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Study the relationship between long non-coding RNA (CCAT1) expression and CDK4 expression levels in Egyptian patients with preeclampsia

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ABSTRACT

Background: Maternal and perinatal mortality is caused by a variety of factors, including preeclampsia. PE's onset and progression may be influenced by lncRNAs. The effect of long non-coding RNA (lncRNA) Colon cancer-associated transcription factor 1 (CCAT1) expression in the placenta of preeclampsia patients on preeclampsia progression was the focus of this investigation.
Objectives: The aim of the current study is to check if the levels of expression of Colon cancer-associated transcription factor 1 (CCAT1) and Cyclin-dependent protein kinase 4 (CDK4) are associated with preeclampsia vulnerability and biogenesis.
Subjects: and methods: This work included the participation of 160 people. Eighty of the patients had preeclampsia. The control group included 80 normal pregnant women. The two groups were almost of the same age. A thorough clinical examination was performed in all groups (including taking a detailed history, concentrating on parity, age and previous background of diabetes or hypertension). The expression levels of CCAT1 and CDK4 in placental tissue were determined using a real-time q PCR technique.
Results: Expression levels of CCAT1 and CDK4 differed significantly between the study groups. preeclamptic patients having the highest level of CCAT1in comparison with control group, However, preeclamptic patients having lower level of CDK4 than controls. There was a strong negative association between CDK4 expression level and DBP, SBP, creatinine, urea and CCAT1 level of expression in the preeclamptic group, whereas there was a positive correlation between CCAT1 level of expression and DBP, SBP, urea and creatinine in patients group.
Conclusion: Based on the findings of this study, it is possible that CCAT1 and CDK4 expression levels could be used to aid in the diagnosis and biogenesis of preeclampsia.

1. Introduction

Preeclampsia is a time-progressing multisystem disease a characteristic of the development of hypertension and proteinuria in late pregnancy or postpartum, severe organ malfunction is sometimes accompanied by proteinuria. Maternal and placental vascular dysfunction causes the disease, which usually resolves spontaneously after birth. Preeclampsia raises the risk of heart disease and stroke later in women's life. The placenta has a vital role to play in PE because PE develops only when placental tissue is present and after the delivery the indicators vanish [1].

Preeclampsia rarely occurs before the 20th week of pregnancy. However, it can occur with triggers such as blister spots, twin pregnancies, fertility and progressive kidney disease [2].

Non-coding RNA having a length of around 200 nucleotides (nt) is known as long non-coding RNA (lncRNA). The disclosure of lncRNAs has opened totally unused areas for quality expression control and epigenetics and lncRNAs have presently been demonstrated to be closely related with different infections, counting tumors.

Furthermore, an increasing number of investigations have found that lncRNA can have a part in the development of preeclampsia by influencing the function of trophoblast cells [3].

Using the differential expression of lncRNA and mRNA, a significant amount of information about lipid metabolism and type 2 immune

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responses was revealed, supporting the hypothesis that PE causes malfunctioning of both metabolic and immunological systems when compared to uncomplicated births [4].

The non-coding RNA molecule CCAT1, which has a length of 2628 nucleotides, was discovered in colon cancer for the first time. CCAT1, a transcription factor related to C Myc, is found at 8q24.21. According to studies, this is the genomic region with the highest rate of genetic alterations. C-Myc ties to *cis*-acting component E-box upstream of CCAT1, coming about within the tall expression of CCAT1 in cells [5].

C-Myc also increases DNA synthesis and transcription, as well as cell proliferation and entry into the S phase, while preventing apoptosis [6]. The link between CCAT1 and preeclampsia has yet to be discovered in the Egyptian population.

CDK4 belongs to the CDK protein family. It's also a crucial serine/threonine protein kinase involved in DNA synthesis and cell division. By attaching to cyclin D (forming a CDK/cyclin D complex), CDK4 has kinase activity and affects cell cycle progression. CDK4 governs the G1transition to S phase of the cell growth cycle. CDK4 interacts to the regulatory subunit Cyclin D1 to form a complex of Cyclin D1-CDK4 that can be activated, causing Rb to be phosphorylated, nuclear factor E2F1 to be released and DNA replication to be promoted [3].

