IL-17A Polymorphism (rs2275913) and Levels Are Associated With Preeclampsia Pathogenesis in Chinese Patients

Xiao Lang
Shanghai Jiao Tong University
Wei Liu
Shanghai Jiao Tong University
Yanyan Hou
Shanghai Jiao Tong University
Wenxia Zhao
Tongji University
Xingyu Yang
Shanghai Jiao Tong University
Lan Chen
Shanghai Jiao Tong University
Qi Yan
Tongji University
Weiwei Cheng (weiweicheng34@yahoo.com)
The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, 200030 2Shanghai Municipal Key Clinical Specialty, Shanghai, China, 200030 https://orcid.org/0000-0002-3999-4378

Research article

Keywords: preeclampsia, IL-17A, IL-23A, gene polymorphism, serum levels

Posted Date: August 24th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-58793/v1

Version of Record: A version of this preprint was published at BMC Medical Genomics on January 6th, 2021. See the published version at https://doi.org/10.1186/s12920-020-00840-8.

Abstract

Background

Preeclampsia (PE) is a pregnant related syndrome affecting both child and mother. Although, the role of various inflammatory molecules in PE has been demonstrated, importance of proinflammatory molecules such as IL-17A, IL-23 are poorly understood. In the present investigation, we tested a possible association of common genetic variants in IL-17A and IL-23 gene with PE.

Methods

Clinically diagnosed 115 PE patients who reported to the International Peace Maternity and Child Health Hospital were enrolled in the present study. Age, sex-matched 102 pregnant women, and 147 healthy Chinese women were also included. ELISA was used to quantify serum levels of IL-17A and IL-23 in all enrolled subjects. Common genetic polymorphisms in *IL-17A* (rs 2275913, rs1974226, and rs1974226), IL-23A (rs11171806), and *IL-12B* (rs3212227) were genotyped by PCR-RFLP or TaqMan probe-based method.

Results

Elevated levels of serum IL-17A was observed in PE patients compared to pregnant (P<0.0001) and healthy women (P<0.0001). However, IL-23 levels were comparable among different clinical categories. Furthermore, heterozygous (GA) and minor allele (A) for *IL-17A* (rs2275913) and *IL-23A* (rs11171806) were more prevalent in PE patients in comparison to pregnant ladies or healthy women suggesting an essential role in predisposition to PE development. Interestingly, IL-17A (rs 2275913) mutants were linked with elevated IL-17A levels in comparison to wildtype (GG).

Conclusions

IL-17A (rs2275913) variants are associated with higher serum levels of cytokine, and predisposed PE development.

Background

Preeclampsia (PE) is a pregnancy-related complication, characterized by high blood pressure and dysfunctions of various organ systems. The PE syndrome has a deleterious effect on both the mother and the developing foetus. Although the exact cause and pathogenesis of this ailment are not known, it is believed that the disease is driven by several factors released from trophoblast induced by placental pressure, and these components promote an overwhelming maternal inflammatory response (Tjoa et al. 2007). An estimate suggested that about 10 million people develop PE every year worldwide. Furthermore, about seventy-six thousand pregnant women lead to death each year due to PE and approximately five lakhs babies die per annum linked with PE (Kuklina et al. 2009). Epidemiological reports on PE in Chinese population are very limited: a retrospective study from three different hospitals

of China showed about 2.35% developed PE from a total of 67,746 pregnant women and the PE was most frequent in nulliparity subjects (81.5%) (Xiao et al. 2014). There is a vast repertoire of immunological modulators and signaling pathways contributing to the onset and progression of PE. Prior evidences suggested that among different immune molecules, cytokines play a critical role in regulating different stages of pregnancy (Bowen et al. 2002; Sargent et al. 2006). The Th1: Th2 dichotomy during pregnancy suggested that a Th2 mediated immunity is involved in maintaining regular events during pregnancy, whereas Th1 type of immune response is associated with pregnancy-related problems such as miscarriage (Raghupathy et al. 2000), premature delivery (Sykes et al. 2012), rupture of fetal membranes before initiation of labor pain (Raghupathy et al. 2001). Previous studies have mentioned a disparity in levels of cytokines in the preeclamptic placenta, leading the complicated conditions such as delayed intrauterine growth and preterm delivery (Raghupathy et al. 2012). The cytokine IL-17 produced from Th17 lymphocytes that were recently discovered as a subset of $CD4^+$ T lymphocytes (Benghiat et al. 2009). Complications such as acute graft rejections reactions and several chronic inflammatory conditions such as cancers, allergic asthma, and other autoimmune disorders were found to be associated with the predominant pathogenic role of IL-17 cytokine (Schnyder-Candrian et al. 2006), (Curtis and Way 2009). Several reports suggested a higher percentage of Th17 cells populations in complicated pregnancies cases, such as miscarriage, preterm birth or PE (Darmochwal-Kolarz et al. 2012; Ito et al. 2010; Nakashima et al. 2010).

Interlukin-23 (IL-23) is a proinflammatory cytokine responsible for the discrimination, expansion, and survival of Th-17 cells (Aggarwal et al. 2003). An upregulation of the Th-17 cell-mediated immune response has been demonstrated in pathogenesis of PE (Darmochwal-Kolarz et al. 2002). IL- 23 is a heterodimeric cytokine consist of IL-12B and IL-23A subunit. Differential levels of IL-23 in preeclamptic patients have been reported earlier (Darmochwal-Kolarz et al. 2012). Based on the importance of IL-23 in controlling inflammation, we hypothesized that IL-23 cytokine could be linked with the clinical condition of PE patients in a Chinese cohort.

