Immunology Letters 192 (2017) 12–19

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Intravenous immunoglobulin (IVIG) treatment modulates peripheral blood Th17 and regulatory T cells in recurrent miscarriage patients: Non randomized, open-label clinical trial

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ARTICLE INFO

Keywords:
Recurrent miscarriage
Intravenous immunoglobulin G
Treg
Th17

ABSTRACT

Background: Th17 cells and Treg cells have been proposed as new risk factors for recurrent miscarriage (RM). In this study, we investigated the effect of Intravenous immunoglobulin G (IVIG) on the levels and function of Th17 and Treg cells and pregnancy outcome in women with RM.

Materials and methods: 94 pregnant women with RM were enrolled in this study. Blood was drawn at the time of positive pregnancy. On the same day, IVIG 400 mg/kg was administered intravenously for 44 patients. 50 other RM patients were included as no IVIG interfering control group. Following the first administration, IVIG was given every 4 weeks through 32 weeks of gestation. Peripheral blood was drawn after the last administration (32 weeks after pregnancy).

Results: IVIG down-regulated Th17 cells population and function and up-regulated Treg cells population and function were significant in the treated group. Pregnancy outcome in IVIG treated subjects was successful in 38 out of 44 RM women (86.3%). However, pregnancy outcome was successful in 21 out of 50 untreated RM women (42%).

Conclusion: Administration of IVIG in RM women with cellular immune cells abnormalities during pregnancy influences Th17/Treg ratio in peripheral blood and enhances Treg and decreases Th17 responses.

1. Introduction

Miscarriage occurs in about 1–2% of human pregnancies and is one of the common pregnancy problems before 12 weeks of pregnancy[1]. Anatomical and chromosomal abnormalities, microbial factors and auto and alloimmune reactions have been speculated to attribute in recurrent miscarriage [2]. Unexplained recurrent miscarriage (URM) is defined as three or more repeated abortions, probably caused by maternal immunological rejection [3]. Given that maternal immune system encounters semi-allogeneic fetus, pregnancy outcome is associated with the interaction between maternal immune system and immuno-regulatory capability of the fetus [3,4].

Pro-inflammatory milieu, either cell mediated or humoral responses, leads to pregnancy complications such as implantation failure, recurrent miscarriage, intrauterine fetal growth restriction and pre-eclampsia. Increased percentage and cytotoxicity of peripheral and endometrial natural killer (NK) cells have been known as poor prognostic factors for unexplained RM [5]. Imbalance of Th1 and Th2 cytokine profile of peripheral lymphocytes were found in women with a history of unexplained RM [6,7]. Therefore, almost 45% of women with unexplained RM show increased number of $CD3^{-}$ CD56+ NK cells, 38% show increased NK cell cytotoxicity, and 28% show increased

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http://dx.doi.org/10.1016/j.imlet.2017.10.003
Received 8 July 2017; Received in revised form 7 October 2017; Accepted 9 October 2017
Available online 10 October 2017

Th1/Th2 cell ratios [8].

T helper 17 (Th17) and T regulatory (Treg) cells are two distinct populations of CD4+ T cells, and both differentiate from naive CD4+ T cells, that represent opposite immunological responses [3].

Th17, an inflammatory Th lymphocyte subpopulation secretes IL-17 as an early phase and pivotal pro-inflammatory cytokine. Therefore, Th17 is involved in pathophysiological immune processes such as pregnancy disorders [9,10]. Recently, higher level of IL-17 has been reported in women with inevitable abortion compared with women with normal pregnancies [11]. In contrast, Treg cells suppress immune response via TGF-β secretion and CTLA-4 expression. The Treg cells specific transcription factor is Forkhead winged helix transcription factor (FOXP3) and Th17 corresponding specific transcription factor is retinoic acid-related orphan receptor gamma or ROR gamma t (RORγt). The first evidence of Treg cells attribution in fetal tolerance was provided by Jasper et al. [12], who found Foxp3 mRNA downregulation in endometrial tissue in the patients experiencing primary unexplained infertility. Later findings confirmed that reduced peripheral Treg −cells and increased number of Th17 are associated with unexplained infertility and pregnancy complications [4,13].

Effectiveness of treatment approaches in RM patients has been controversial and remained to be discovered [14]. Immunomodulatory agents such as corticosteroids and allogeneic lymphocyte immunization showed variable success rates in RM patients [15]. Therapeutic effects of IVIG in unexplained RM is controversial and most positive results were obtained from the trials in RM women with cellular immune abnormalities, such as increased NK cell level and/or cytotoxicity, and T cell abnormalities [16,17].

In the present study, we investigated Th17/Treg cells ratio and corresponding transcription factors and cytokines, IL-17, IL-23, IL-10 and TGF-β, and pregnancy outcome in RM women with abnormal cellular immune profile, treated with IVIG.

2. Materials and methods

2.1. Study population

Patients were recruited from Dr Majidi Center of Infertility, Alzahra Hospital of Tabriz University of Medical Sciences between February 2015 and March 2016. The study was approved by the Research Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1396.130). An informed consent was signed by the participants before the enrollment. Sample size calculations were based on the results of the two pilot studies before of the onset of the trial. In order to demonstrate a difference between live-birth rates of 40% in the control group and 75% in the IVIG group, using a one-sided significance test with a $\alpha = 0.05$ and a power of 85%, 44 subjects were required in each group.