The purpose of this investigation was to examine the relationship between CCAT1, CDK4 levels of expression and the vulnerability and etiology of preeclampsia.

1.1. Subjects and method

1. **Subjects**

This case–control study included 160 people: 80 patients with PE and 80 healthy pregnant women of comparable ages like a control group. Cases were collected from Department of Gynecology and Obstetrics in the Outpatient Department of Menoufia University Hospital in Egypt.

3. **The approval of ethics**

All individuals were given consent in writing (approved through the committee of Human Ethics and Rights in Medicine Faculty Research, Menoufia University) prior to sample collection. Each participant was subjected to a full laboratory evaluation, ultrasound and clinical assessment (SGPT, SGOT as a liver enzymes), blood urea and creatinine (renal function), and physical examination with serum uric acid and protein in urine was measured by using test strips to determine [7]. CCAT1 and CDK4 gene expression levels were estimated.

4. Blood sample:

Four milliliters of blood were taken from each individual and divided into two tubes: one was used to estimate the total blood count using a Pentra-80 automatic blood counter, and the other was placed in a plain tube to clot for 30 min. The serum was centrifuged for 10 min at 3000 rpm and put away at −80 °C for renal function tests (urea [8] & creatinine [9]), uric acid [10] and liver function tests (AST& ALT) [11].

Proteinuria is measured using a test strip that had been submerged in fresh urine for 1 s. Check strip was compared to the colour scale after 30–60 s [12]. A heat coagulation test was used to validate the assessment.

For RNA extraction, approximately a total of 40 mg of placental tissue was obtained and kept at 80 °C until the process of the extraction of RNA was done.

4. Placental tissue isolation of RNA

The expression of (CCAT1 and CDK4) in placental tissue was measured using quantitative real-time PCR in four steps: 1- Placental tissue samples were used to isolate RNA. 2- PCR (polymerase chain reaction): cDNA synthesis (reverse transcription step). 3- PCR (real-time PCR step): Amplification of cDNA, preparing the plate document, and starting the PCR run. Applied Biosystems 7500 software version 2.0.1 was used for data analysis.

1 RNA extraction from placental tissue:

LncRNA was separated from placental tissues utilizing QIAamp RNA. (MiniKit, Qiagen, USA).

2 Obtaining on complementary DNA (cDNA) by utilizing miScript II RT Kit (Qiagen, USA)

cDNA synthesis (RT-step) was performed employing a High Capacity cDNA reverse transcription kit, Applied Biosystems, USA. Every reaction was done in a total volume of 20 μl (10 μl template RNA, 4 μl reverse transcriptase buffer, 1 μl reverse transcriptase enzyme, in addition to 5 μl nuclease-free water were placed on ice). The samples were incubated using the 2720 thermal cycler from Applied Bio Systems (Singapore) for one cycle as shown: 10 min at 42 °C, then comes 5 min at 95 °C to deactivate the reverse transcriptase enzyme, followed by 5 min at 4 °C. The CDNA was stored at 20 °C for the analysis of real-time PCR.

3 Amplification of cDNA through Real time PCR step by using SYBR Green PCR Kit (Qiagen, USA)

LncRNA expression was measured using real-time PCR. Using the Quanti Test SYBR Green PCR Kit, Applied Biosystems, USA, and real-time PCR was employed to detect the expression of (CCAT1 and CDK4) genes. Each reaction was done at a volume of 20 μl (10 μl of SYBR green Master Mix, 4 μl of Nuclease-free water, 4 μl of template cDNA, 1 μl of forward primer and 1 μl of reverse primer). The next primer (Midland, Texas) was employed:

Forward primer of CCAT1 5′-CATTGGGAAAGGTGCCGAGA-3′′: reverse primer of.

CCAT1 5′-ACGCTTAGCCATACAGAGCC-3'; Forward primer of CDK4:5′-CTTCCCGTCAGCACAGTTC-3'; reverse primer of CDK4:5′-GGTCAGCATTTCCAGTAGC-3'; and forward & reverse primers of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) respectively 5′-CGGAGTCAACGGATTGGTCGTAT-3'and 5′AGCCTTCTCCATGGTGGTGAAGAC-3'. For CCAT1 and CDK4 amplification, the following PCR conditions were used: a 30-s activation step at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C, 1 min, and a 10-min extension phase at 72 °C. For fluorescence detection and data processing, the 7500 ABI PRISM (Applied Biosystems, USA) v.2.0.1 was eventually employed. The relative quantification (RQ) of both genes expression was measured using the comparative Ct technique [13]. As shown in Fig. 1, the total of the CCAT1 gene is normalised to an endogenous Housekeeping gene called glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and compared to a control. As shown in Fig. 1, the CDK4 gene sum is normalised to an endogenous Housekeeping gene called glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and compared to a control. A melting curve was used to validate the specificity and identification of the PCR products.

