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The Correlation of *SKA2* with Cortisol, IL-1β and Anxiety in Pregnant Women with the Risk of Preterm Delivery

Janat Ijabi[1], Reza Afrisham[2], Hemen Moradi-Sardareh[2], Parisa Roozehdar[3], Fatemeh Seifi[4], and Roghayeh Ijabi[4] ✉

[1]Department of Hematology, School of Allied Health, Iran University of Medical Sciences, Tehran, Iran
[2]Department of Clinical Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Medical Veterinary, Azad University, Garmsar Branch, Garmsar, Iran
[4]Counseling and Reproductive Health Research Centre, Golestan University of Medical Sciences, Gorgan, Iran

Objective The association between preterm birth (PTB), Spindle and Kinetochore Associated Complex Subunit 2 gene (*SKA2*), cortisol and anxiety have been shown, but in this study, we aimed to clarify whether the expression of the *SKA2* gene plays a role in interleukin-1β (IL-1β) level since increasing level of IL-1β is linked with PTB.

Methods The case-control study was conducted on 49 and 51 women with preterm and term delivery, respectively. The score of anxiety was ranked according to the Spielberger state trait Anxiety Inventory. The concentration of cortisol and IL-1β was determined by the ELISA method. The expression of *SKA2* gene was assessed by the quantitative real time real time polymerase chain reaction (qRT-PCR). The western blot analysis was also performed to confirm the expression of *SKA2* at the levels of protein.

Results The results showed that the gene/protein expression of *SKA2*, the concentrations of cortisol and IL-1β were significantly higher in the preterm than the term group. In the preterm group, the expression of *SKA2* was positively correlated to the other factors including cortisol, IL-1β, and the degree of anxiety.

Conclusion Our findings suggest that the expression of *SKA2* was correlated positively to the levels of cortisol, IL-1β and the rate of anxiety in women with PTB.
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Key Words Anxiety, Cortisol, IL-1β, Premature birth, Pregnancy, *SKA2*.

INTRODUCTION

The preterm birth (PTB) is defined as birth occurring before 37 completed weeks of gestation, which accounts for two-thirds of infant death worldwide.[1] The prevalence of preterm delivery varies over the world as the most incidence rate belongs to countries with poor socio-economic conditions. The prevalence of premature birth is inversely associated with the socioeconomic status of countries. The cost of care is very high for premature infants.[2]

Preterm delivery has multifactorial etiology in which various factors synergistically result in premature birth in pregnant women include genetic, environmental and hormonal factors. It has been reported that placental Hypothalamic-Pituitary-Adrenal (HPA) axis plays a significant role in the development of preterm delivery.[3-5] The highest concentration of corticotropin-Releasing Hormone (CRH) is secreted by pituitary and placenta of pregnant women just near delivery.[3-5] Scientific reports also show that cortisol is produced at a significant level in the third quarter, and it would be increased as the delivery time approaches.[6] Placental CRH is different from maternal CRH and not affected by the negative feedback of adrenal glucocorticoids. The increased production of cortisol induces the secretion of placental CRH which, in turn, leads to an increase in the concentration of cortisol by affecting the fetal adrenal gland.[3-5]

A growing body of evidence proves that cortisol levels are also increased during anxiety.[7-9] Anxiety influences maternal adrenal glands through an increase in maternal CRH, followed by an increment in cortisol level, eventually leading to increased

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✉ **Correspondence:** Roghayeh Ijabi, MSc, PhD
Counseling and Reproductive Health Research Centre, Golestan University of Medical Sciences, Shast Kola Road, Gorgan, Iran
Tel: +981732426900, **Fax:** +981732455171, **E-mail:** R.ijabi@goums.ac.ir

fetal cortisol mediated by cortisol-induced placental CRH.[10,11] Some studies have suggested that placental CRH is probably related to stress[12-14] and there is a significant relationship between anxieties with preterm delivery.[7-9]

As mentioned above, high levels of CRH lead to increased cortisol production from the adrenal gland of the mother and the fetus, as well as increased the production of steroids. Higher cortisol levels may result in changes in other hormones. It is approved that placental CRH increase the production of estrogen.[15] There is a hypothesis suggesting that the increased estrogen levels may alter the homeostasis, change the progesterone receptors, and lead to delivery. Progesterone is considered as a tranquilizer factor in the uterus which is sometimes prescribed as a drug for the management of preterm delivery.[16-20] CRH also increases the contraction of the uterus by increasing the level of prostaglandin.[19] Therefore, CRH and cortisol are used as placental clock.[3]