Totally, 94 pregnant women with a history of unexplained RM were consecutively enrolled in the present study, 39 women had primary and 55 women had secondary RM. All subjects represented abnormal flow cytometry result for one or more immune cell population such as $CD3^{-}CD56^{+}$ NK cell, NK cell cytotoxicity assay, and Th1/Th2 cytokine producing T cell ratio as earlier reported [18]. Women with anatomical, infectious, endocrine or genetic etiologies relating to their abortion were excluded. Partners of all participants had normal semen status according to World Health Organization criteria, no chromosomal disorders and were non-smokers. Demographic data of patients are summarized in Table 1. Blood was drawn at the time of positive pregnancy and Th17 and Treg frequencies, corresponding transcription factors (RORγt and FOXP3) and cytokines were assessed in all participants. On the same day, IVIG, 400 mg/kg was administered intravenously. 50 out of the 94 RM patients (voluntarily) with abnormal cellular immune profile were included as no IVIG interfering control group. Following the first administration, IVIG was given every 4 weeks through 32 weeks of gestation. Peripheral blood was drawn after the last administration in the IVIG group and same time in pregnant women of the control group, Th17 and Treg cell population were assessed. The outcome of IVIG treatment was successful as live birth was considered in both groups. Routine high-risk pregnancy care including periodic uterine ultrasonography and fetal heart monitoring was provided in all women. Patients were also monitored for side effects of the IVIG therapy.

2.2. Peripheral blood mononuclear cells (PBMC) isolation

10 ml of heparinized blood samples were collected and PBMCs were isolated using standard Ficoll (lymphosep) 1.077 g/ml (Biosera, UK) centrifugation (25 min, 450*g*). Cells were washed twice with phosphate buffered saline (PBS) (Sigma, Germany) and 10^6 cells were cultured in 1 ml medium containing 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 200 mML-glutamine. 10 ng/mL of PMA (eBioscience, San Diego, CA, USA) was added to the medium, and incubated for 5 h at 37 °C and 5% CO_2. The cultured cells were used for flow cytometry analysis, and RNA extraction. Supernatant of the cultured cells were collected for measurement of cytokine concentrations by Enzyme linked immunosorbent assay (ELISA).

2.3. Flow cytometry analysis

The frequency of IL-17+ and Foxp3+ T cells were determined by using flow cytometry as described earlier. Briefly, 5×10^6 of PBMCs were incubated with 10 ng/mL PMA (Sigma) and 0.5 μM ionomycin (Sigma) for 5 h at 37 °C in a 5% CO_2 humidified incubator. One microliter of Monensin (eBioscience, San Diego, CA, USA) was used to enhance intracellular cytokine staining. The cells were washed in phosphate-buffered saline (PBS), 0.09% w/v sodium azide (Sigma), and stained with the anti-CD4-APC (BD Biosciences) for 15 min at 4 °C. The cells were washed in PBS and fixed using fixation buffer (eBioscience). Afterwards, the cells were washed twice with 1 × permeabilization buffer (eBioscience) and incubated with 0.25 μg conjugated anti-human IL-17A-PE or anti-Foxp3- Alexa Fluor 488 antibody (eBioscience) at room temperature for 20 min. The cells were again washed with 1 × permeabilization buffer and resuspended in 0.5 ml of permeabilization buffer. The percentage of IL-17+ T cells in peripheral blood lymphocytes was counted by flow cytometry. Alexa Fluor 488- and APC rat IgG2a was used as an isotype control.

For $IL\text{-}17^{+}$ T/$Foxp3^{+}$ T cell ratio calculation, percentage of $IL\text{-}17^{+}$ T cells was divided by percentage of $Foxp3^{+}$ T cells. The prepared staining cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences). Data analysis was done using CellQuest Pro software (BD Biosciences). A total of 1,000,000 cells were counted. Viable lymphocytes were gated based on their forward and side scatters profile.

Also triple-color immunofluorescence analyses of lymphocyte markers using anti-CD3, anti-CD16 and anti-CD56 antibodies was performed for PBMC staining. CD56 + CD16 + CD3- cells were reported as NK population in peripheral blood. NK cell values of > 12% were defined as abnormal NK cell levels.

NK cytotoxicity was assessed by flow cytometric quantification of the relative proportion of killed-K562 target cells assessed by propidium-iodide uptake following co-incubation with isolated patient mononuclear cells [19]. Percentage of killed target cells was then measured by flow cytometry. Cytotoxicity was regarded as increased when target cell killing exceeded 15% [20].

2.4. Real Time PCR

Total RNA was isolated using RNX-PLUS Solution (SinaClon, Tehran, Iran), and complementary DNA (cDNA) was synthesized by using RevertAid Reverse Transcriptase kit (Thermo Fisher, Waltham, MA, USA). SYBR Green method was employed for FoxP3, RORγt, IL-23, IL-17, IL-10, and TGF-β analyses. The relative quantity of the genes

Table 1
Age and clinical data of pregnant women with Unexplained Recurrent Miscarriage (n = 94).

