Induction of ICAM-1 and ICAM-3 in Women with Recurrent Pregnancy Loss

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Abstract

Background: Recurrent pregnancy loss (RPL) has been found to be associated with increase in the pro-inflammatory cytokines which cause up-regulation of inflammatory mediators including cell adhesion molecules (CAMs) that might act in aggravation of this pathological process.

Objective: To find out whether there is a relation between the pathology of RPL and the expression of intracellular adhesion molecule-1 (ICAM-1) and ICAM-3 at the feto-maternal interface in these patients.

Methods: Immunohistochemistry technique was performed to detect and determine the expression of ICAM-1 and ICAM-3 using paraffin embedded sections of curate samples obtained from 40 women, who where divided into three groups: 24 women with RPL, 10 women with abortion for the first time, and 6 women with induced abortion.

Results: The levels of the expression of both endothelial ICAM-1 and leukocytes ICAM-3 at the feto-maternal interface were found to be significantly up-regulated in the first group as compared with the second and the third groups (p=0.001), with a highly significant positive correlation between these two parameters (r=0.927, p<0.01).

Conclusion: ICAM-1 and ICAM-3 might play an important role in the pathology of RPL by increase adherence and recruitment of inflammatory cells at the feto-maternal interface ending with a pregnancy failure.

Key words: Inter Cellular Adhesion Molecule-1 and Inter Cellular Adhesion Molecule -3, Recurrent pregnancy loss.

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Introduction

Cell adhesion molecules (CAMs) mediate cell–cell interactions and play an important role in cell differentiation [(1)], in the organization of the extracellular matrix and in the recruitment and aggregation of leukocytes from the circulation [(2)]. The immunoglobulin superfamily of which intercellular adhesion molecule (ICAM)-1 and ICAM-2 are members is the most widely distributed family of cell adhesion molecules. ICAM-1 is expressed on leukocytes, epithelial and endothelial cells, ICAM-2 is mainly found on resting endothelial cells and ICAM-3 is constitutively expressed by all resting leukocytes [(3)].

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Cell adhesion molecules are present in human endometrium, where they may play a role in regulating leukocyte trafficking into this tissue [(2, 4, 5)]. It is recognized that the normal endometrium has a population of leukocytes, including macrophages, T-lymphocytes and granulocytes, which are important in the physiology of the endometrium. Furthermore, T cells form 10-15% of lymphocytes in early pregnancy deciduas [(6)], while B cells are professional cells that produce immunoglobulines; their count and population in the endometrium do not change through out menstruation and during pregnancy, and the second major decidual leukocyte population consists of the monocytes/macrophages [(7)].

The expression of ICAM-1 in human endometrium can be stimulated by cytokines including interferon (IFN)-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-1β [(4, 8, 9)].

However, endothelial, fibroblastic and epithelial cells differ in their response to ICAM-1-inducing cytokines [8, 9]. In contrast to ICAM-1; ICAM-2 expression is not normally augmented by cytokine activation [3].

It was found that these type1 cytokines (like IFN-γ and TNF-α) are up regulated in women with RPL [10, 11]. And as the target of these pro-inflammatory cytokines was found to be mainly vascular associated with inflammatory cells infilterate[7,12,13] . We attempted in this study to explore the expression of endothelial ICAM-1 which is inducible by these cytokines [4], and the expression of leukocytes' ICAM-3, at the feto-maternal interface in these patients to find out whether or not these adhesion molecules play a role in the pathology of pregnancy loss.

Patients and Methods

Patients were divided into three groups; **Group A:** 24 pregnant ladies presented with incomplete first trimester abortion, all of whom gave a history of previous 3-6 consecutive first trimester abortions, with no medical diseases, family history of genetic diseases or uterine anatomical anomaly. Also all of them were negative for acute infection with rubella, HCMV or toxoplasmosis. **Group B:** 10 pregnant ladies presented with incomplete first trimester abortion and had at least three previous normal pregnancies with no previous abortion, and no history of any medical illness, and **Group C:** 6 pregnant ladies with elective termination of pregnancy in the first trimester for a maternal indication under approved consent of two senior gynecologists and a physician. Curate samples of the feto-maternal interface were taken from all these women at the end of evacuation curate operation.

Samples were embedded in paraffin and subjected for immunohistochemistry technique using DAKO cytomation detection kit (Denmark). Refer to the immunohistochemistry procedure in reference [14], and signal evaluation using CD31 as baseline endothelial marker in blood vessel counting in reference [15], dilution of the monoclonal antibodies was 1:50 for both ICAM-1 and ICAM-3 (DAKO cytomation-Denmark). Negative controls were obtained by omitting the monoclonal antibody and using antibody diluent alone to verify the signal specificity.

Statistical analysis

ANOVA test was used to determine the difference in the expression of ICAM-1 and ICAM-3 among the three groups, and the relationship between these two parameters was measured using the correlation coefficient (*r*). Values of $p<0.05$ were considered as statistically significant.