Also, an increase in the level of cortisol for the long-term period can induce inflammatory factors including IL-1β;[21,22] and IL-1β which is indirectly linked with preterm delivery by increasing the levels of Toll-like receptors (TLRs). On the other hand, the lack of TLRs is correlated with delayed or post-term delivery.[23-25] Cortisol and IL-1β have a synergistic effect on the preterm delivery; it has been shown that cortisol can cause collagen atrophy and Premature Rupture of the Membranes (PROM), moreover, increased IL-1β results in the elevation of metalloproteinases, collagen deformation, and PROM.[26-28]

Recent studies demonstrated that kinetochore associated complex subunit 2 (*SKA2*) gene could regulate the secretion of cortisol via chaperoning glucocorticoid receptors in order to transport them from the cytoplasm into the nucleus[29] and the *SKA2* gene expression is associated with the increase in cortisol level.[29] Also, an association between anxiety and the expression of *SKA2* has been reported in some studies,[30] and in another study there is a relationship between PTB and increasing cortisol level and *SKA2* gene single-nucleotide polymorphism (SNP) (NC_000017.11: g.59110368G>A) which supposed as predictive PTB biomarker in pregnant women.[31]

Regarding the effect of cortisol and IL-1β on the risk of premature birth and the impact of the *SKA2* expression on the regulation of cortisol; we decided to investigate the role of the *SKA2* expression on IL-1β in the susceptibility of premature birth in pregnant women. Hence, the question is whether the expression of the *SKA2* gene plays a role in preterm delivery through the changes in the degree of anxiety, as well as the expression of cortisol and IL-1β. In some researches have shown that IL-1β could possibly serve as a marker for preterm delivery[32,33] but in the current study, we evaluated the relationship of *SKA2* with IL-1β in preterm delivery for the first time.

METHODS

Ethical approval

The study was approved by the ethics committee of the Golestan University of Medical Science, Iran (NO: EC/IR.GOUMS.rec. 1394.79). Written informed consent was obtained from all pregnant women.

Study design and the collection of samples

An analytical case-control study was carried out in pregnant women who referred to Shariati and Arash Hospitals, affiliated with Tehran University of Medical School (TUMS), for giving birth. Sample collection was performed between Feb 2014 and Feb 2017. A total number of 100 pregnant women were divided into two groups. The experimental group consisted of 49 pregnant women with preterm delivery who gave birth during 34−37 weeks of pregnancy. The control group included 51 pregnant women with term delivery. Both groups of pregnant women were adjusted considering the confounding factors such as age, history of conception (1−3 times), academic education, and their employment. The inclusion criteria for the selection of pregnant women were age range between 18−40 years, singleton pregnancy, medium family income, and routine serial weighing during pregnancy. The exclusion criteria were depression, mental disorders leading to hospitalization, cervical failure, hypertension, preeclampsia, acute fatty liver, vaginal infections, pyelonephritis, antepartum hemorrhage, inability of mother to gain weight during the gestation period, placental abruption, placenta Previa, low maternal weight as a cause of intrauterine growth restriction (IUGR), oligohydramnios, use of tobacco, alcohol, and cocaine during pregnancy, cardiovascular diseases, diabetes, and kidney failure. By referring to the patient's file, we extracted the information such as psychiatric screening that was evaluated every three months during pregnancy and recorded in the file, and even the screening for diabetes, hypertension and acute fatty liver etc. using the diagnostic methods. The rest of information was got from interviews.

Evaluation of serum cortisol and IL-1β

About 10 mL of blood samples were collected from all individuals after 24 hours of delivery at 8 A.M. while pregnant women were fasting (about 10 hours). Each blood sample was aliquoted into two parts. One part was specified for the isolation of serum and another for the isolation of peripheral blood mononuclear cells (PBMCs). To separate serum from the whole blood sample, tubes were immediately centrifuged at 3,500 rpm for 10 minutes, and the obtained sera were stored at -80°C until the measurements. The concentrations of IL-1β and cortisol were measured using the ELISA commercial kits [for IL-

1β (Quantikine HS ELISA Kit HSLB00D, Minneapolis, MI, USA) with an intra-assay coefficient of variation (CV)=2.4%, inter-assay %CV=6.3% and for cortisol (IBL international, Hamburg, Germany) with an intra-assay CV=2.57%, inter-assay %CV=3.41%], and finally the absorbance of the samples (450 nm) was read with an ELISA Reader (Bio-Tek ELX800, Winooski, VT, USA). All the tests were carried out as duplicate measurements.