Variable	IVIG group	Control group	P value
Number	44	50	NS
Age(year ± SD)	33.8 ± 3.6	34.1 ± 3.4	NS
Recurrent miscarriage(number ± SD)	3.3 ± 1.4	3.2 ± 1.1	NS
Primary RM	18	21	NS
Secondary RM	26	29	NS
Gestational ages of previous miscarriages(weeks ± SD)	12.9 ± 6.2	13.2 ± 5.9	NS
NK cell levels(mean ± SD)	18.23 ± 2.45%	17.95 ± 3.35%	NS
Abnormal NKcell levels(number, percent)	22, 50%	28, 56%	NS
NKcell cytotoxicity(mean ± SD)	19.12 ± 2.75	20.2 ± 3.43	NS
Abnormal NK cell cytotoxicity(number, percent)	31, 62%	33, 66.%	NS
Th1/Th2 cell ratio(mean ± SD)	14.72 ± 3.17	14.25 ± 1.95	NS
Abnormal Th1/Th2 cell ratio(number, percent)	18, 40.9%	21, 42%	NS

were normalized to the relative quantity of Beta-actin, as fold change of gene expression.

The polymerase chain reaction conditions for SYBR Green method were as follows: holding at 95 °C for 10 s, followed by 40 cycles of denaturation at 95 °C for 10 s, followed by annealing and extension at 60 °C for 30 s.

Sequences of the primers are summarized in Table 2. Amplification was confirmed by using electrophoresis analysis on 2% agarose gel followed by DNA sequencing performed by Bioneer (Bioneer corporation, Daejeon, South Korea).

2.5. Enzyme linked immunosorbent assay (ELISA)

IL-17, IL-23, IL-10 and TGF-β secretion in PBMCs culture supernatants were measured using ELISA, according to the manufacturer's instructions (Biosource, Nivelles, Belgium). All sample conditions were measured in duplicate.

2.6. Statistical analysis

Statistical analysis was performed using SPSS PC Statistics (version 19.0; SPSS Inc., Chicago, IL, USA). Paired *t*-test was applied to compare the results of immunologic studies before and after IVIG treatment. Linear-by-linear association chi-square test was carried out to test linear trend among the groups of immune variables, response rate of the variables and pregnancy outcome. P-values < 0.05 were reported to be statistically significant.

Table 2
Primer sequences.

Gene	Primer	Sequence (5′ → 3′)	Amplican size(bp)
TGF- β1	Forward	CGA CTA CTA CGC CAA GGA	150
	Reverse	GAG AGC AAC ACG GGT TCA	
FoxP3	Forward	CAC CTG GCT GGG AAA ATG G	63
	Reverse	GGA GCC CTT GTC GGA TGA	
Beta-actin	Forward	GCA TGG GTC AGA AGG ATT CCT	106
	Reverse	TCG TCC CAG TTG GTG ACG	
IL-17	Forward	CAT AAC CGG AAT ACC AAT ACC AAT	104
	Reverse	GGA TAT CTC TCA GGG TCC TCA TT	
IL-23	Forward	GGA CAA CAG TCA GTT CTG CTT	115
	Reverse	CAC AGG GCT ATC AGG GAG C	
IL-10	Forward	CAT CGA TTT CTT CCC TGT GAA	74
	Reverse	TCT TGG AGC TTA TTA AAG GCA TTC	
RORγt	Forward	GCA GCG CTC CAA CAT CTT CT	111
	Reverse	ACG TAC TGA ATG GCC TCG GT	

3. Results

3.1. mRNA expression level of Th17/Treg cytokines in activated PBMC isolated from the IVIG treated and untreated pregnant women

IL-17, IL-23, RORγt, IL-10, TGF-β and FoxP3 mRNA levels were measured in the PBMC from IVIG treated and untreated pregnant RM patients with abnormalities in the blood immune cell composition. TGF-β, IL-10 and FoxP3 mRNA levels in IVIG group significantly increased following treatment with 400 mg for 32 weeks. However, IL-17 and RORγt mRNA levels significantly reduced following IVIG treatments (Figs. 1 and 2 and Table 3).

In contrast, IL-17 and RORγt mRNA levels significantly increased in untreated RM pregnant women following 32 weeks after pregnancy (Figs. 1 and 2 and Table 3).

3.2. Th17/Treg cytokine secretions in pregnant RM women treated with IVIG

Measurement of Th17/Treg cytokine secretions in the supernatant of PBMCs isolated from the treated and untreated subjects indicated a significant increase of anti-inflammatory cytokines, TGF-β and IL-10, and decrease of inflammatory cytokines, IL-17 in treated patients (Fig. 3 and Table 3). Moreover, no significant difference was observed in cytokines levels in the untreated control group after 32 weeks (Fig. 3 and Table 3).