Variance of serum cytokines levels in subjects have been associated with functional single nucleotide polymorphisms (SNPs). Although several SNPs are reported in the IL-17A gene, common polymorphisms like rs2275913 (-197G > A), rs1974226 (3'UTR C > T), and rs3748067 (1249C > T) are widely investigated on genetic association studies. Similarly, variants of IL-23A (rs11171806: A > G, exon 106Ser > Ser) and IL-12B (rs3212227 A > C 3'UTR) also investigated in various reports and their association with susceptibility to a wide range of diseases has already been established. Since functional SNPs in IL-17A, IL-23A, and IL-12B associated with levels of cytokines, we hypothesized that common variants would be linked with the development of PE in a Chinese cohort.

Methods

Study subjects

The present study was performed between a period from March 2017 to December 2019 at the International Peace Maternity and Child Health Hospital after the approval of study protocol by the Institutional Review Board of Shanghai Jiao Tong University. Primarily, three study groups were recruited in the investigation. Before the investigation, all enrolled subjects were submitted signed consent form as per the ethical committee guidelines. The first category included 115 patients with PE. The primary inclusion criteria for these patients was third-trimester pregnancy complicated with PE, blood pressure > 140/90 with proteinuria > 300 mg in 24 hours (According to ACOG) (Bulletins–Obstetrics 2002). The second category included 102 women in third-trimester pregnancy without PE. The third category included 147 healthy non-pregnant women and was used as controls. The exclusion criteria included patients with microvascular complications, co-existing autoimmune, chronic/acute inflammatory diseases, multiple gestations, diabetes mellitus, sickle cell disease, and HIV infection. Data for clinical characteristics were collected from hospital records.

Collection of serum

Three milliliters of blood samples (without anticoagulant) collected intravenously from pregnant women and non-pregnant controls before starting therapy. The serum was separated from each sample by centrifuging blood at 950 g for 5 minutes. The supernatant was collected and kept at -80^{0}C for future quantification of cytokines.

Cytokines (IL-17A and IL-23A) quantification

Serum levels IL-17A or IL-23 were quantified in all subjects enrolled for the present investigation by enzyme-linked immunosorbent assays (ELISA) using the predesigned kit as per the instructions of the producer's (R&D Systems, Inc, USA).

Genomic DNA isolation

200 ul whole blood was mixed with anticoagulant was used for separation of genomic DNA. The whole genomic DNA was isolated using the whole blood by SIGMA mini Genomic DNA extraction kit.

Genotyping of IL-17A, IL-12B and IL-23A polymorphisms

A polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) technique was employed for the genotyping of IL-12B (rs3212227), as described by an earlier report(Chen et al. 2009). Briefly, two primers (forward: GATATCTTTGCTGTATTTGTATAGTT and reverse: AATATTTAAATAGCATGAAGGC) were used for amplification of a 118 bp gene fragment flanking the polymorphic site. The thermal cycler conditions were as follows: early denaturation at 95^{0}C for 5 minutes followed by 35 cycles of 95^{0}C for 40 s, 55^{0}C for 35 s, and at 72^{0}C for 25 s. The final extension reaction was performed at 72^{0}C for 10 minutes. The amplicon was digested with TaqI restriction enzyme and fragments were analysed for rs3212227 polymorphism. Amplicon with mutant allele has a restriction site for TaqI, thus produces two fragments (92 bp + 26 bp); on the other hand, for wildtype allele, the amplicon remained undigested (118 bp). Similarly, the PCR-RFLP technique was also used for genotyping of IL-17A (rs2275913, rs1974226, and rs3748067) polymorphisms, as described in an earlier report(Keramat et al.

2019). Following primers were used for amplification of IL-17A gene bordering SNPs sites and yield different amplicons (rs2275913: forward- GCTCAGCTTCTAACAAGTAAG, reverse- AAGAGCATCGCACGTTAGTG, amplicon size-338 bp; rs1974226: forward- AAAGGAGCTGATGGGGCAGTA, reverse- GGTCTTTCAAGAAGCAGGGAG, amplicon size-211; rs3748067: forward- GGGCTGAACTTTTCTCATACTTAGA, reverse- GAGACATTGTCTTCAGACTACAATG, amplicon size-212 bp). The annealing temperature for genotyping of IL-17A polymorphisms was fixed at 58^0C, and other conditions were like those of IL-12B. Different restriction enzymes were used (rs2275913: EarI, rs1974226: RsaI and rs3748067: EcoRI) for digestion of the amplicon and based on differential digested DNA fragments, genotypes of subjects were determined as follows (rs2275913: A = 259 + 79 bp, G = 338 bp; rs1974226: A = 221 bp, G = 191 + 20 bp and rs3748067:G = 212 bp, A = 198 + 24 bp). As described earlier(Jia et al. 2015), the IL-23A (rs11171806) gene polymorphism was genotyped by the TaqMan PCR assay by using a TaqMan probe.

Statistical Analysis

Graphpad prism v8.2 was employed for all statistical analyses. Serum levels of IL-17A and IL-23A in different clinical groups were compared by one-way analysis of variance (ANOVA) and mean cytokine levels were compared in between all clinical categories by Tukey's post-test. Genotype and allele frequencies were calculated by manual counting. Genotypes distribution of all studied SNPs were tested for Hardy Weinberg equilibrium with in house developed Microsoft excel file. Distribution of genotypes and alleles were compared with Fisher exact test in different clinical categories. A P value of less than 0.05 was taken as significant.