Western blot

To extract the total cellular protein contents, 100 mL of the blood samples were homogenized in 1 mL ice-cold extraction buffer (PBS). After the incubation for 20 min at 4°C, two centrifugation steps were performed at 4°C at 14,000×rpm for 20 min to remove cell debris. Afterward, 20 μg of the extracted proteins were loaded in each lane on 7.5% (w/v) polyacrylamide gel and then transferred to a PVDF membrane (Amersham Biosciences, Freiburg, Germany). After the incubation in blocking solution (5% skim milk powder in 1×PBS), the membrane was incubated with primary antibodies against *SKA2* (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a 1:300 dilution and β-actin at a 1:1,000 dilution (as a loading control) at 4°C overnight. After washing the membrane, it was probed with anti-rabbit IgG-HRP (Santa Cruz Biotechnology) at a 1:3,000 dilutions for 1−2 h at room temperature for 3−5 hours. The bound proteins were visualized by chemiluminescence using enhanced electrochemiluminecence (ECL) reagents and subsequent autoradiography. Densitometry analysis was performed using scanning immunoblots and quantitating protein bands using ImageJ software (National Institutes of Health, imagej.nih.gov/ij).

RNA extraction and reverse transcription

About 5 mL of whole blood was collected and poured into two K2 ethylenediaminetetraacetic acid (EDTA) tubes. The tubes were stored at -20°C. The EDTA tubes were transferred into a 50 mL polypropylene conical centrifuge tube, and then RBC Lysis Buffer was added until the volume brought to 45 mL. The mixtures were left at room temperature for 10 minutes. Then, 1,200 μL of TRIzol solution was added to each tube. The extracted RNA was assessed by the measurement of the absorbance at 260 nm using NanoDrop ND-1000 (NanoDrop Technologies, Montchanin, DE, USA). After mixing and centrifugation, the extracted RNA was stored on ice. Next, 5 μg of the total RNA was added to 1 μL Random Hexamer primer, and then the volume was brought to 12 μL with distilled water according to the Ampliqon Kit (Ampliqon, Odense, Denmark). RNA template was mixed, centrifuged, and incubated at 65°C for 5 minutes and immediately placed on ice. In the next step, 4 μL of 5X reaction buffer, 1 μL of Ribolick RNase Inhibitor, 2 μL of 10 Mm dNTP Mix, and 1 ML of Revert Aid M-MuL-VRT (200 U/μL) were added to the final product, and the volume was brought to 20 μL with distilled water (Ampliqon Kit Denmark).

Quantitative real-time PCR

The expression of *SKA2* gene was measured by real-time polymerase chain reaction. In this method, 5 μL of Master Mix SyberGreen (10 μmol/L) was added to 0.5 μL of the forward primer (*SKA2*) with the sequence of 5'-GCCCAACAG-GAAAATGTGTC-3' and 0.5 μL of reverse primers with the sequence of 5'-CCGCCTCCATGTTGAATAGT-3'. The same amount of Master Mix SyberGreen was added to 0.5 μL of the forward primer (GAPDH) with the sequence of 5'-GAAGGT-GAAGGTCGGAGTC-3' and 0.5 μL of the reverse primer with the sequence of 5'-GAAGATGGTGATGGGATTTC-3'. Next, 0.8 μL of complementary DNA (cDNA) was added and the volume was brought to 10 μL with distilled water. The PCR was performed by the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The threshold cycle (CT) is defined as a fraction of the cycle at which the fluorescence is emitted from denatured DNA. The PCR products were identified using ethidium bromide with electrophoresis on 1.2% agarose gel.

Anxiety detection

In this study, the anxiety scores were examined by Spilberger Inventory. About 40 items in two sections of the state and trait Anxiety Inventory were used. Items in a four-point Likert scale were evaluated. Each item was categorized from 1 to 4 followed by the rate of overall scores which was varied from 20 to 80 (Cronbach alpha 0.94 and 0.87, respectively).[34]

Statistical analysis

Baseline data and demographic characteristics of participants were compared using chi-square tests. The independent t-test was applied to compare differences between term and preterm groups. Pearson correlation coefficient was separately calculated between the expression of the *SKA2* gene and the concentrations cortisol and IL-1β, as well as the score of the state and trait anxiety. All of the statistical analyses were performed by the SPSS software, version 21 (IBM Corp., Armonk, NY, USA). The p-value of less than 0.05 was considered statistically significant.