3.3. Th17 and Treg cell frequencies in the IVIG treated RM pregnant women

In a complementary set of experiment, flow cytometry analysis was applied for enumeration of peripheral Th17 and Treg cells in the IVIG treated RM pregnant women with immune cell abnormalities. The results revealed that the frequency of $CD4^+$ $FOXP3^+$ Treg and $CD4^+$ $IL\text{-}17A^+$ Th17 cells, and Th17/Treg cell ratios were significantly affected following IVIG treatment in all the pregnant RM women (n = 38) (Fig. 4 and Table 3).

IVIG treatment significantly reduced the frequency of Th17 cells from 33.92 ± 1.46

% to 2.38 ± 0.99%. IVIG treatment also led to an increase in Treg cell number from 4.18 ± 1.92% to 9.28 ± 3.01% in all of the treated patients (Fig. 4).

Moreover, ratio of $CD4^+$ $IL\text{-}17A^+/CD4^+$ $FOXP3^+$, Th17/Treg ratio, was 0.93 ± 0.76% before intervention. Th17/Treg ratio was significantly reduced to 0.25 ± 0.23% following IVIG intervention. In contrast, no significant difference in Th17 and Treg cell frequencies was observed in untreated subjects before and after pregnancy (data not shown).

We also compare Th17 and T reg frequencies in two group of patient that have normal NK cell levels/NK cell cytotoxicity and abnormal NK cell levels/NK cell cytotoxicity before and after IVIG therapy, we

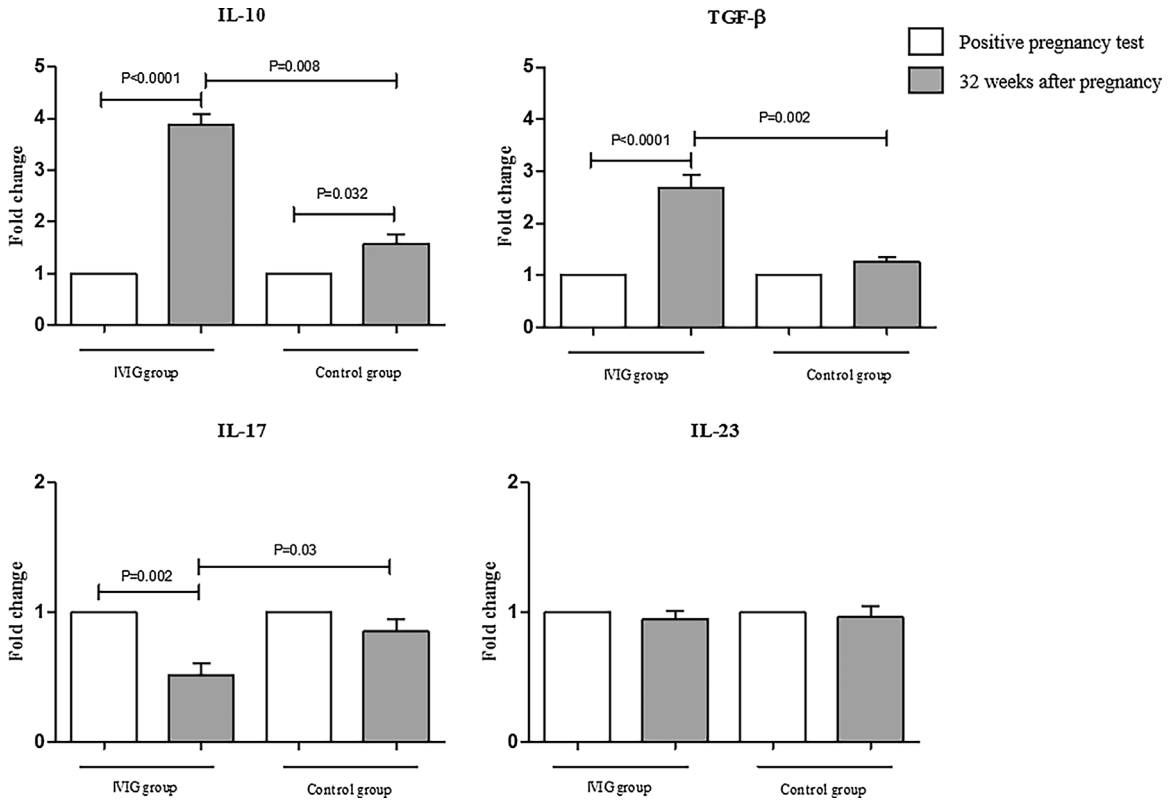

Fig. 1. Cytokines mRNA expression level in PBMC from IVIG group and control group in time of positive pregnancy test and 32 weeks after pregnancy was measured by RT-PCR. The result was normalized to beta actin.

investigate that some different of Th17 and Treg in two group before and after treatment (Fig. 5). Therefore modulation of Th17/Treg imbalance has therapeutic effect of IVIG and this matter don't NK cell and NK cell cytotoxicity effect.

3.4. Pregnancy outcome in IVIG treated and untreated women

Pregnancy outcome was followed up in the IVIG treated and untreated RM patients through 32 weeks of gestation. Pregnancy outcome in IVIG treated subjects was successful in 38 out of 44 RM women (86.3%). 38 women gave a live birth at term and six women ended with miscarriage (normal fetal karyotypes were found in four abortuses, and no fetal chromosomal study was performed in two).