1.1.1. Statistical analysis

The software program SPSS (version 20.0) was utilized for the analysis of data. The Kolmogorov-Smirnov test was utilized to ensure that the distribution of data was normal, and the Chi-square test was utilized to check comparisons of group variables. Quantitative variables with a regular distribution were assessed utilizing the Student t-test to compare two groups. Mann Whitney test was exploited for the comparison of the two groups that have quantitative variables not normally distributed. The Pearson coefficient was utilized to detect a correlation between quantitative variables that are normally distributed. The markers' diagnostic performance was evaluated utilizing a receiver

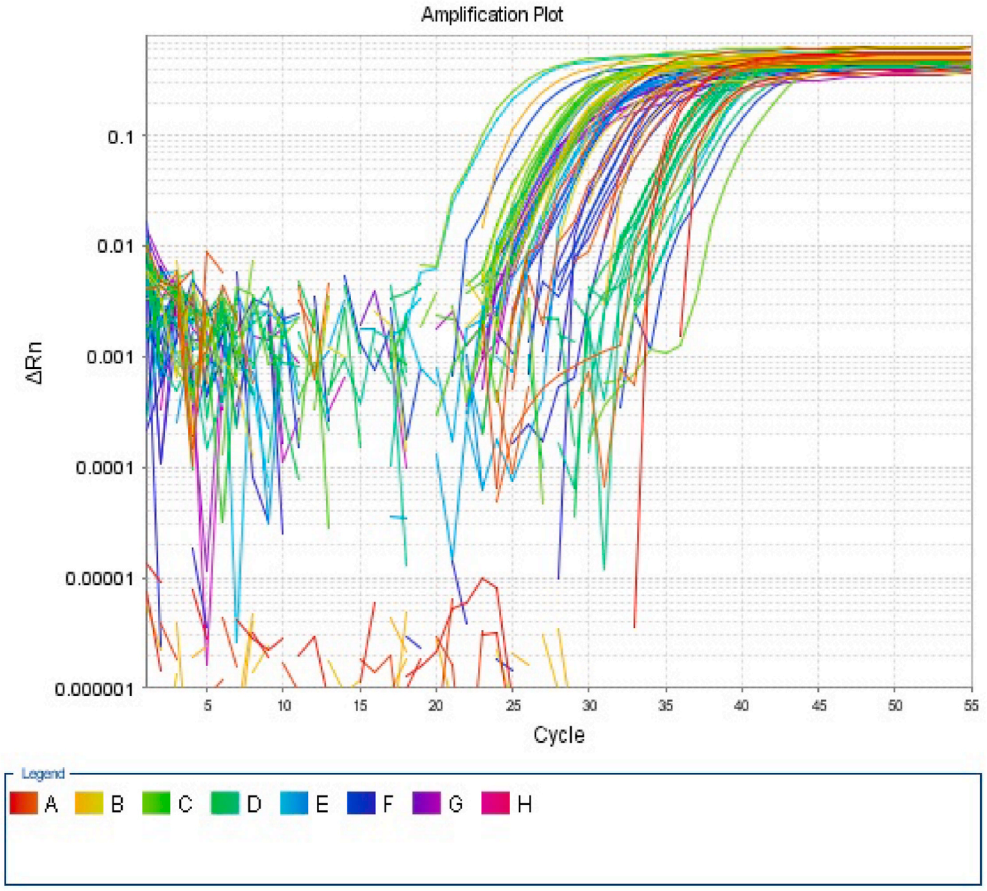

Figure (1). Amplification plot of CCAT1 gene expression (normalised fluorescence signal (ΔRn) plotted versus cycle number).

operating characteristic curve (ROC), with an area greater than 50% indicating adequate performance and an area around 100% indicating the most effective performance for the test. The significance level of 5% was the basis for the assessment of the acquired results. Regression analysis assesses the consequences of various risk factors as independent Odds ratios after other confounders are eliminated.