RESULTS

The comparison of the relative gene expression of *SKA2*, as well as the levels of cortisol and IL-1β, and last menstrual period (LMP), baby weight, and APGAR score between the two

preterm and term groups, showed significant differences. The anxiety scores (trait and state) in preterm group slightly more than term group but not significant (Table 1, Figure 1).

As shown in Table 2, there is a significantly positive correlation between the increase in the expression of *SKA2* gene and cortisol, IL-1β, and state anxiety, as well as *SKA2* expression has significant negative correlation with LMP, baby weight, and APGAR. There is also a significant relationship between increased levels of cortisol and the concentration of IL-1β, as well as the score of state and trait anxiety. An increment in the levels of IL-1β was also significantly associated with state and trait anxiety. There is significantly negative correlation between the increase of both cortisol and IL-1β levels with LMP, baby weight, and APGAR.

Table 1. The comparison of all variables in preterm and term delivery groups

Variables	Groups	N	Mean	SD	p-value
Age (years)	Preterm	49	25.21	0.29	0.682
	Term	51	26.37	0.34	
SKA2	Preterm	49	2.9167	0.41706	<0.001
	Term	51	1.7016	0.52210	
Cortisol (nmol/L)	Preterm	44	270.7045	73.55320	<0.001
	Term	42	207.3333	53.37610	
IL-1β (pg/mL)	Preterm	49	2.5316	0.53172	0.046
	Term	51	2.3112	0.55673	
Trait anxiety	Preterm	49	50.4490	13.85349	0.956
	Term	51	50.2941	14.23137	
State anxiety	Preterm	49	50.8163	14.54400	0.964
	Term	51	49.1176	14.75757	

According to Table 3, it was shown that parallel with an increase in the expression of *SKA2* gene in the preterm group, the rate of anxiety levels, as well as the levels of IL-1β and cortisol was heightened. Accordingly, an increase in the level of cortisol is significantly associated with the increase in the concentration of IL-1β and the score of state and trait anxiety. Notably, increased level of IL-1β is significantly correlated with the increase in the score of state and trait anxiety. The present data demonstrate that the increase of *SKA2* expression, cortisol, IL-1β, and anxiety scores significantly negatively correlated with LMP, baby weight, and APGAR

As shown in Table 4, in term group expression of the *SKA2* gene has not significant correlation with the concentration of cortisol and IL-1β, as well as the score of trait anxiety. An increase in the expression of *SKA2* leads to a reduction in the levels of factors which were mentioned earlier. On the other hand, parallel with the increase in cortisol, a significant increase was observed in the level of IL-1β and the degree of state anxiety and trait anxiety. An increase in the level of IL-1β is also significantly correlated with the increase in state and trait anxiety.

As indicated in Figure 2 and Table 5, the western blot analysis showed that the level of *SKA2* protein was significantly higher (p=0.008) in the preterm group compared with the term group.

DISCUSSION

There is some evidence on the association of anxiety and premature birth. However, causing why anxiety lead to preterm delivery is not clear, but the rate of anxiety is high in pregnant women, causing many adverse effects such as hyperten-