However, pregnancy outcome was successful in 21 out of 50 untreated RM women (42%). 21 women gave a live birth at term and 29 women ended with miscarriage (normal fetal karyotypes were found in 19 abortuses, and no fetal chromosomal study was performed in 10) (Table 4).

4. Discussion

IVIG therapy for RM patients began in the late 1980s, and is indicated for women with miscarriages associated with antiphospholipid antibodies (APA) [21]. IVIG therapy was then recommended for patients with repeated unexplained miscarriages [22]. However, the molecular and cellular mechanisms underlying IVIG effects on the prevention of abortions are not completely understood, although it is expected to be its immunosuppressive properties including inhibition of

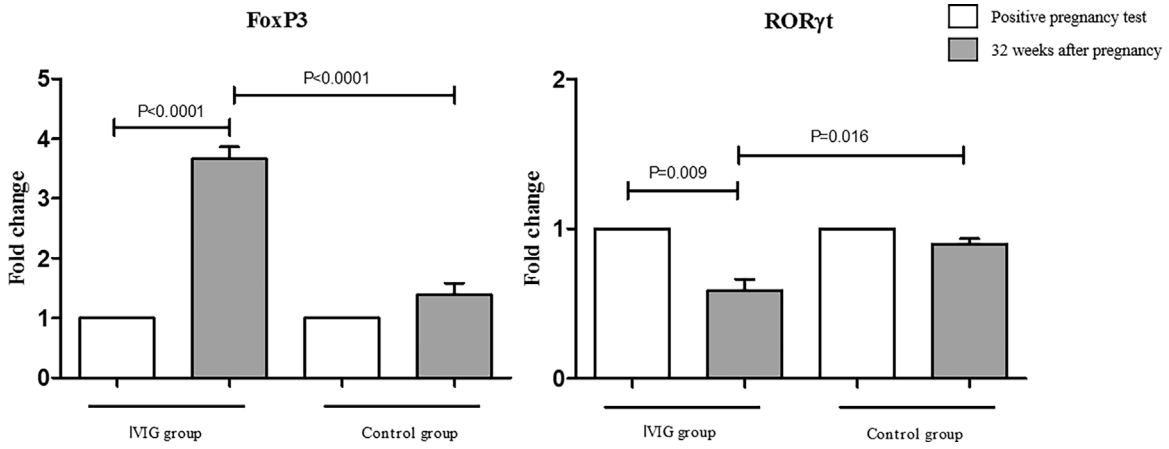

Fig. 2. mRNA expression of transcription factors in PBMC from IVIG group and control group in time of positive pregnancy test and 32 weeks after pregnancy was measured by RT-PCR. The result was normalized to beta actin.

Table 3
Th17 and Treg cell alteration in pregnant women (treated and untreated) with a history of Recurrent Miscarriage and cellular immune abnormalities.

	IVIG group		P-value	control group P-value		P-value
	Before	After		Before	After	
mRNA expression of cytokines in activated PBMC						
IL-17	1	0.51 ± 0.48	0.002	1	0.85 ± 0.43	0.03
IL-23	1	0.94 ± 0.34	NS	1	0.96 ± 0.37	NS
IL-10	1	3.88 ± 0.99	0.0001	1	1.58 ± 0.54	0.032
TGF-β	1	2.67 ± 1.29	0.0001	1	1.26 ± 0.39	0.002
RORγt	1	0.58 ± 0.24	0.009	1	0.9 ± 0.1	0.016
FoxP3	1	3.66 ± 1.01	0.0001	1	1.38 ± 0.56	NS
Cytokine secretions (pg/ml)						
IL-17	107.8 ± 20.62	55.6 ± 16.1	0.009	113.3 ± 19.64	102.7 ± 23.95	0.007
IL-23	221 ± 60.57	208.2 ± 65.84	NS	207.3 ± 70.83	206.9 ± 55.64	NS
IL-10	440 ± 205.7	1075 ± 517	0.0024	392.1 ± 197.6	498.9 ± 213	0.0078
TGF-β	152.7 ± 52.54	262.7 ± 122.9	0.004	162.3 ± 66	176.2 ± 70.9	NS
Cell frequency in PBMC						
Th17($CD4^+$ $IL\text{-}17A^+$)cells	3.92 ± 1.46	2.38 ± 0.99	0.0001	3.58 ± 1.4	3.24 ± 0.88	NS
Treg ($CD4^+$ $FOXP3^+$) cells	4.18 ± 1.92	9.28 ± 3.01	0.001	4.51 ± 1.9	4.85 ± 2.56	NS
Th17/Treg cells ratio	0.93 ± 0.76	0.18 ± 0.23	0.001	0.79 ± 0.18	0.66 ± 0.22	NS

Fc receptor-mediated phagocytosis [23], anti-idiotypic suppression of auto-antibodies [24], augmentation of regulatory function of T-cell [25], down-regulation of B-cell function [26], blockade of complement fixation [27] and modulation and tuning of cytokines and cytokine receptors expressions, signaling and functions [28].