2. Results

This study included 80 preeclamptic patients and 80 pregnant women of similar ages who appeared to be in good health. The differences with statistical significance were identified in diastolic blood pressure and systolic, serum urea, uric acid, creatinine, odema, SGOT, proteinuria and birth weight when preeclamptic individuals were compared to controls (see Table 1).

In terms of CCAT1 expression levels, there was a substantial statistical difference between the two studied groups, with preeclamptic patients having the highest level in comparison with control group. However, when the two groups were compared, a statistically significant difference existed in CDK4 expression levels. As demonstrated in Fig. 1, the expression level of CDK4 was lower for the PE group than for the control group (Table 2).

There was a strong negative association between CDK4 expression level and DBP, SBP, creatinine, urea and CCAT1 level of expression in the preeclamptic group, whereas there was a positive correlation between CCAT1 level of expression and DBP, SBP, urea and creatinine in the preeclamptic group, as shown in Fig. 1 (Table 3 and Fig. 2). **The diagnostic usefulness of CCAT1 and CDK4 expression levels in preeclamptic versus control individuals was assessed using the ROC curve.** It was discovered that the level >4 is the ideal cutoff point for CCAT1. CCAT1 had a diagnostic sensitivity of 93.75, a specificity of 87.50, a positively predictive value of 88.24%, and a negatively predictive value of 93.33%. In the case of CDK4, the optimal cutoff value for CDK4 expression level was ≤1.5, according to the ROC curve. CDK4 had a demonstrative affectability of 91.25%, specificity of 86.25%, with a positive predictive value of 86.90% and negative predictive value of 90.79% (Fig. 3 and Table 4). **Univariate logistic regression to analyze the parameters impacting preeclampsia revealed that** CDK4 is a factor that protects against preeclampsia if its level rises and a risk factor if it falls in patients compared to controls. **Alternatively, CCAT1 increases the risk of preeclampsia to 8.932 CI;(4.011–19.889) as shown in the study (Table 5).** CCAT1 increases risk for preeclampsia odds ratio 9.862 CI; (2.314–42.019) when adjusted against CDK4 and increases the risk for PE odds ratio 42.512 CI; (1.28–1414.32) when adjusted against diastolic blood pressure and systolic in a multivariate logistic regression to analyze the parameters impacting preeclampsia. While CDK4 raises the risk of PE (odds ratio: 1.770, 95% confidence interval: 1.09–2.87), when the systolic and diastolic blood pressures are taken into account, as illustrated in (Table 6).

3. Disscussion

Around 10% of all pregnancies in the globe are affected by hypertensive disorders of pregnancy, which includes the 3%–5% of all pregnancies complicated by preeclampsia. Preeclampsia is a form of hypertension that develops following the 20-week of gestation and is joined by indications of uteroplacental malfunction, maternal organ or proteinuria. Preeclampsia may be a driving cause of maternal and fetal morbidity [14].

During placental morphogenesis and substantial angiogenesis, multipotent trophoblast stem cells develop into multiple trophoblast lineages. In the placenta, trophoblast cells have a range of specialized roles

Table (1)
Demographic, clinical and laboratory data in the studied groups.