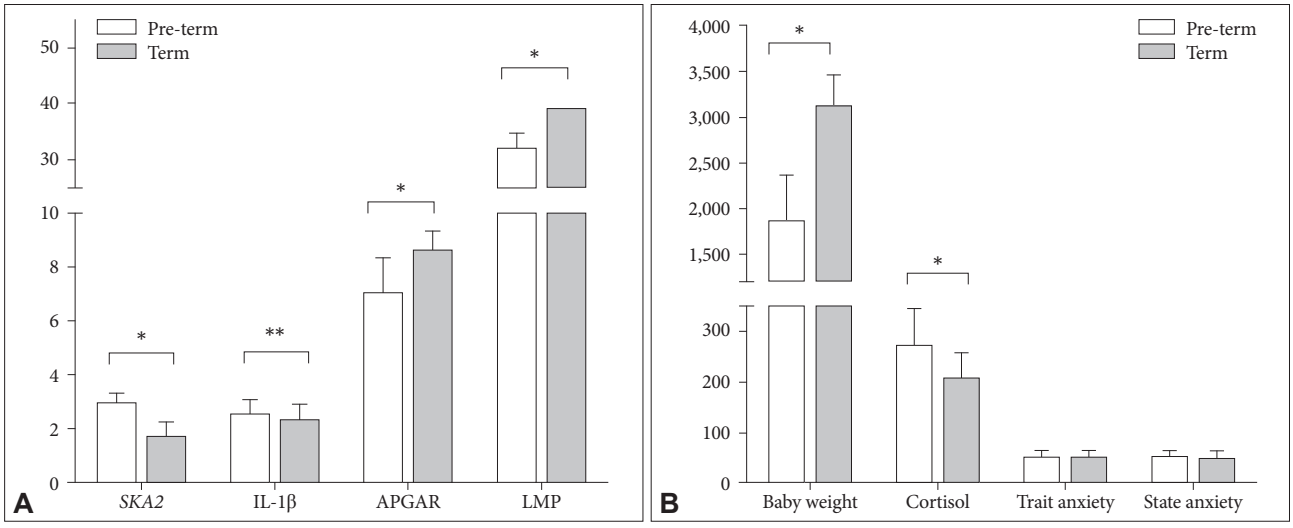

Figure 1. The comparison of all variables (A. *SKA2*, IL-1β, APGAR and LMP as well as B. Baby weight, cortisol, Trait anxiety and State anxiety) between preterm and term groups. All variables except for both parameter of anxiety showed a significant difference between the term and preterm groups. Data are presented as the mean±SD (preterm N=49, term N=51) and were analyzed by independent sample t-test. *p<0.0001, **p<0.05. Units: nmol/L for cortisol, pg/mL for IL-1β, weeks for LMP, grams for body weight.

Table 2. Correlation analysis between *SKA2* expression and other variables in all participants

SKA2	Cortisol	IL-1β	Latent anxiety	Apparent anxiety	LMP	Baby weight	APGAR
SKA2	R=0.551	R=0.266	NS	R=0.202	R=-0.765	R=-0.744	R=-0.571
	p<0.001	p=0.008		p=0.040	p<0.001	p<0.001	p<0.001
	Cortisol	R=0.916	R=0.729	R=0.697	R=-0.589	R=-0.559	R=-0.428
		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
		IL-1β	R=0.735	R=0.690	R=-0.331	R=-0.294	R=-0.243
			p<0.001	p<0.001	p<0.001	p=0.003	p=0.015
			Latent anxiety	R=0.879	NS	NS	NS
				p<0.001			
				Apparent anxiety	NS	NS	R=-0.203
							p=0.042
					LMP	R=0.944	R=0.778
						p<0.001	p<0.001
						Baby weight	R=0.756
							p<0.001

LMP: last menstrual period, NS: not significant

Table 3. Correlation analysis between *SKA2* expression and other variables in preterm group

SKA2	Cortisol	IL-1β	Latent anxiety	Apparent anxiety	LMP	Baby weight	APGAR
SKA2	R=0.835	R=0.827	R=0.669	R=0.644	R=-0.421	R=-0.419	NS
	p<0.001	p<0.001	p<0.001	p<0.001	p=0.003	p=0.003	
	Cortisol	R=0.913	R=0.694	R=0.698	R=-0.527	R=-0.520	NS
		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	
		IL-1β	R=0.650	R=0.663	R=-0.540	R=-0.532	R=-0.327
			p<0.001	p<0.001	p<0.001	p<0.001	p=0.022
			Latent anxiety	R=0.906	R=-0.398	R=-0.408	R=-0.324
				p<0.001	p=0.005	p=0.004	p=0.023
				Apparent anxiety	R=-0.418	R=-0.443	R=-0.351
					p=0.003	p=0.001	p=0.014
					LMP	R=0.957	R=0.704
						p<0.001	p<0.001
						Baby weight	R=0.722
							p<0.001

LMP: last menstrual period, NS: not significant

Table 4. Correlation analysis between *SKA2* expression and other variables in term group

	Cortisol	IL-1β	Latent anxiety	Apparent anxiety	Baby weight	APGAR
SKA2	NS	NS	NS	NS	NS	NS
	Cortisol	R=0.895	R=0.812	R=0.700	NS	NS
		p<0.001	p<0.001	p<0.001		
		IL-1β	R=0.831	R=0.718	NS	NS
			p<0.001	p<0.001		
			Latent anxiety	R=0.857	NS	NS
				p<0.001		
				Apparent anxiety	NS	NS
					Baby weight	NS

