Author's response to reviews

Title: Determination of Interleukin-6 and Tumor Necrosis Factor-alpha concentrations in Iranian-Khorasanian patients with preeclampsia.

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Author's response to reviews: see over
Dear Sirs,

I have sent you the revised manuscript as an attached file along with changes to the original manuscript. We are grateful to Dr Hennessy and Dr Lopez-Jaramillo for their comments. We have made major changes to the original manuscript. Point by point changes is presented. The revised sentences are typed in red.

Dr Hennessy

Abstract

The conclusion in the abstract has been changed as follows;

“Increased IL-6 concentration in patients with preeclampsia suggests its contribution in pathophysiology of the disease.”  Has been changed to

“The results of this study show that IL-6 as a pro-inflammatory cytokine is present in higher concentration in women with preeclampsia. The study was undertaken in women with established preeclampsia and it is not possible to determine whether the increased concentration of IL-6 is a cause or consequence of the disease.”

“Furthermore, these findings suggest that serum TNF- α level does not associate with preeclampsia.” Has been changed to

“Furthermore, these findings suggest that serum TNF- α level is not associated with preeclampsia.

Introduction

“Preeclampsia is a relatively common, yet mysterious, disease pregnancy, of which the aetiology has not yet been fully elucidated. Has been changed to
“Preeclampsia is a critically important disease of pregnancy, one of the major causes of fetal and maternal morbidity and mortality throughout the world. In spite of its importance for public health, the etiology of preeclampsia has not yet been fully elucidated.”

TNF-α is a potent modulator of immune and inflammatory responses that is produced by macrophages, lymphocytes and trophoblasts and contributes to the trophoblast growth and invasion. Has been changed to

“TNF-α is a potent modulator of immune and inflammatory responses that are produced by macrophages, lymphocytes and trophoblasts and contribute to the trophoblast growth and invasion.

Methods

The following paragraph was added to this section as indicated by the reviewer;

“Briefly, IL-6 or TNF-α present in the samples or standard binds to anti-IL-6 or anti-TNF-α monoclonal antibody adsorbed to the microwells. A biotin-conjugated monoclonal anti-IL-6 or anti-TNF-α antibody was added and binds to IL-6 or TNF-α captured by the first antibody. Following incubation unbound biotin-conjugated anti-IL-6 or anti-TNF-α is removed during a wash step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-IL-6 or anti-TNF-α; following incubation unbound Streptavidin-HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of IL-6 or TNF-α present in the sample. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve was prepared from seven IL-6
and TNF-α standard dilutions and IL-6 or TNF-α sample concentrations determined. The detection limit for IL-6 and TNF-α were 1.4 pg/ml and 3.83 pg/ml respectively. The overall inter-assay coefficient of variation for IL-6 and TNF-α were 5.2% and 6.9% respectively. “

“The distribution of IL-6 and TNF-α serum levels are given as medians (range). Independent-samples T test was used to compare the mean levels between patients and control group. P-value <0.05 were considered statistically significant.” Has been changed to

“All serum IL-6 and TNF-α analyses were performed at the same time, in the same batch, and in duplicate according to manufacturer's instructions. IL-6 and TNF-α serum levels are given as normal distribution. Independent-samples t test was used to compare the mean levels between patient and control groups. P-value <0.05 were considered statistically significant.”

Discussion

“Although the pathogenesis of preeclampsia is still unknown, immunologic and inflammatory causes may play an important role. IL-6 and other cytokines are important components of immune response, and therefore can participate in the immune aspects of the pathophysiology of this disease.” Has been changed to

“Although the pathogenesis of preeclampsia is still unknown, immunologic and inflammatory causes may play an important role. IL-6 and other cytokines are important components of immune response, and therefore can participate in the immune aspects of the pathophysiology of this disease. Proinflammatory cytokines appear to be involved in
cellular events that establish and maintain pregnancy (18); however, their role has not yet been well defined.”

“Tumor necrosis factor- α (TNF- α) is another pro-inflammatory cytokine that its contribution in the pathogenesis of preeclampsia has been suggested in recent studies (8, 10). TNF- α may contribute to abnormal placental invasion (15), endothelial cell damage (4) and oxidative stress (3). TNF- α can stimulate IL-6 production (16), since IL-6 inhibits TNF- α release (17). In contrast to IL-6, no increased serum concentration of TNF- α was found in preeclamptic patients compared to control pregnant women. This finding is consistent with reports by Greer et al in 1994 (6), Heyl et al in 1999 (18) and Ellis et al in 2001 (19). Our findings support the hypothesis that immune activation is involved in preeclampsia, and that IL-6 may participate in the abnormal immune response. However, we cannot determine whether IL-6 is an active mediator in preeclampsia or a marker of immune activation, and it seems necessary to perform further studies, including longitudinal studies before the onset of preeclampsia, to elucidate the role of this cytokine in the pathogenesis of preeclampsia.”