In the current study, we explored whether IVIG therapy in pregnant women with history of RM and abnormalities in the blood immune cell composition will lead to modulation of Th17/Treg cells ratio. Therefore Kim et al. showed intravenous immunoglobulin G treatment modulated imbalance of Th17 and Treg cells in pregnant RPL women with cellular immune abnormality [29].

IVIG has already been shown to modulate APC-derived cytokines and APC mediated stimulation of T cells [30]. In vivo inhibitory effect of IVIG on Th17 cells function and expansion might results in differentiation and expansion of natural or inducible Treg cells [31,32]. In this respect, antigen-specific Treg cells have also been shown to regulate Th17-mediated inflammatory processes [33,34].

There are increasing evidences suggesting that Th17 and Treg cell imbalance is involved in clinicopathological feature of RM patients [10,35,36].

IVIG is one of the well-studied therapeutic approaches for RM women [37]. Several clinical studies have performed to investigate therapeutic effect of IVIG in idiopathic RM, although its benefit still remained controversial [37,38] A recent meta-analysis revealed that IVIG resulted in significantly higher live birth rate in RM women with immune abnormalities as compared to those normal subjects [39]. In addition, the most recent and comprehensive meta-analysis of IVIG in RM suggests that women with secondary RM seemed most probable to obtain a potential beneficial effect of IVIG, furthermore, they cannot recommend or refute IVIG in women with RM [40]. Thus, inclusion of

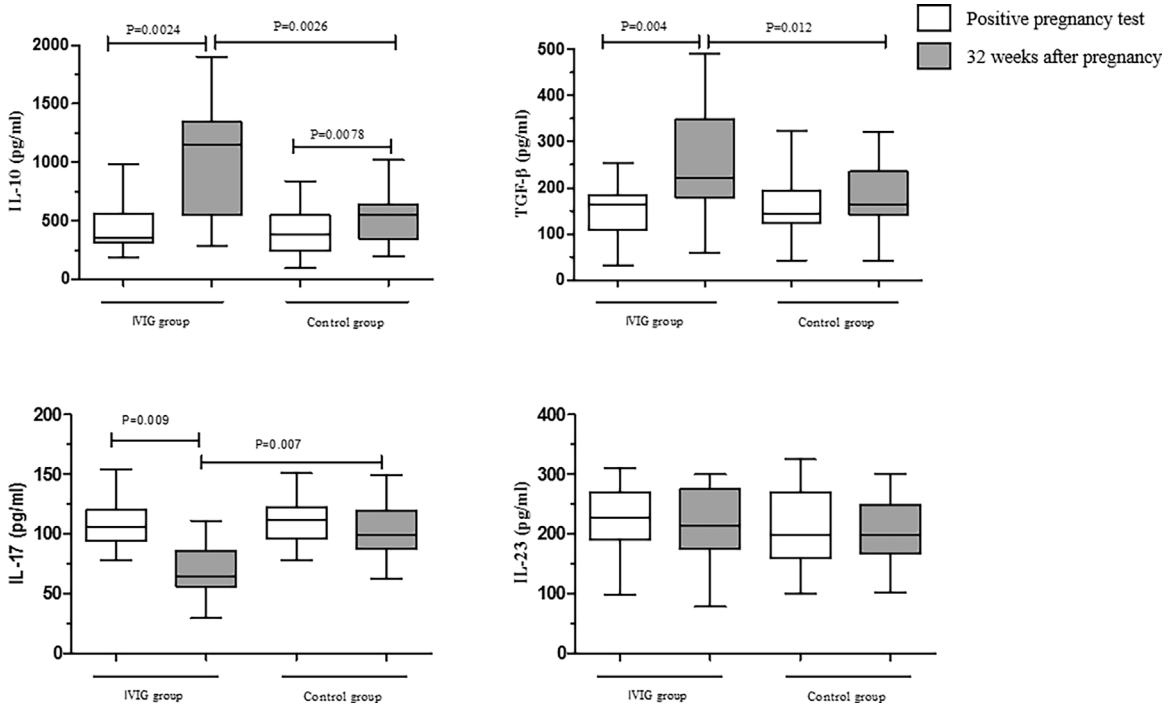

Fig. 3. Cytokine concentrations were determined in supernatant of PMA ionomicin stimulated PBMC from IVIG group and control group in time of positive pregnancy test and 32 weeks after pregnancy. The concentrations of cytokine were measured using specific cytokine ELISA detection kits.