NS: not significant

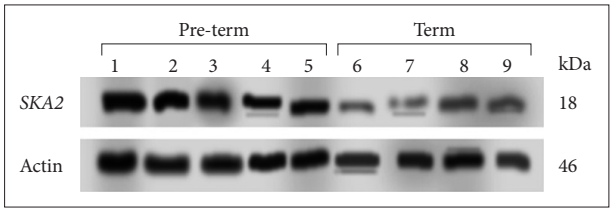

Figure 2. Representative Western blots showing the immune labeling of spindle and kinetochore associated complex subunit 2 (*SKA2*) and beta-actin in the membrane fraction of 2 groups: preterm labor, term labor. No. 1 to 5 is related to preterm delivery, with cortisol levels of 469, 335, 425, 341, and 388, respectively. Numbers 6 to 9 are related to term delivery, with cortisol levels 282, 268, 318, and 299, respectively.

Table 5. Comparison of protein *SKA2* synthesis between preterm and term groups

	Groups	Mean±SD	p value
Protein *SKA2*	Preterm	1.18±0.16	0.008
	Term	0.63±0.28	

SD: standard deviation

sion, preeclampsia, and growth restriction during gestation period.[35-38] The prevalence of anxiety in gestation period varies in different populations. An epidemiological study indicated that the incidence of anxiety in pregnant women was 20% in Pakistan, while it was 11% in South Africa. The severity of anxiety is a spectrum from the moderate to low grades in pregnant women.[39] In a study performed by Shapiro et al.,[40] it was shown that premature birth was linked with the stress-related infections, but did not correlate with mental stress. In line with this, Ravid et al.[41] demonstrated that anxiety was not associated with pregnancy complications. Contrarily, Ding et al.[38] showed that maternal anxiety has a strong linkage with preterm delivery.

It seems that biological studies are warranted to elucidate the association of anxiety with premature birth. In accordance with our results, the mean trait anxiety was partially higher in women with preterm delivery in comparison with those with term delivery. The current study results include maternal anxiety which has a significant positive correlation with *SKA2* expression, cortisol concentration, and IL-1β concentration. As well as, these mentioned factors have significant negative correlation with LMP, AGPAR and baby weight. From these results, it can be concluded that anxiety, the expression of *SKA2* gene, and the production of cortisol and IL-1β were correlated to the pregnancy period and the weight of infants.

A study carried out by Giurgescu;[42] revealed that anxiety and mental stress in pregnant women, as a consequence of increased levels of cortisol, could result in premature birth. Notably, preterm delivery is associated with the early increase in CRH.[41] Besides, elevated levels of CRH can increase the concentration of cortisol and estrogen. Heightened ratios of estrogen to progesterone may disturb the uterus tranquility, which could increase the risk of premature birth. An early increase in cortisol can increase the uterus contraction and subsequently cause premature birth mediated by the production of prostaglandins (as an uterotonins).[43] Our data indicated that an increase in the levels of cortisol results in a reduction in birth weight and LMP, considering that the levels of cortisol were elevated in women with preterm delivery compared with term delivery group.

It has been implicated that *SKA2* protein is able to increase the levels of glucocorticoids via chaperoning the glucocorticoid receptors and transporting them from the cytoplasm into the nucleus.[29] In 2016, Boks et al.[44] reported that the rate of *SKA2* gene methylation is reduced in patients with stress disorders, which is eventually resulted in an increase in the levels of cortisol. Also, Ijabi et al.[31] carry out a study which has evaluated *SKA2* gene SNP (NC_000017.11: g.59110368G>A) as a predictive biomarker for the prediction of preterm delivery. In the present study, *SKA2*, at the level of protein and gene expression, was significantly elevated in premature birth compared with term delivery. There were significant positive correlations between the *SKA2* gene expression and other variables (cortisol, IL-1β and anxiety) in all participants (Table 2). Our findings indicated that the increased expression of *SKA2* results in decreased LMP, APGAR, and birth weight in preterm group (Table 2) but not in term group (Table 3). Following an increase in the expression of *SKA2* gene, the concentration of the corresponding protein would be increased and we also have shown that *SKA2* protein is higher in women with preterm birth than term.