“TNF- α is another pro-inflammatory cytokine that its contribution in the pathogenesis of preeclampsia has been suggested in recent studies (8, 10). In healthy pregnant women, TNF- α is thought to modulate the growth and invasion of tropoblasts in maternal spiral arteries (22). TNF- α may contribute to abnormal placental invasion (23), endothelial cell damage (4) and oxidative stress (3). TNF- α can stimulate IL-6 production (24), since IL-6 inhibits TNF- α release (25). In contrast to IL-6, no increased serum concentration of TNF- α was found in preeclamptic patients compared to control pregnant women.
Several investigators (26-28) have reported that serum concentration of TNF-α was significantly higher in the first and second trimester among pregnant women who subsequently developed preeclampsia compared to those in the control group. This finding is consistent with reports by Greer et al in 1994 (6), Heyl et al in 1999 (29) and Ellis et al in 2001 (30). Furthermore, the levels of IL-6 and TNF-α that we detected in the Khorsanian women studied were significantly higher than those reported for European and North American women (26-28). Whether these differences are related to genetic (inflammatory response and L-arginine;NO pathway) and/or environmental factors (e.g., infection) remains to be determined. Several evidence links infection and inflammatory processes with preeclampsia (31, 32). The role of infection in the pathogenesis of preeclampsia is particularly relevant in developing countries, where the high incidence of chronic subclinical infection may contribute to the high incidence of preeclampsia.

Our findings support the hypothesis that immune activation is involved in preeclampsia, and that IL-6 may participate in the abnormal immune response. This study was undertaken in women with established preeclampsia, therefore, it cannot be determined whether the increase IL-6 was a cause or a consequence of the disease (10, 26-28). We cannot determine whether IL-6 is an active mediator in preeclampsia or a marker of immune activation, and it seems necessary to perform further studies, including longitudinal studies before the onset of preeclampsia, to elucidate the role of this cytokine in the pathogenesis of preeclampsia.

Figures
The source of cytokine was added to the legends.
Tables

Table 1 has been changed; t and p values were calculated and were added to the data.

References

The following references have been added.


MAJOR COMPULSORY REVISIONS

1. Introduction. Describe that IL-6 and TNF-α are also produced in the adipose tissue, and the importance of this tissue in endothelial dysfunction.

Both IL-6 and TNF-α are expressed in adipose tissue (11, 12) and in vitro release of TNF-α by adipocytes has been reported (13). Among the known effects of these cytokines are inhibition of insulin signaling (14) and induction of both hypertriglyceridemia (15) and endothelial activation (16).

2. Materials and Methods: Provide more data about the within assay coefficients of variation and limits of sensitivity for each of the cytokine assays.

Briefly, IL-6 or TNF-α present in the samples or standard binds to anti-IL-6 or anti-TNF-α monoclonal antibody adsorbed to the microwells. A biotin-conjugated monoclonal anti-IL-6 or anti-TNF-α antibody was added and binds to IL-6 or TNF-α captured by the first antibody. Following incubation unbound biotin-conjugated anti-IL-6 or anti-TNF-α is removed during a wash step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-IL-6 or anti-TNF-α; following incubation unbound Streptavidin-HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount
The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve was prepared from seven IL-6 and TNF-α standard dilutions and IL-6 or TNF-α sample concentrations determined. The detection limit for IL-6 and TNF-α were 1.4 pg/ml and 3.83 pg/ml respectively. The overall inter-assay coefficient of variation for IL-6 and TNF-α were 5.2% and 6.9% respectively.

3. Results: Describe in the text or in the table 1 the weigh, body mass index and the weigh gain during pregnancy of the women included in the study by groups.

This study was undertaken in women with established preeclampsia, therefore, we did not have data for the weight, BMI and weight gain during pregnancy.

4. Discussion. Discuss the possible reasons of the discrepancie with previous reports that have shown higher levels of TNF-alpha; in preeclampsia.

Several investigators (26-28) have reported that serum concentrations of TNF-α was significantly higher in the first and second trimester among pregnant women who subsequently developed preeclampsia compared to those in the control group. Furthermore, the levels of IL-6 and TNF-α that we detected in the Khorsanian women studied were significantly higher than those reported for European and North American women (26-28). Whether these differences are related to genetic (inflammatory response and L-arginine;NO pathway) and/or environmental factors (e.g., infection) remains to be
Several evidence links infection and inflammatory processes with preeclampsia (31, 32). The role of infection in the pathogenesis of preeclampsia is particularly relevant in developing countries, where the high incidence of chronic subclinical infection may contribute to the high incidence of preeclampsia.

5. Discussion. Discuss about the eventual role of overweight in the increased levels of IL-6 and not only the role of immune activation.

Since IL-6 and TNF-α are both expressed in adipose tissues, then in overweight women with pre-eclampsia, the role of IL-6 may be considered. In the present study, the subject group was diagnosed as established pre-eclampsia.

DISCRETIONARY REVISION

1. Will be nice to have the data of fasting glicemia and insuline by groups.

We did not measure FBS and insulin in the serum of subject and control groups.

References

The following references have been added.