Age (years)	PE Cases (n = 80)	Control (n = 80)	Test of Sig.	p
Mean ± SD.	27.3 ± 4.8	26 ± 4.5	t = 1.745	0.083
Gestational age (weeks)				
Mean ± SD	36.1 ± 3.2	34.5 ± 4.2	t = 2.858*	0.005*
Past history of diabetes	6 (7.5%)	0 (0%)	$\chi 2$ = 6.234*	^{FE}p = 0.028*
Birth weight (Kg)				
Mean ± SD	3 ± 0.4	3.2 ± 0.3	t = 4.786*	<0.001*
Systolic (mmhg)				
Mean ± SD.	148.4 ± 16	114.6 ± 6.9	t = 17.291*	<0.001*
Diastolic (mmhg)				
Mean ± SD.	97.8 ± 13.5	73.4 ± 6.4	t = 14.613*	<0.001*
Oedema	80 (100%)	0 (0%)	$\chi 2$ = 160.0	<0.001*
Proteinuria				
+1	38 (47.5%)	–	–	–
+2	27 (33.8%)	–		
+3	6 (7.5%)	–		
+4	9 (11.3%)	–		
Urea (mg/dl)				
Mean ± SD.	26.2 ± 4.6	22.4 ± 2.4	U= 1256.50*	<0.001*
Creatinine (mg/dl)				
Mean ± SD.	1.4 ± 0.2	0.8 ± 0.1	U= 77.50*	<0.001*
Uric acid (mg/dl)				
Mean ± SD.	5.6 ± 0.9	4.3 ± 0.7	t = 10.006*	<0.001*
SGOT (U/L)				
Mean ± SD.	34.4 ± 32.6	23.5 ± 4.7	U = 1660.0*	<0.001*
SGPT (U/L)				
Mean ± SD.	23.6 ± 22.3	17.3 ± 5.5	U = 2697.0	0.085
HB (g/dl)				
Mean ± SD.	11.1 ± 1	11.6 ± 1.2	t = 2.422*	0.017*
Platelets number (cm3)				
Mean ± SD.	206 ± 76.7	216.8 ± 50.4	t = 2955.50	0.400

SD: Standard deviation.
t: Student t-test.
U: Mann Whitney test.
χ^2: **Chi square test**.
FE**: Fisher Exact**.
p: p value for comparing between the studied groups.
*: Statistically significant at $p \leq 0.05$.

Table (2)
Comparison between the two studied groups according to CCAT1 expression and CD4.

CCAT1 expression	PE Cases (n = 80)	Control (n = 80)	Test of Sig.	p
Mean ± SD.	7.4 ± 1.9	3.3 ± 0.8	U = 182.50*	<0.001*
Median (Min. – Max.)	7.9(4–9.8)	3(1–5)		
CDK4				
Mean ± SD.	0.8 ± 0.4	1.7 ± 0.2	t = 20.175*	<0.001*
Median (Min. – Max.)	0.7(0.4–1.6)	1.7(1.5–2.5)		

SD: **Standard deviation**.
t: Student t-test.
U: Mann Whitney test.
p: p value for comparing between the studied groups.
*: Statistically significant at $p \leq 0.05$.

Table (3)
correlation between long non coding RNA level and demographic, clinical and laboratory data in patients' group.

	CCAT1 expression		CDK4	
	r_s	P	r	p
Age (years)	0.162	0.151	−0.050	0.660
Gestational age (weeks)	0.141	0.211	0.072	0.526
Birth weight (Kg)	−0.060	0.598	0.084	0.458
Systolic (mmhg)	0.364	0.001*	−0.255	0.023*
Diastolic (mmhg)	0.349	0.002*	−0.225	0.045*
Proteinuria	0.066	0.563	−0.087	0.442
Urea (mg/dl)	0.227	0.043*	−0.240	0.032*
Creatinine (mg/dl)	0.237	0.034*	−0.221	0.049*
Uric acid (mg/dl)	0.122	0.280	−0.193	0.086
SGOT (U/L)	−0.044	0.696	0.086	0.449
SGPT (U/L)	0.047	0.679	0.058	0.611
HB (g/dl)	−0.030	0.791	0.066	0.558
Platelets number (cm3)	−0.025	0.828	−0.070	0.540

r_s: Spearman coefficient.
r: Pearson coefficient.
*: Statistically significant at $p \leq 0.05$.

throughout pregnancy. A lack of trophoblast cell growth leads to a low pregnancy yield for both the foetus and the mother. They include a number of disorders of the placenta, like preeclampsia and restriction of intrauterine growth, which have unknown etiology. Long non-coding RNAs are thought to participate in the proliferation of trophoblasts and the establishment of a functional placenta since it has a different somatic tissues in the body's epigenome [15].

Long non-coding RNAs are in this manner likely to have a major role in the improvement of the placental development and trophoblastic growth [15]. lncRNAs play a variety of roles in cell biology (for example, stem cell transformation, cell differentiation, proliferation, and gene imprinting, apoptosis, and inactivation of X-chromosome) [16].