Results

Baseline characteristics of patients and controls

Three hundred sixty-four females were enrolled in the present study comprising 115 PE patients, 102 pregnant women, and 147 non-pregnant women. Different baseline characteristics were compared among clinical categories (Table-1). A significant difference was observed in different parameters while comparing PE cases and pregnant women, such as duration of gestation (days), body mass index (Kg/m2), vaginal delivery (%), caesarian section (%), fetal birth weight (gram), systolic/diastolic blood pressure (mmHg), WBC count ($x10^9$/ L), levels of urea and uric acid (mg/dL). However, % of pre-eclamptic patients with primiparas were not significantly altered in comparison to healthy women.

PE patients displayed higher serum IL-17 compared to controls

Previous reports (Darmochwal-Kolarz et al. 2012; Poordast et al. 2017) have revealed the importance of IL-17 and IL-23 on the clinical condition of PE in different populations, however, the number of patients enrolled was very low. To validate the link between IL-17 and IL-23 with the pathological process of PE in Chinese patients, women from three different clinical categories (healthy, pregnant, and PE) were enrolled

in the present investigation, and serum levels of IL-17 and IL-23 were quantified. IL-17 levels in three different study groups are shown in figure-1A. Mean level of IL-17 was 59.1 ± 0.93 pg/ml in healthy women without pregnancy, whereas healthy pregnant women and subjects with PE had 61.13 ± 1.43 pg/ml and 746.7 ± 17.16 pg/ml of IL-17 level, respectively. Although a comparable level in IL-17 was observed between healthy pregnant and non-pregnant women, subjects with PE demonstrated a noticeably higher cytokine level as compared to two other study groups, suggesting an essential role of this molecule in promoting pathogenesis during PE. Further, to assesses the importance of IL-23 in regulating the pathologic condition of PE, the titer of the cytokine was measured in sera and the results are shown in figure-1B. The results showed a relatively similar level of this cytokine in all the three groups.

Distribution of IL-17A, IL-23A and IL-12B polymorphisms in healthy non-pregnant women

A total of 147 healthy non-pregnant women were enrolled in the present investigation. All subjects were genotyped for IL-17A (rs2275913, rs1974226, and rs3748067), IL-23A (rs11171806) and IL-12B (rs3212227) polymorphisms by appropriate methods, and the frequency of genotypes are given in the Table-2. The distribution of genotypes for all SNPs are in Hardy-Weinberg equilibrium (rs2275913: X^2 = 2.58, P = 0.10; rs1974226: X^2 = 2.83, P = 0.08; rs3748067: X^2 = 0.17, P = 0.67; rs11171806: X^2 = 1.93, P = 0.16; rs3212227: X^2 = 0.01, P = 0.90)

IL-17A (rs2275913) and IL-23A (rs11171806) polymorphisms are associated with predisposition to PE

Various factors are responsible for cytokine levels in humans, and differential cytokines levels in subjects have been linked with individuals' genetic makeup(Li et al. 2016). In the current investigation, we detected higher levels of IL-17A in PE patients compared to healthy controls and pregnant women. However, serum IL-23 levels were comparable among different clinical groups. Furthermore, IL-23 and IL-12B are interlinked and responsible for inflammations. Based on these observations, we hypothesized that variants in IL-17A, IL-23A, IL-12B gene would be associated with predisposition to PE development. Common functional polymorphisms in IL-17A (rs2275913, rs1974226, and rs3748067), IL-23A (rs11171806) and IL-12B (rs3212227) were genotyped in PE patients, healthy controls and pregnant women by appropriate methods and the results are shown in Table-2. Heterozygous (GA) and minor allele (A) of IL-17A (rs2275913) polymorphism were significantly more prevalent in PE patients compared to healthy women (GA: P = 0.005, OR = 2.35; A: P = 0.05, OR = 1.41) and pregnant women (GA: P = 0.007, OR = 2.40; A: P = 0.02, OR = 1.54). However, the distribution of mutant genotypes (GA and AA) and minor allele (A) in healthy women and pregnant women were comparable. Similarly, no significant genetic association was observed in the distribution of other IL-17 polymorphisms (rs1974226 and rs3748067) and IL-12B (rs3212227) in PE patients in comparison to healthy women or pregnant women. Interestingly, when we analyzed association of IL-23A polymorphism (rs11171806) with predisposition to development of PE, a significant link of heterozygous variants and minor allele were noticed with susceptibility to PE

development irrespective of pregnant status of women (healthy non-pregnant women; GA: P = 0.007, OR = 2.93, A: P = 0.009, OR = 2.51; pregnant women; GA: P = 0.008, OR = 3.24, A: P = 0.004, OR = 3.27).

Genotype-phenotype association of IL-17A and IL-23A polymorphisms

As IL-17A and Il-23A polymorphisms are associated with predisposition to PE development, we hypothesized the possible role of those variants with the determination of serum concentration of IL-17A and Il-23A. Serum levels of IL-17 were compared among different genotypes of IL-17A polymorphisms (rs2275913, rs1974226, and rs3748067). As shown in Figure-2, AA and GA genotypes of rs2275913 polymorphisms displayed significantly higher serum IL-17A compared to wildtype (GG). Serum IL-17A levels were comparable among different genotypes of rs1974226 and rs3748067 polymorphisms (data not shown). Furthermore, variants of IL-23A (rs11171806) also failed to demonstrate any functional relevance on serum levels of IL-23A.

Discussion

Observations from the last few years, it is evident that PE is related to unrestricted proinflammatory activation of the maternal immune system. Earlier studies have indicated a substantial role of many cytokines in mediating the pathogenesis of PE. However, the pathogenic role of IL-17 and IL-23, two crucial proinflammatory cytokines, has never been seriously looked at in the context of PE, and we aimed at fulfilling this lacuna.

