH[40]. Meanwhile, these observations, contradicted those of

Haldar et al., stating that there were substantial differences between the preeclamptic group compared with the control group in terms of coagulation factor-like PTT and PT, in addition to PCV and HB [41]. Also, according to *Shetty et al.,* a considerable lengthening of PT in PIH is a sign that coagulation factors, particularly factor VIII, have been consumed [42].

Comparing the preeclamptic group to the control group in the current study revealed highly substantial increases in ALT, AST, urea, and creatinine levels (Table 3 and Figures 3&4).

These findings confirmed those of *Tok et al.,* who found that the preeclamptic group's mean serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine were substantially greater than the healthy group[43]. *Ekun et al.* found the preeclamptic group had a statistically significant increase in levels of urea, creatinine, uric acid, serum AST, ALT, and plasma potassium activities when compared to the normotensive control group[44].

Myers et al. reported that HELLP syndrome is a preeclampsia complication characterized by hemolysis, elevated liver enzymes, and low platelet count. HELLP starts in the final three months of pregnancy[45]. Meanwhile, these findings contradicted the findings of *Moussa et al.,* who stated that there was no clear differentiation between the preeclamptic and the normal pregnant groups regarding AST and ALT[46]. *Tolba et al.* reported no substantial difference in serum creatinine and urea levels between preeclamptic cases and normal pregnant cases[47].

In the current study, the preeclamptic group's serum albumin level significantly dropped compared to the control group (Table 3 and Figure 3). These outcomes supported the conclusion reported by *Ekun et al.,* who discovered that preeclamptic women's serum albumin was considerably lower than that of the normotensive control women[44].

Kinoshita et al. explain that albumin is the most abundant protein in plasma. It is associated with exposure to oxidative damage in preeclampsia and it is suspected to be the main circulating antioxidant in plasma leading to decreased serum albumin levels in preeclamptic cases compared with normal pregnant cases[48].

In the current study, urine albumin was absent in the control cases as it was present in the PE cases, and the intensity of the test strip color indicated how much albumin was present in the urine (Figure 5). According to *Magee et al., (2014)* and *Szczepanski et al., (2020),* the cause of high urine albumin in the preeclamptic group is that the vital proteins leak into the mother's urine as a result of renal lesions, decreased renal perfusion, and glomerular filtration rate produced by renal vasospasm in preeclampsia[49-50].

Human miR-149, which has polymorphisms and is encoded by a signal exon, is found at 2q37.3[51]. During pregnancy, four of the most prevalent uterine miRNAs, miR-149, miR-141, miR-135b, and miR-299-5p were detected in the mother's plasma and their levels dropped after delivery. The role of miR-149 in PE has not been broadly investigated, and its role in preeclampsia pathogenesis is still unclear. However, many studies have shown that miR-149 is significantly down-regulated in preeclampsia [52]. The studied groups showed a highly marked decline in miR-149 concentration in the preeclamptic group when compared to the normal pregnant group (Table 4 & Figure 6).

According to *Whigham et al.,* the women who would later evaluate preeclampsia at 36 weeks had reduced expression of miRs 363,18a, 149, 1283, and 424[53]. Similar observations were reported by *Zhao et al.,* who found that 4hydroxyglutamate and miR-149-5p were predictive of the unfavorable prognosis of preeclamptic patients. Preeclampsia results in high levels of 4hydroxyglutamate expression and low levels of MiR-149-5p can be utilized for 4hydroxyglutamate single or combination detection for preeclampsia prediction and diagnosis[54].

Using gene set enrichment analysis, *Xiaobo et al.,* discovered that miR-149 was significantly down-regulated in preeclampsia using predicted microRNAs[55]. Consequently, miR-149 is an effective therapeutic target in human PE. However, these findings were opposed by *Salimi et al,* who discovered no significant difference between the preeclamptic and the normal pregnant groups in the prevalence of placental miR-149 rs2292832 genotypes. These genotypes also were not linked to an increased risk of PE or severe PE[56].

In the current study, the preeclamptic group's copeptin levels substantially increased as compared to the control group (Table 4, Figure 6).

These findings corroborated those of *Santillan et al*., who discovered that copeptin levels were noticeably greater in preeclamptic pregnancies than in control pregnancies. They argued that a 24 ng per hour continuous infusion of arginine vasopressin during gestation is adequate to cause preeclampsia[57]. Additionally, according to *Sandgren et al*., AVP secretion is increased in women who eventually develop PE beginning in the sixth week of pregnancy[58].

Furthermore, it was discovered by *Cornelius et al.* that severe preeclampsia is more strongly associated with elevated copeptin levels than moderate preeclampsia[15].

Zulfikaroglu et al., (2011) claimed that higher maternal levels of copeptin may be playing a role in the progression of preeclampsia and assist in determining the disease's severity[59].

According to *Bellos et al., (2020),* preeclampsia was linked to dramatically increased serum copeptin levels throughout the entire pregnancy. Although there was no difference between early and late-onset preeclampsia, it was estimated that the increase in copeptin values was more apparent in the severe form of the condition[60].

Akinlade et al., (2015) found that an elevated blood copeptin level during the third trimester was connected to a worse perinatal outcome and could be employed to predict preeclampsia[25].

Additionally, *Yeung et al.* reported that pregnancies that progress to preeclampsia have greater serum copeptin levels. However, they claimed that women with additional pregnancy problems such as premature birth, gestational hypertension, and diabetes mellitus, did not yet have higher copeptin levels. This contradicts our findings, which showed a dramatic rise in copeptin level in the preeclamptic group when compared to contro[28].

Marek et al., (2021) the obtained results confirm that copeptin concentration increased beginning in the second trimester of pregnancy in the group of women with pregnancy-induced hypertension. Copeptin has been demonstrated to be ineffective as a standalone marker for predicting PIH. Copeptin measurement coupled with a Doppler examination of the uterine arteries during the first trimester of pregnancy may be a helpful marker in predicting the development of PIH. On the contrary hand, the combination of copeptin with the Doppler investigation of the uterine arteries during the first trimester of pregnancy is distinguished by high specificity and sensitivity[61].

5. CONCLUSIONS

The present study suggests that copeptin may serve as a prognostic biomarker of preeclampsia in women who suffer from pregnancy-induced hypertension. The detection of expressed microRNA 149 in maternal blood provides a chance to establish a preeclampsia indicator that is readily accessible. The potential for discovering both therapeutic and diagnostic strategies for preeclampsia is greatly enhanced by a good knowledge of the function of microRNA 149 in the genesis of preeclampsia. Moreover, concurrent usage of copeptin and microRNA 149 could provide a clearly differentiate between the preeclamptic and normal pregnant groups and could detect the pathogenesis of preeclampsia.

Funding: No specific grant was given to this research by financial institution in public, private, or not- for- profit sectors.

Acknowledgments: We would like to acknowledge El-Kaser Al- Ainy hospital for facilitating the laboratory work.

Conflicts of Interest: The authors declare they have no conflict of interest.

Ethical Statement: Study protocol was approved by the Kaser Al- Ainy hospital ethical committee, Egypt and Faculty of Pharmacy, girls, Al-Azhar University (REC number: 256). All participants provided written informed consent for the screening and the participation phase.

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