Specific lncRNA upregulation and downregulation can affect important mechanisms in the progression of PE, resulting in changes in trophoblast invasion, proliferation, apoptosis, and migration. PE is defined by severe placental malfunction resulting from invasion, dysregulation, and ultimately remodeling of the spiral arteries of trophoblast differentiation. Disruptions in placentation have serious implications later in pregnancy, leading to a widespread inflammatory response that has a negative influence on maternal and foetal health [17].

In our work, we discovered a substantial statistical difference between preeclampsia and control in terms of CAAT1 expression, with PE having the highest level of expression than the control group. In preeclampsia group, a substantial positive connection existed between the levels of CCAT1 and SBP, DBP, urea, and creatinine. Higher lncRNA CCAT1 expression was reported in preeclampsia patients according to J.-L et al., 2018 [3].

CCAT1 stimulates hepatocyte proliferative activity and activation, cholangiocarcinoma, gastric cancer, and liver cancer; it was also a classic biomarker for the analysis of breast cancer, and preeclampsia. CCAT1 overexpression inhibits the cyclin D1-P16-CDK4 pathway, which reduces trophoblast proliferation [3].

The early cause of PE is a lack of uterine spiral artery remodeling. The ability of trophoblasts to migrate and invade is closely linked to the epithelial-mesenchymal transition (EMT), which is linked to placental development. The key factors that lead to PE are decreased trophoblast proliferation, migration, invasion, and stimulated apoptosis. LncRNAs have the ability to impact the incidence as well as progression of PE through altering the activities of trophoblast cells. In PE, lncRNAs were identified to be on the rise [18].

When comparing control groups and PE, there was a statistical significant difference in CDK4 levels, with the highest level in the control

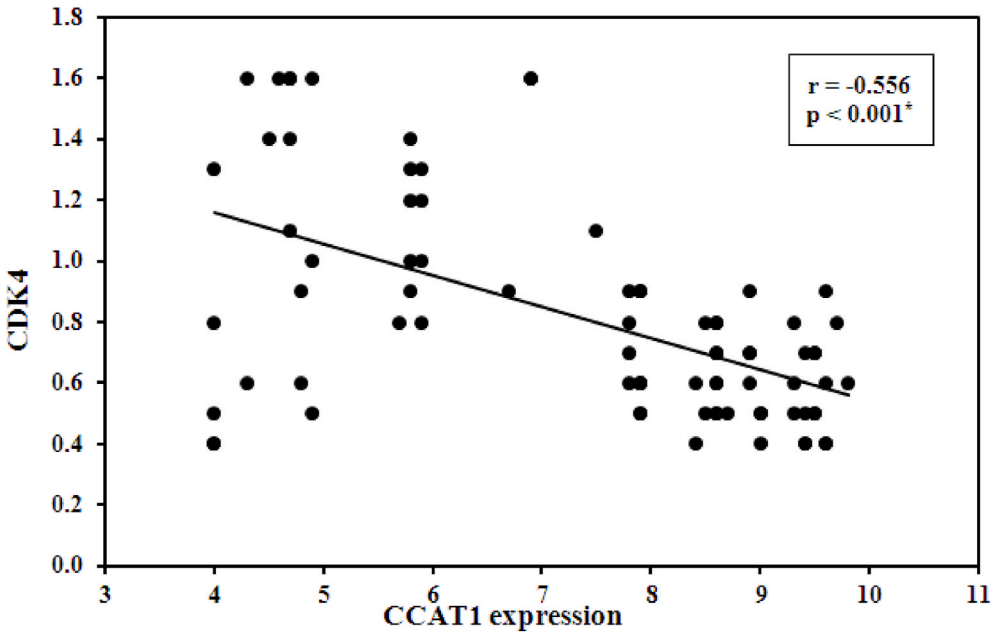

Figure (2). Correlation between CCAT1 expression and CDK4 in PE Cases (n = 80).