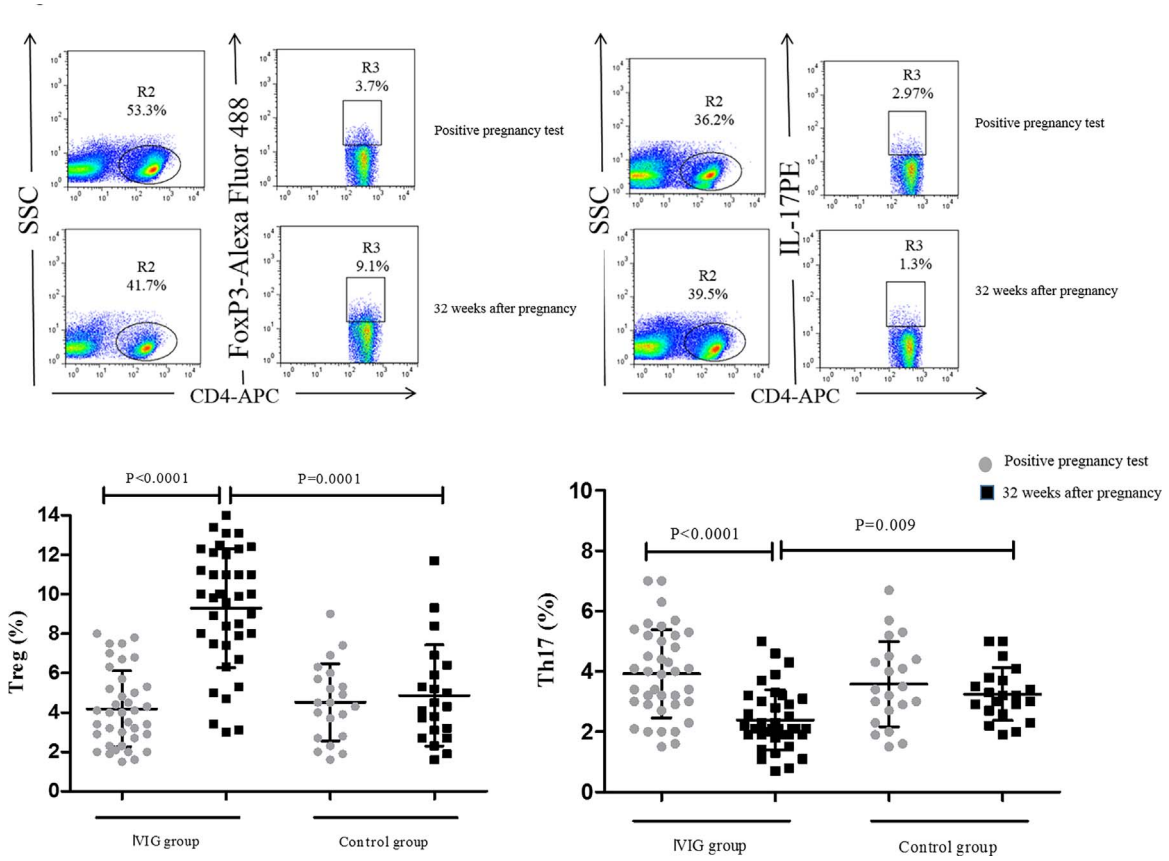

Fig. 4. The effects of IVIG therapy on the frequency of peripheral IL-17+ Th17 and Foxp3+ Treg cells in pregnant women following IVIG therapy.

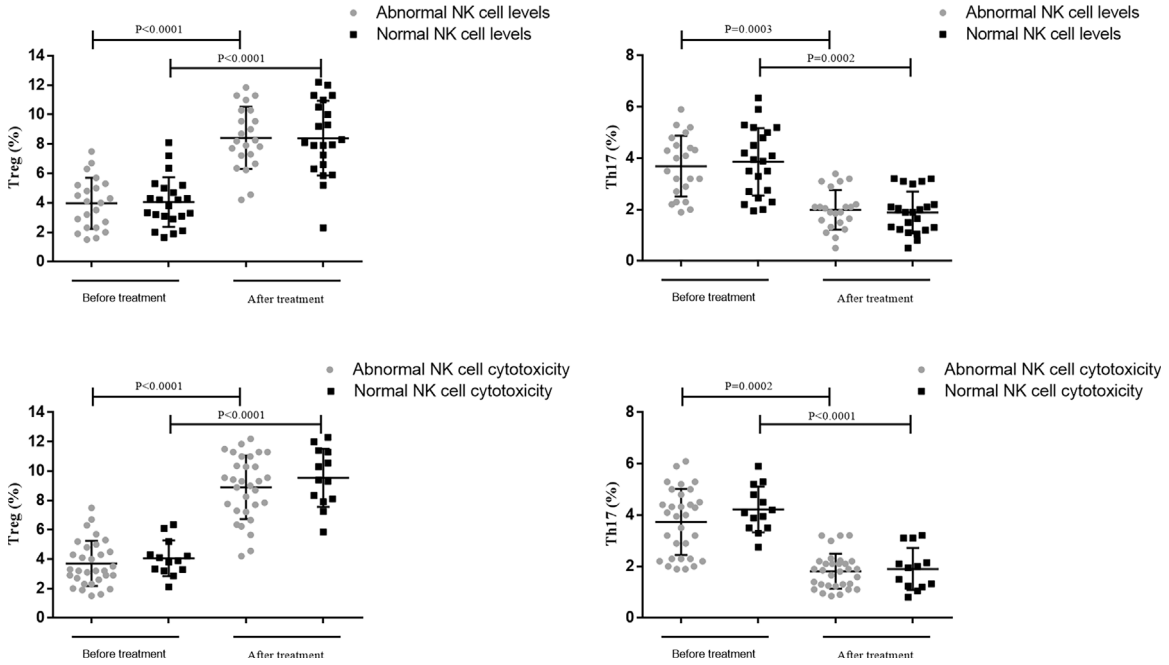

Fig. 5. The effects of IVIG therapy on the frequency of peripheral IL-17+ Th17 and Foxp3+ Treg cells in patient with normal and abnormal NK cell levels and cytotoxicity following IVIG therapy.

patients with secondary RM and immune cell abnormalities may significantly enhance therapeutic index of IVIG administration in pregnant RM women.