Before examining the biological relevance of proinflammatory cytokines in PE, we compared different clinical characteristics among the three groups, i.e., PE patients, pregnant cases, and healthy non-pregnant women. Data clearly showed a reduction in duration of gestation, % vaginal delivery, and fetal birth weight in subjects with PE as compared to pregnant women without any disease. On the other hand, pre-eclamptic women demonstrated an increased body mass index (BMI), % caesarian section, blood pressure, WBC count, serum urea, and uric acid levels as compared to healthy controls. All these data were consistent with an earlier report (Darmochwal-Kolarz et al. 2017).

We observed elevated IL-17A levels in PE patients in comparison to the pregnant ladies and healthy cases. However, earlier reports remained controversial concerning the importance of IL-17 in PE. For example, a study by Jonsson et al. (Jonsson et al. 2006) showed no evidence of possible links between cytokine levels and PE pathologic conditions. On the contrary, our result is corroborated with earlier observations indicating a remarkably higher titer of IL-17A in the sera of pregnant subjects complicated by fetal growth restriction (FGR) and PE as compared to healthy pregnant normotensive women (Darmochwal-Kolarz et al. 2017). These results were further strengthened by several other reports mentioning that there was a higher prevalence of Th17 cells in peripheral blood and enhanced expression of RORγt mRNA (transcription factor of Th17 cells) in placentas of pre-eclamptic subjects as compared normal healthy ones (Darmochwal-Kolarz et al. 2012), (Toldi et al. 2011), (Jianjun et al. 2010). Overall, all these studies possibly indicate a pathogenic role of IL-17A in PE.

In contrast, we did not observe a possible difference in serum level IL-23A levels among three different studied groups. These results are contradictory to an earlier report (Poordast et al. 2017) where a significantly lower level of this cytokine was demonstrated in pregnant groups (with and without PE) in comparison to healthy non-pregnant subjects. However, in line with our observations, a report by Darmochwal-Kolarz et al. (Darmochwal-Kolarz et al. 2017) also failed to demonstrate the difference of Il-23A among pregnant subjects with placental insufficiency (fetal growth restriction and PE) and healthy pregnant women. Our result showing a comparable level of IL-23 in subjects with PE and healthy pregnant women matched with the report, as mentioned above. Moreover, a comparable level of this cytokine in both the pregnant groups and healthy non-pregnant subjects indicate a negligible role of IL-23 in the context of pregnancy and its related complication in PE.

Common polymorphisms in the IL-17A gene have been associated with hypertension (Huang et al. 2017)and various organ dysfunctions(Domanski et al. 2019). As the primary clinical characteristics of PE are high blood pressure and dysfunction of kidney and liver, we hypothesized that variants in the IL-17A gene would be associated with predisposition to the development of PE. Out of three SNPs investigated in the present study, we observed a significant association of rs2275913 polymorphism with a predisposition to PE: heterozygous and minor allele was more frequent in PE cased when compared to pregnant women and healthy women. In contrast, the previous reports in Brazilian(Tanaka et al. 2019), Han Chinese (Wang et al. 2015), and the Iranian population (Anvari et al. 2015) failed to demonstrate such association. Similarly, other variants of IL-17 polymorphism (rs1974226 and rs374806) were also not associated with susceptibility to PE. Furthermore, IL-23A (rs11171806) variants were also more frequent in PE compared to healthy and pregnant women indicating a susceptible genetic factor for PE development. The possible mechanism of how IL-23A rs11171806 is associated with PE susceptibility is not known. Mutation at 703 nucleic acids (G > A) position leads to a synonymous Ser106Ser, not affecting the three-dimensional structure of the IL-23A protein. Furthermore, in the present study, we also failed to observed differential IL-23A serum levels among different clinical categories. Also, the distribution of IL-12B (rs3212227) variants was comparable in PE patients and other clinical categories, indicating no significant role of IL-12B polymorphism in predisposition to PE development.

In the current report, a strong association of IL-17A (rs2275913) and IL-23A (rs11171806) polymorphism with susceptibility to PE was observed. Also, we noticed elevated IL-17A levels in PE patients in comparison to healthy women and pregnant women, and IL-23A remained comparable. Based on these results, we hypothesized that common polymorphisms in IL-17A and IL-23A would be correlated with serum levels of IL-17A and IL-23A, respectively. Serum levels of IL-23A were not associated with different genotypes of IL-23 gene (rs11171806), as the mutation (G > A) lead to no change in amino acids (Ser106Ser). Interestingly, IL-17A (rs2275913) polymorphism was observed to contributing serum levels of IL-17A: homozygous (AA) and heterozygous mutant (GA) displayed higher serum IL-17 compared to GG genotype. In line with the present report, earlier studies(Huang et al. 2017; Tang et al. 2018) have also demonstrated the functional relevance of IL-17A (rs2275913) with plasma or serum levels of IL-17A. Genetic variation at the promoter region of IL-17A gene would possibly enhance the binding of transcription factor and increased production of IL-17A cytokine.

Conclusions

The current report revealed an important role of IL-17A in the pathogenesis of PE in Chinese patients. Furthermore, heterozygous mutant and minor allele of IL-17A (rs2275913) and IL-23A (rs11171806) polymorphisms predisposed subjects for the development of PE. Interestingly, the current report further re-validated the functional relevance of IL-17A (rs2275913) variants and demonstrated the association of mutants with elevated IL-17A levels. However, further studies, including more significant sample-sized in the different populations, are required to validate the observations of the present study.