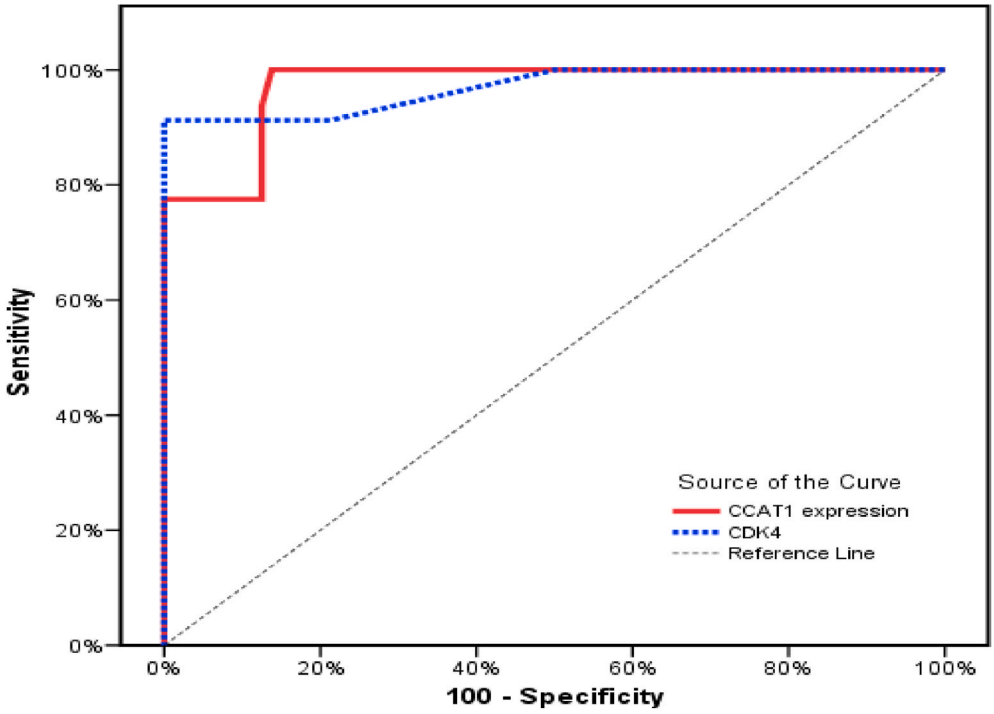

Figure (3). ROC curve for CCAT1 expression and CDK4 to discriminate PE patients (n = 80) from control (n = 80).

Table (4)
validity (AUC, sensitivity, specificity) for CCAT1 expression and CDK4 to discriminate PE patients (n = 80) from control (n = 80).

	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
CCAT1 expression	0.971	<0.001*	0.951–0.992	>4	93.75	87.50	88.24	93.33
CDK4	0.969	<0.001*	0.944–0.993	≤1.5	91.25	86.25	86.90	90.79

AUC: Area Under a Curve p value: Probability value.
CI: Confidence Intervals.
NPV: Negative predictive value.
PPV: Positive predictive value.
*: Statistically significant at p ≤ 0.05.

Table (5)
logistic regression analysis of demographic, clinical and laboratory data affecting preeclampsia.

	Univariate	
	P	OR (95%C.I)
age (years)	0.084	1.062(0.992–1.136)
Gestational age (weeks)	**0.006***	1.130(1.036–1.233)
Birth weight (Kg)	**<0.001***	0.134(0.053–0.341)
Systolic (mmhg)	**0.991**	6.371(0.0–2.116)
Diastolic (mmhg)	**<0.001***	1.684(1.362–2.083)
Urea (mg/dl)	**<0.001***	1.538(1.329–1.780)
Creatinine (mg/dl)	**<0.001***	8.721(3.590–21.183)
Uric acid (mg/dl)	**<0.001***	7.801(4.041–15.062)
SGOT (U/L)	**<0.001***	1.166(1.091–1.247)
SGPT (U/L)	**0.014***	1.056(1.011–1.103)
HB (g/dl)	**0.018***	0.708(0.531–0.943)
Platelets	**0.291**	0.997(0.993–1.002)
CCAT1 expression	**<0.001***	8.932(4.011–19.889)
CDK4	**<0.001***	0.0(0.0–0.001)

OR: Odd's ratio.
C.I: Confidence interval LL: Lower limit UL: Upper Limit.
#: All variables with $p < 0.05$ was included in the multivariate.
*: Statistically significant at $p \leq 0.05$.

Table (6)
Univariate and multivariate logistic regression analysis for the parameters affecting PE patients.