In 2012, Christian[45] demonstrated that chronic mental stress has detrimental effects on cell-mediated immunity, which could be resulted in serious complications. Fast et al.[46] showed that increased levels of cortisol could elevate the concentrations of IL-1β in Salmon fish. Prinz et al.[22] in a study, evaluate the influencing factors such as stress, cortisol, and IL-1β in healthy aged people. They highlighted that an increase in cortisol could result in elevated levels of IL-1β secretion. There is still ambiguity on how glucocorticoids can shift the balance in favor of either inflammatory or anti-inflammatory responses. In this context, Straub and Cutolo[21] showed that chronic increase of glucocorticoids in blood circulation could increase inflammation in patients, making them obliged to use immunosuppressive/immunomodulatory agents to quench the elevated inflammatory reactions. Cytokines, produced in the decidua, increase the secretion of uterotonins such as prostaglandins and they are capable of initiating the uterus contraction. A number of studies demonstrated that anti-inflammatory compounds play an axial role in the prevention of premature birth. It has been reported that elevated levels of prostaglandins would increase the risk of premature birth.[47,48] A higher degree of cell death and apoptotic index was observed in pregnant women PROM

when compared with those with term delivery. It has been shown that TNF-α and IL-1β play a regulatory role in apoptosis. Moreover, increased amounts of cytokines bring about the elevation in metalloproteases and deformation in collagen.[49] In line with previous studies, we showed that the levels of IL-1β were significantly higher in preterm delivery in comparison with term delivery (Figure 1) and also indicated that IL-1β had a significant inverse correlation with LMP, APGAR, and birth weight. Our current results show that there is a direct relationship between the expression of *SKA2* and each early-mentioned factors such as cortisol, IL-1β, and anxiety in preterm women. The over-expression of the *SKA2* gene can result in the elevation of IL-1β and the risk of preterm delivery by increasing the levels of cortisol. Understanding the biological pathways involved in anxiety could shed light on the role of chronic stress in the increase of the levels of cortisol and IL-1β, which indirectly predispose pregnant women to develop premature birth.

The present investigation has the following limitations: the lack of evaluation of molecular signaling pathways, failure to follow the participants in subsequent pregnancies as well as the low sample size. Furthermore, we evaluated the relation of *SKA2* to IL-1β in preterm delivery for the first time that it was the strengths of the study. According to our findings, we suggest further studies are warranted to illuminate the role of *SKA2* gene in preterm delivery.

In conclusion, the results revealed that the *SKA2* gene expression was significantly higher in preterm delivery compared with pregnant women with term delivery. Additionally, the correlation analysis showed that increased expression of *SKA2* in the preterm group was positively correlated with cortisol, IL-1β, and anxiety while in the term group, such correlations were non-significant. According to the results obtained in the present study, it can be inferred that perhaps, *SKA2* gene could trough increasing of cortisol and IL-1β level lead to preterm delivery.

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Janat Ijabi, Roghayeh Ijabi. Data curation: Roghayeh Ijabi. Formal analysis: Hemen Moradi-Sardareh. Funding acquisition: Roghayeh Ijabi. Investigation: all authors. Methodology: Hemen Moradi-Sardareh, Janat Ijabi, Reza Afrisham, Roghayeh Ijabi. Projectadministration: Janat Ijabi, Roghayeh Ijabi. Resources: Janat Ijabi, Roghayeh Ijabi, Parisa Roozehdar. Software: Reza Afrisham, Janat Ijabi, Roghayeh Ijabi, Hemen Moradi-Sardareh. Supervision: Roghayeh Ijabi. Validation: Janat Ijabi, Roghayeh Ijabi. Visualization: Janat Ijabi, Roghayeh Ijabi. Writing—original draft: all authors. Writing—review & editing: Hemen Moradi-Sardareh, Janat Ijabi, Reza Afrisham, Roghayeh Ijabi.

ORCID iDs

Janat Ijabi	https://orcid.org/0000-0002-4945-5757
Reza Afrisham	https://orcid.org/0000-0001-8064-118X
Hemen Moradi-Sardareh	https://orcid.org/0000-0002-5522-4445
Parisa Roozehdar	https://orcid.org/0000-0003-4056-9341
Fatemeh Seifi	https://orcid.org/0000-0001-9698-8575
Roghayeh Ijabi	https://orcid.org/0000-0002-1659-761X

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