Abbreviations

Not Applicable

Declarations

Funding: This study was supported by The Interdisciplinary Program of Shanghai Jiao Tong University(No. ZH2018QNB16, No. ZH2018ZDA31), Tradational Chinese and Western medicine in Shanghai (No. 18411963500), and Important disease of Xuhui District Health Planning Commission (No. XHLHGG201805). The funders has no role in reporting of the manuscript.

Competing interest: authors declear no conflict of interest

Ethics and consent to participate: The Institutional Review Board of Shanghai Jiao Tong University has approved the study and written informed consent was obtained from all participants.

Consent for publication: Not applicable

Authors contributions: XL and WL: performed experiments and prepare the first draft the manuscript; YH, WZ, XY, LC and QYinvolved in data analysis and interpretation; WC: made a contribution in the design, data interpretation, work supervision and critically revising the manuscript. All authors read and approved the manuscript

Availability of data and materials: Data will be available up on request to the corresponding author.

Code availability: Not applicable to this report.

Acknowledgement: Authors would like to thanks all participants for the voluntary contribution for the present investigation.

References

1. Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL (2003) Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17 J Biol Chem 278:1910-

1914 doi:10.1074/jbc.M207577200

2. Anvari F, Dabagh-Gorjani F, Soltani-Zangbar M-S, Kamali-Sarvestani E, Malek-Hosseini Z, Gharesi-Fard B (2015) Investigating the Association of IL-17A and IL-17F with Susceptibility to Pre-eclampsia in Iranian Women Iran J Immunol 12:117-128
3. Benghiat FS, Charbonnier LM, Vokaer B, De Wilde V, Le Moine A (2009) Interleukin 17-producing T helper cells in alloimmunity Transplant Rev (Orlando) 23:11-18 doi:10.1016/j.trre.2008.08.007
4. Bowen JM, Chamley L, Mitchell MD, Keelan JA (2002) Cytokines of the placenta and extra-placental membranes: biosynthesis, secretion and roles in establishment of pregnancy in women Placenta 23:239-256 doi:10.1053/plac.2001.0781
5. Bulletins--Obstetrics ACoP (2002) ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002 Obstet Gynecol 99:159-167 doi:10.1016/s0029-7844(01)01747-1
6. Chen X et al. (2009) Interactions of IL-12A and IL-12B polymorphisms on the risk of cervical cancer in Chinese women Clin Cancer Res 15:400-405 doi:10.1158/1078-0432.CCR-08-1829
7. Curtis MM, Way SS (2009) Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens Immunology 126:177-185 doi:10.1111/j.1365-2567.2008.03017.x
8. Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, Oleszczuk J (2012) The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia J Reprod Immunol 93:75-81 doi:10.1016/j.jri.2012.01.006
9. Darmochwal-Kolarz D, Michalak M, Kolarz B, Przegalinska-Kalamucka M, Bojarska-Junak A, Sliwa D, Oleszczuk J (2017) The Role of Interleukin-17, Interleukin-23, and Transforming Growth Factor-beta in Pregnancy Complicated by Placental Insufficiency Biomed Res Int 2017:6904325 doi:10.1155/2017/6904325
10. Darmochwal-Kolarz D, Rolinski J, Leszczynska-Goarzelak B, Oleszczuk J (2002) The expressions of intracellular cytokines in the lymphocytes of preeclamptic patients Am J Reprod Immunol 48:381-386 doi:10.1034/j.1600-0897.2002.01089.x
11. Domanski L et al. (2019) IL17A and IL17F genes polymorphisms are associated with histopathological changes in transplanted kidney BMC Nephrology 20:124 doi:10.1186/s12882-019-1308-z
12. Huang H-T et al. (2017) The association of IL-17A polymorphisms with IL-17A serum levels and risk of ischemic stroke Oncotarget 8:103499-103508 doi:10.18632/oncotarget.21498
13. Ito M et al. (2010) A role for IL-17 in induction of an inflammation at the fetomaternal interface in preterm labour J Reprod Immunol 84:75-85 doi:10.1016/j.jri.2009.09.005
14. Jia H et al. (2015) Both interleukin-23A polymorphism and serum interlukin-23 expression are associated with Graves' disease risk Cell Immunol 294:39-43 doi:10.1016/j.cellimm.2015.01.015
15. Jianjun Z, Yali H, Zhiqun W, Mingming Z, Xia Z (2010) Imbalance of T-cell transcription factors contributes to the Th1 type immunity predominant in pre-eclampsia Am J Reprod Immunol 63:38-45 doi:10.1111/j.1600-0897.2009.00763.x