	Univariate		#Multivariate	
	P	OR (95%C.I)	p	OR (95%C.I)
CCAT1 expression	**<0.001***	8.932 (4.011–19.889)	0.002*	9.862 (2.314–42.019)
CDK4	**<0.001***	0.0(0.0–0.001)	0.012*	0.0(0.0–0.045)
CCAT1 expression	**<0.001***	8.932 (4.011–19.889)	0.036*	42.512 (1.28–1414.32)
Diastolic (mmhg)	**<0.001***	1.684 (1.362–2.083)	0.056	1.966(0.98–3.93)
Gestational age (weeks)	**0.006***	1.130 (1.036–1.233)	0.174	1.350(0.87–2.08)
CDK4	**<0.001***	0.0(0.0–0.001)	0.021*	1.770(1.09–2.87)
Diastole (mmhg)	**<0.001***	1.684 (1.362–2.083)	0.086	1.879(0.91–3.87)
Gestational age (weeks)	**0.006***	1.130 (1.036–1.233)	0.054	0.0(0.0–1.42)

OR: Odd's ratio.
C.I: Confidence interval.
LL: Lower limit.
UL: Upper Limit.
#: All variables with $p < 0.05$ was included in the multivariate.
*: Statistically significant at $p \leq 0.05$.

group than in PE. When CDK4 was compared to CCAT1, SBP, and DBP, there was a substantial negative correlation. The following explanation can be used to demonstrate this. The findings revealed that upon the comparison to the control group, low CCAT1 expression can dramatically boost cell proliferation as well as promoting cell cycle, but the overexpression of CCAT1 showed the opposite effect, as seen in our findings. After CCAT1 was overexpressed, the levels of cyclin D, E2F1, CDK2, and CDK4 in JEG3 cells were considerably reduced. All of this suggests that CCAT1 may be involved in the development and pathophysiology of PE. LncRNA CCAT1 can hasten the onset of preeclampsia by decreasing the production of CDK4, which aids cell proliferation by crossing the G1-S limit and entering the S phase, allowing for faster cell proliferation [3].

Multivariate logistic regression for the risk of PE in this analysis showed that high CDK4 expression was a factor of protection against preeclampsia. This is because the levels significantly reduced in these patients in comparison with healthy group. PE diagnosis Multivariate logistic regression analysis of PE risk showed that large expression of CCAT1 was a preeclampsia risk factor for diagnosis. This is consistent with LI et al., 2018 [3] and Xiuhua Yang et al., 2019 results [18].

Several studies have suggested that PE is linked to decidualization dysplasia. Furthermore, inadequate decidualization can contribute to extravillous trophoblasts' lower invasive potential, uterine spiral artery dysplasia, and decreased blood flow at the maternal-fetal interface. Increased expression of harmful cytokines in the maternal peripheral circulation occurs as a result of placental ischemia, further weakening endothelial cells. Glycolysis is thought to be critical for endothelial cell development and decreased glycolysis could contribute to impaired decidualization [19].

PE patients demonstrated a statistical significant increase in serum creatinine in comparison with controls, regardless insignificant statistical difference urea between controls and PE. Severe preeclampsia is indicated by a great creatinine level, especially when in combination with oliguria, according to Greer, 2005, and Herse et al., 2007 [20,21].

Preeclampsia patients reported a significant elevation in the levels of uric acid in comparison with controls in the current study. Which may aid in the development of proteinuria, that has a tendency to be concurrent with the increase in blood pressure. The elimination of uric acid has been decreased when PE developed. As preeclampsia advances, the concentration of uric acid in the blood rises. The level of greater than 5.5 mg/dL is a strong predictor of the disorder [22].

4. Conclusion

preeclamptic patients having the highest level of CCAT1and lowest level of CDK4. Also there was a strong negative association between CDK4 expression and CCAT1 level of expression in the preeclamptic group.

So the abnormal expression of CAAT1 and CDK4 levels takes part in the biological functions of trophoblast proliferation, migration, angiogenesis and invasion, as well as the pathogenesis of complications of PE.

Authors contribution

Eman masoud: Idea, writing, revision and methodology**,** Heba Maged Abo Shady: writing and revision,Mohammed Elhelbawy: writing ,revision and statistical analysis**, Eman Salah El Deen**:writing, revision and statistical analysis**.**

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Declaration of competing interest

No conflict of interest.

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