In the present study, we enrolled pregnant RM women with abnormalities in the peripheral immune cells, such as increased $CD3^-$ $CD56^+$ NK cells, NK cell cytotoxicity, and TNF-α/IL-10 expressing CD4+ T cell ratio, which were determined by cut-off values of those immune markers recently reported [8].

Ramos Medina et al. study showed that immunomodulation with IVIG in recurrent reproductive failure patients with immunologic

Table 4
Pregnancy outcome in IVIG treated and untreated RM patients.

	No. of patient	No. of live birth (%)
Treated	44	38(86.3%)
Untreated	50	21(42%)
P value	NS	0.0006

alterations particular expansion of blood NK cells and NKT-like cells according to previous studies enhanced clinical pregnancy and live birth rates [41].

Our findings showed an abnormal Th17 and Treg cell profiles in RM women before and after conception which is line with the others [3,35,38,42,43].

The results also demonstrated a significant modulatory effect of IVIG therapy on IL-17+ T and Foxp3+ T cells phenotypes and frequencies in the subjects. Significant decrease in IL-17+ T cell and increase FoxP3+ T cell were observed following IVIG treatment (Fig. 2). Furthermore, the ratios of IL-17+ T/Foxp3+ T cells dropped following IVIG therapy.

We postulate that IVIG-responding Th17 and Treg cells may express receptors that are sensitive to IVIG or differ in their binding affinity and signaling pathways to IVIG. Our findings are consistent with the recent reports demonstrating the modulatory effect of IVIG on Th17 cells and Treg cells frequencies and functions. IVIG inhibits Th17 cell proliferation and IL-17 secretion ex vivo, which was not modulated by anti-IL-17 antibody [44].

IVIG treatment leads to proliferation of CD4+ Foxp3+ Treg cells and modulates experimental autoimmune encephalitis [32]. Also, IVIG stimulates the expansion of natural Treg cells without induction of induced Treg (iTreg) cells from naive T cells [45]. CD4+ Foxp3+ T cells increase in the feto-maternal interface in the first half of pregnancy [46]. Therefore, increase of Treg cells following IVIG therapy may be in favor of pregnant RM patient with reduced Treg cells.

However our result showed that modulation of Th17/Treg is mediated through therapeutic effects of IVIG and it is independent of NK cell and NK cell cytotoxicity effect (Fig. 5) but it can be consider that treatment with IVIG had inhibitory effects on NK cell number and cytotoxicity in the peripheral blood. Moreover it is evident that IVIG can have beneficial clinical effect on successful pregnancy in women with RM and RM women with suppressed NK cytotoxicity and down-regulated NK cells show more successful pregnancy in treatment with IVIG. Thus, further studies should be done to clarify that which effects of IVIG is responsible for successful pregnancy in RM women.

TGF-β, IL-10 and FoxP3 mRNA levels were significantly increased following IVIG treatment. In contrast, IVIG treatments led to significant down-regulation of IL-17, and RORγt mRNA but not IL-23. Previous studies have demonstrated a significantly higher level of IL-23 in patients with unexplained RM [47,48]. However our results revealed that IVIG therapy is unable to reduce the amount of IL-23 in RM patients significantly. IL-17 produced by Th17 cells has a protective role against bacterial and fungal pathogens via neutrophil infiltration and stimulation of other pro-inflammatory cytokines and chemokines. Higher expression of IL-17 in women who have experienced unexplained RM has also been previously reported [10,36,49]. Here, we showed that IVIG therapy could significantly reduce the amount of IL-17 in RM patients.

It is well-accepted that Treg cell function lead to IL-17 down-regulation, however a diminished suppressive function of Treg cells in unexplained RM women was found [10]. As Th17 cells is the main resource of IL-17, a significant negative correlation was observed between IL-17 and the number of Treg cells, which supports the results of previous studies [36].

It has been known that differentiation and function of Treg and Th17 cell subsets are controlled by a Foxp3 and RORγt specific master transcription factors, respectively. Our results indicated that IVIG treatment decreases the expression of not only IL-17 but also RORγt in PBMC of the RM women with cellular immune cell abnormalities. Additionally, our study showed that IVIG treatment increases the expression of not only IL-10 and TGF-β, but also Foxp3.

In the current study, it was found that the administration of IVIG in RM women with cellular immune cells abnormalities during pregnancy influences Th17/Treg ratio in peripheral blood and enhances Treg and decreases Th17 responses. This immune modulatory effect of IVIG on imbalance of IL-17+ T and Treg cells may be associated with successful pregnancy outcome. However further studies are needed to further clarify and elucidate this important issue.

Acknowledgement

This study was supported by a grant of Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

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