16. Jonsson Y et al. (2006) Cytokine mapping of sera from women with preeclampsia and normal pregnancies J Reprod Immunol 70:83-91 doi:10.1016/j.jri.2005.10.007
17. Keramat F et al. (2019) Association of interleukin-17 gene polymorphisms and susceptibility to brucellosis in Hamadan, western Iran 63:139-146 doi:10.1111/1348-0421.12675
18. Kuklina EV, Ayala C, Callaghan WM (2009) Hypertensive disorders and severe obstetric morbidity in the United States Obstet Gynecol 113:1299-1306 doi:10.1097/AOG.0b013e3181a45b25
19. Li Y et al. (2016) Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi Nat Med 22:952-960 doi:10.1038/nm.4139
20. Nakashima A, Ito M, Shima T, Bac ND, Hidaka T, Saito S (2010) Accumulation of IL-17-positive cells in decidua of inevitable abortion cases Am J Reprod Immunol 64:4-11 doi:10.1111/j.1600-0897.2010.00812.x
21. Poordast T, Najib FS, Baharlou R, Bijani A, Alamdarloo SM, Poordast A (2017) Assessment of T helper 17-associated cytokines in third trimester of pregnancy Iran J Immunol 14:172-179 doi:IJIv14i2A8
22. Raghupathy R, Al-Azemi M, Azizieh F (2012) Intrauterine growth restriction: cytokine profiles of trophoblast antigen-stimulated maternal lymphocytes Clin Dev Immunol 2012:734865 doi:10.1155/2012/734865
23. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R (2000) Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion Hum Reprod 15:713-718 doi:10.1093/humrep/15.3.713
24. Raghupathy R, Makhseed M, El-Shazly S, Azizieh F, Farhat R, Ashkanani L (2001) Cytokine patterns in maternal blood after premature rupture of membranes Obstet Gynecol 98:122-126 doi:10.1016/s0029-7844(01)01408-9
25. Sargent IL, Borzychowski AM, Redman CW (2006) Immunoregulation in normal pregnancy and pre-eclampsia: an overview Reprod Biomed Online 13:680-686 doi:10.1016/s1472-6483(10)60659-1
26. Schnyder-Candrian S et al. (2006) Interleukin-17 is a negative regulator of established allergic asthma J Exp Med 203:2715-2725 doi:10.1084/jem.20061401
27. Sykes L, MacIntyre DA, Yap XJ, Teoh TG, Bennett PR (2012) The Th1:th2 dichotomy of pregnancy and preterm labour Mediators Inflamm 2012:967629 doi:10.1155/2012/967629
28. Tanaka SCSV, Hortolani ACC, Pissetti CW, Paschoini MC, Cintra-Ruiz MT, Jr. VR, Balarin MAS (2019) Polymorphisms in the IL17A gene are not involved in the development of preeclampsia in the Brazilian population J Bras Patol Med Lab 55:170-181
29. Tang H, Pei H, Xia Q, Tang Y, Huang J, Huang J, Pei F (2018) Role of gene polymorphisms/haplotypes and serum levels of interleukin-17A in susceptibility to viral myocarditis Exp Mol Pathol 104:140-145 doi:10.1016/j.yexmp.2018.03.002
30. Tjoa ML, Levine RJ, Karumanchi SA (2007) Angiogenic factors and preeclampsia Front Biosci 12:2395-2402 doi:10.2741/2241

31. Toldi G, Rigo J, Jr., Stenczer B, Vasarhelyi B, Molvarec A (2011) Increased prevalence of IL-17-producing peripheral blood lymphocytes in pre-eclampsia Am J Reprod Immunol 66:223-229 doi:10.1111/j.1600-0897.2011.00987.x
32. Wang H et al. (2015) Role of IL-17 Variants in Preeclampsia in Chinese Han Women PLoS One 10:e0140118-e0140118 doi:10.1371/journal.pone.0140118
33. Xiao J et al. (2014) Is ethnicity a risk factor for developing preeclampsia? An analysis of the prevalence of preeclampsia in China J Hum Hypertens 28:694-698 doi:10.1038/jhh.2013.148

Tables

Table-1 Baseline characteristics of study subjects

Parameters	Subjects with PE (n=115)	Pregnant women (n=102)	Non-pregnant women (n=147)
Age (years)	31 ± 5	33 ± 6	29 ± 4
Primiparas (%)	59.3	54.9	NA
Duration of gestation (days)	249 ± 14*	269 ± 19	NA
BMI at blood draw (Kg/m2)	30.1 ± 5.2*#	26.2 ± 4.1#	21.1 ± 3.8
Vaginal delivery (%)	12.3*	56.6	NA
Caesarian section (%)	87.7*	43.4	NA
Fetal birth weight (grams)	2651*	3216	NA
Systolic blood pressure (mmHg)	149 ± 13*#	109 ± 09	113 ± 11
Diastolic blood pressure (mmHg)	89 ± 11*#	75 ± 09	80 ± 10
White blood cell (x 10^9/L)	10.1 ± 3.5*#	9.6 ± 3.2#	8.8 ± 2.9
Uric acid (mg/dL)	7.1 ± 0.9*#	4.2 ± 1.3	3.9 ± 1.9
Urea (mg/dL)	24.09 ± 13.6*#	18.6 ± 3.9	17.6 ± 4.4

Note: Data are presented as either mean % or mean % ± SD. NA – Not applicable. *: P < 0.05- Subjects with PE versus healthy pregnant women; #: P < 0.05 - Subjects with PE versus healthy non pregnant women.

Table-2 Distribution of IL-23A, IL-12B and IL-17A gene polymorphisms in healthy controls, pregnant woman and preeclampsia patients.

Genotype/Allele	HW (n=147)	PW (n=102)	PE (n=115)	HW vs PW (P value, OR, 95% CI)	HW vs PE (P value, OR, 95% CI)	PW vs PE (P value, OR, 95% CI)
IL-23A (rs11171806)						
GG	135 (92)	95 (93)	92 (80)	1, ref	1, ref	1, ref
GA	11 (7)	7 (7)	22 (19)	1, 0.90, 0.35 to 2.41	**0.007, 2.93, 1.32 to 6.60**	**0.008, 3.24, 1.31 to 8.53**
AA	1 (1)	0	1 (1)	--	1, 1.46, 0.07 to 28.06	--
G	281 (96)	197 (97)	206 (90)	1, ref	1, ref	1, ref
A	13 (4)	7 (3)	24 (10)	0.64, 0.76, 0.29 to 2.01	**0.009, 2.51, 1.22 to 5.23**	**0.004, 3.27, 1.44 to 8.18**
IL-12B (rs3212227)						
AA	125 (85)	89 (87)	93 (81)	1, ref	1, ref	1, ref
AC	21 (14)	12 (12)	20 (17)	0.70, 0.80, 0.37 to 1.66	0.49, 1.28, 0.66 to 2.45	0.25, 1.59, 0.75 to 3.47
CC	1 (1)	1 (1)	2 (2)	1, 1.40, 0.07 to 26.87	0.57, 2.68, 0.30 to 39.23	1, 1.91, 0.21 to 28.01
A	271 (92)	190 (93)	206 (90)	1, ref	1, ref	1, ref
C	23 (8)	14 (7)	24 (10)			
IL-17A (rs2275913)						
GG	54 (37)	39 (38)	24 (21)	1, ref	1, ref	1, ref
GA	62 (42)	44 (43)	65 (56)	1, 0.98, 0.54 to 1.70	**0.005, 2.35, 1.32 to 4.21**	**0.007, 2.40, 1.29 to 4.47**
AA	31 (21)	19 (19)	26 (23)	0.72, 0.84, 0.43 to 1.70	0.10, 1.88, 0.92 to 3.80	1, 0.96, 0.47 to 1.96
G	170 (58)	122 (60)	113 (49)	1, ref	1, ref	1, ref
A	124 (42)	82 (40)	117 (51)	0.71, 0.92, 0.64 to 1.31	**0.05, 1.41, 1.00 to 2.00**	**0.02, 1.54, 1.05 to 2.25**
IL-17A						

(rs1974226)						
CC	129 (88)	87 (85)	102 (89)	1, ref	1, ref	1, ref
CT	16 (11)	13 (13)	13 (11)	0.68, 1.20, 0.56 to 2.67	1, 1.02, 0.48 to 2.26	0.83, 0.85, 0.37 to 1.94
TT	2 (1)	2 (2)	0	1, 1.48, 0.22 to 9.58	--	--
C	274 (93)	187 (92)	217 (94)	1, ref	1, ref	1, ref
T	20 (7)	17 (8)	13 (6)	0.60, 1.24, 0.62 to 2.39	0.71, 0.82, 0.40 to 1.68	0.34, 0.65, 0.32 to 1.36
IL-17A (rs3748067)						
CC	119 (81)	82 (80)	91 (79)	1, ref	1, ref	1, ref
CT	26 (18)	20 (20)	24 (21)	0.74, 1.11, 0.59 to 2.13	0.63, 1.20, 0.65 to 2.20	0.86, 1.08, 0.56 to 2.08
TT	2 (1)	0	0	--	--	--
C	264 (90)	184 (90)	206 (90)	1, ref	1, ref	1, ref
T	30 (10)	20 (10)	24 (10)	1, 0.95, 0.52 to 1.71	1, 1.02, 0.57 to 1.83	0.87, 1.07, 0.58 to 2.04

Note: data are presented in number (%). HW: healthy non-pregnant woman, PW: pregnant woman, PE: preeclampsia patients, OR: odds ratio, CI: confidence interval.

Figures

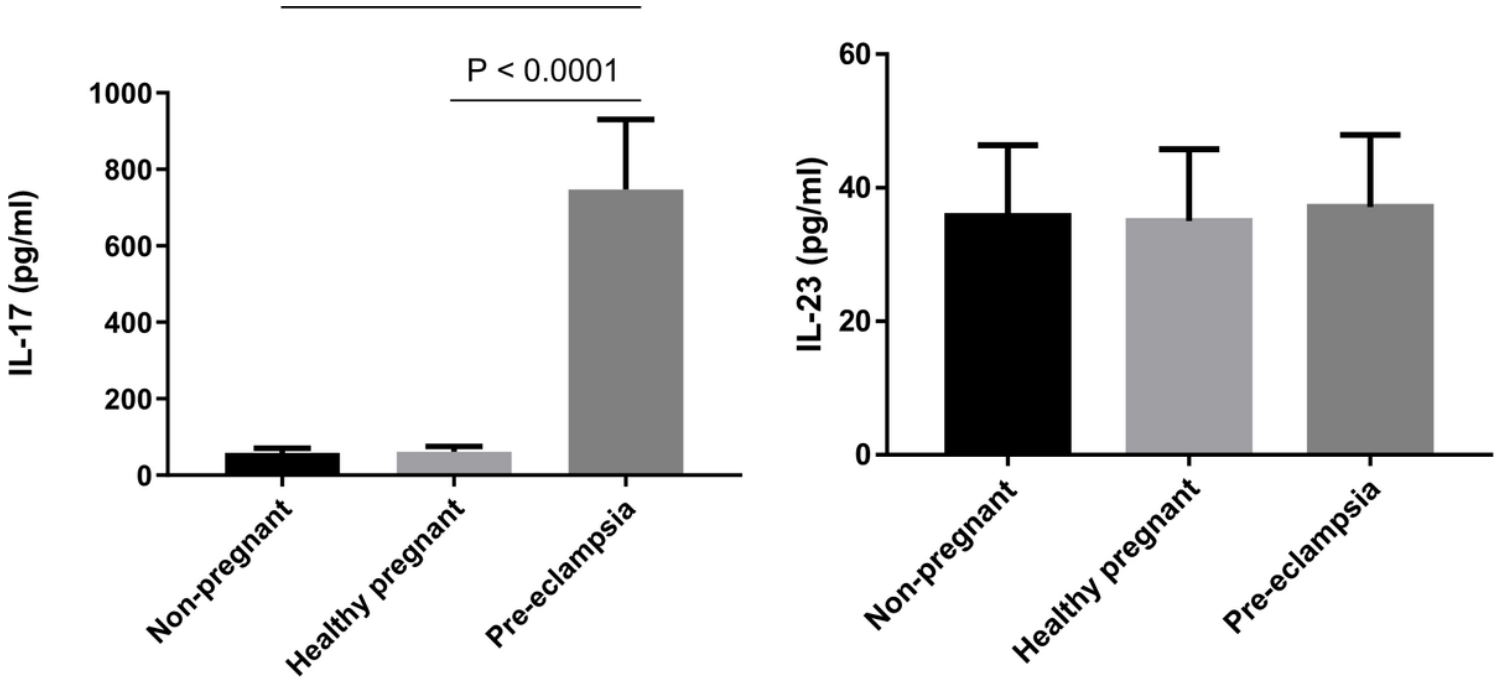

Figure 1

Serum level IL-17 (A) and IL-23 (B) in subjects with PE (n=115), healthy pregnant women (n=102) and healthy non pregnant controls (n=147). Data represent Mean pg/ml ± SE and were analyzed with one way ANOVA for comparison. P < 0.05 was considered statistically significant.

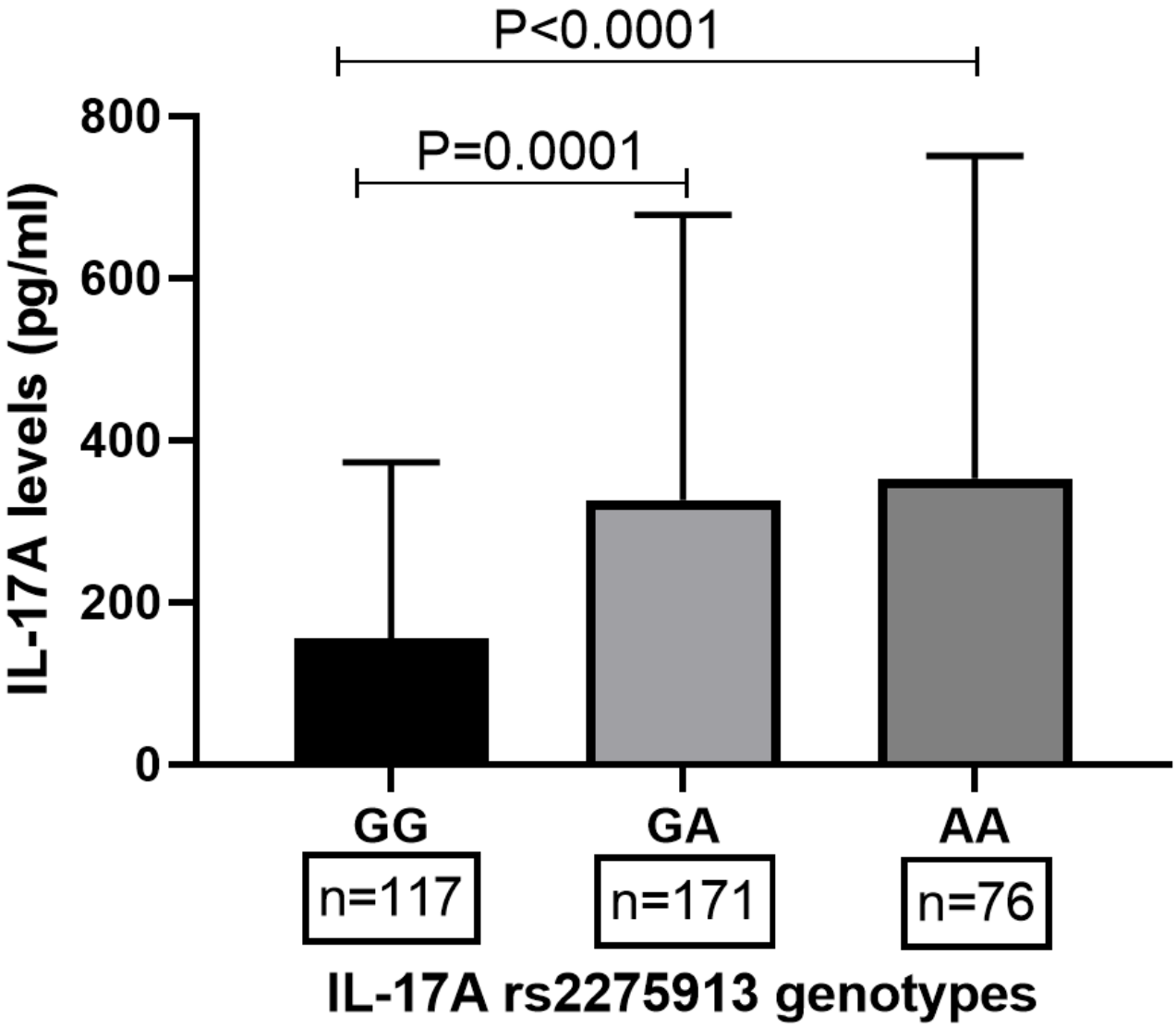

Figure 2

Association of IL-17A rs2275913 polymorphism with serum levels of IL-17A. Serum levels of IL-17A were quantified by ELISA and rs2275913 polymorphism was typed by PCR-RFLP in a total of 364 subjects comprising of healthy females, pregnant woman, and subject with preeclampsia. Mean IL-17A levels in the different genotype of rs2275913 polymorphism were compared by ANOVA followed by Tukey's post-test. A P value of less than 0.05 was taken as significant.