Results

Figures 1 and 2 shows the percentages of ICAM-1 and ICAM-3 expression respectively in terms of mean ± SE. As shown in figure 1 that the mean percentage of ICAM-1 expression in the first group, which is significantly higher (P=0.001) than that of the second and third groups (using ANOVA analysis), as demonstrated in (Figure 1), and the same was found for ICAM-3 (P=0.001) (Figure2). Additionally, the study showed a highly significant positive correlation between the expression of ICAM-1 and ICAM-3 ($r=0.927$, $p\leq0.01$) in the investigated groups.

Table 1: The expression of ICAM-1 among the studied groups.

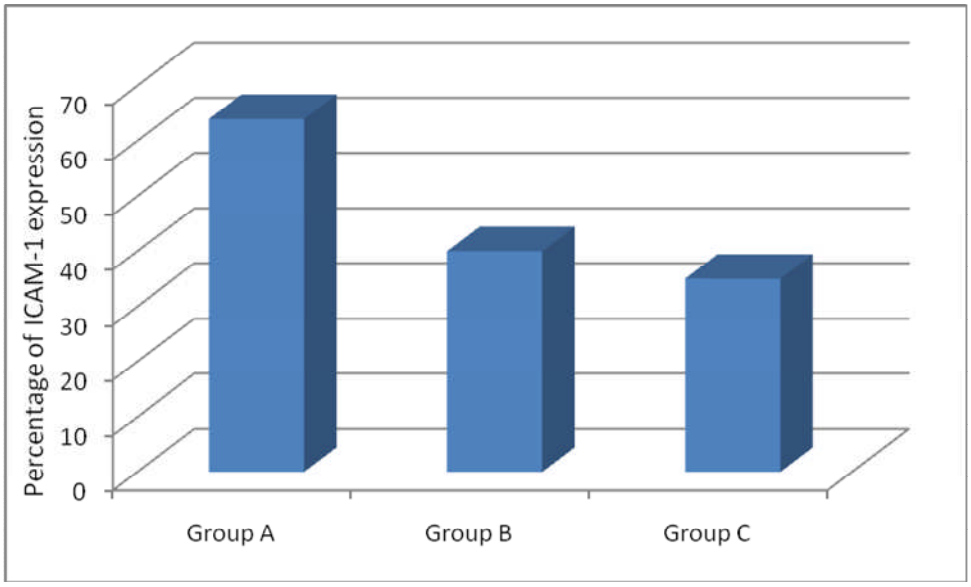
ICAM-1	n	Percentage of expression		
		Mean ± S.E.$^{\Psi}$	Min. Value	Max. Value
Group A	24	63.96 ± 3.38	40	90
Group B	10	40.00 ± 3.33	30	55
Group C	6	53.00 ± 1.83	30	40

Different letters: significant difference (P<0.05) between means.

Table 2: The expression of ICAM-3 among the studied groups.

ICAM-3	n	Percentage of expression		
		Mean ± S.E.$^{\Psi}$	Min. Value	Max. Value
Group A	24	55.63 ± 3.41	30	80
Group B	10	31.00 ± 3.06	20	55
Group C	6	25.00 ± 2.58	20	35

Different letters: significant difference (P<0.05) between means.



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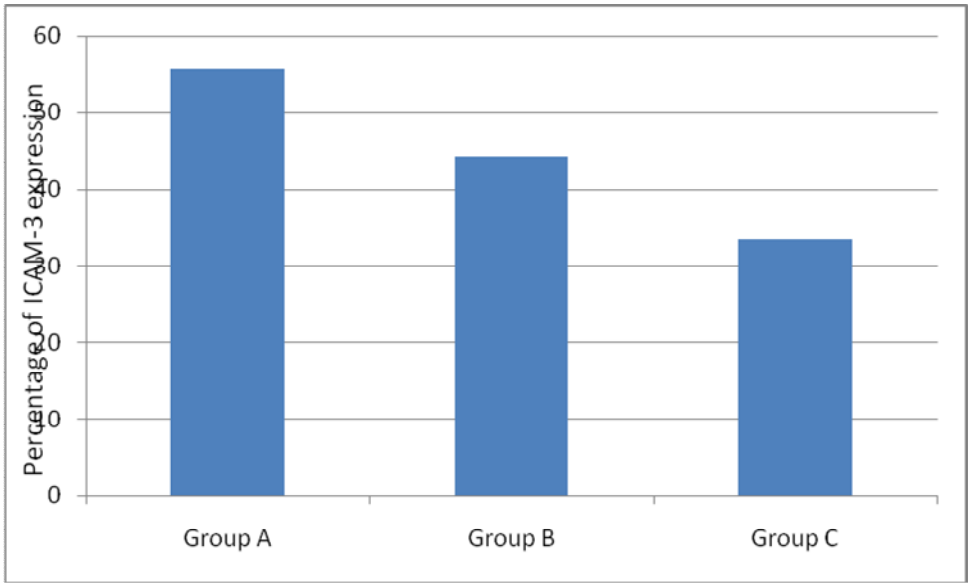

Figure 2: The expression of ICAM-3 among the studied groups

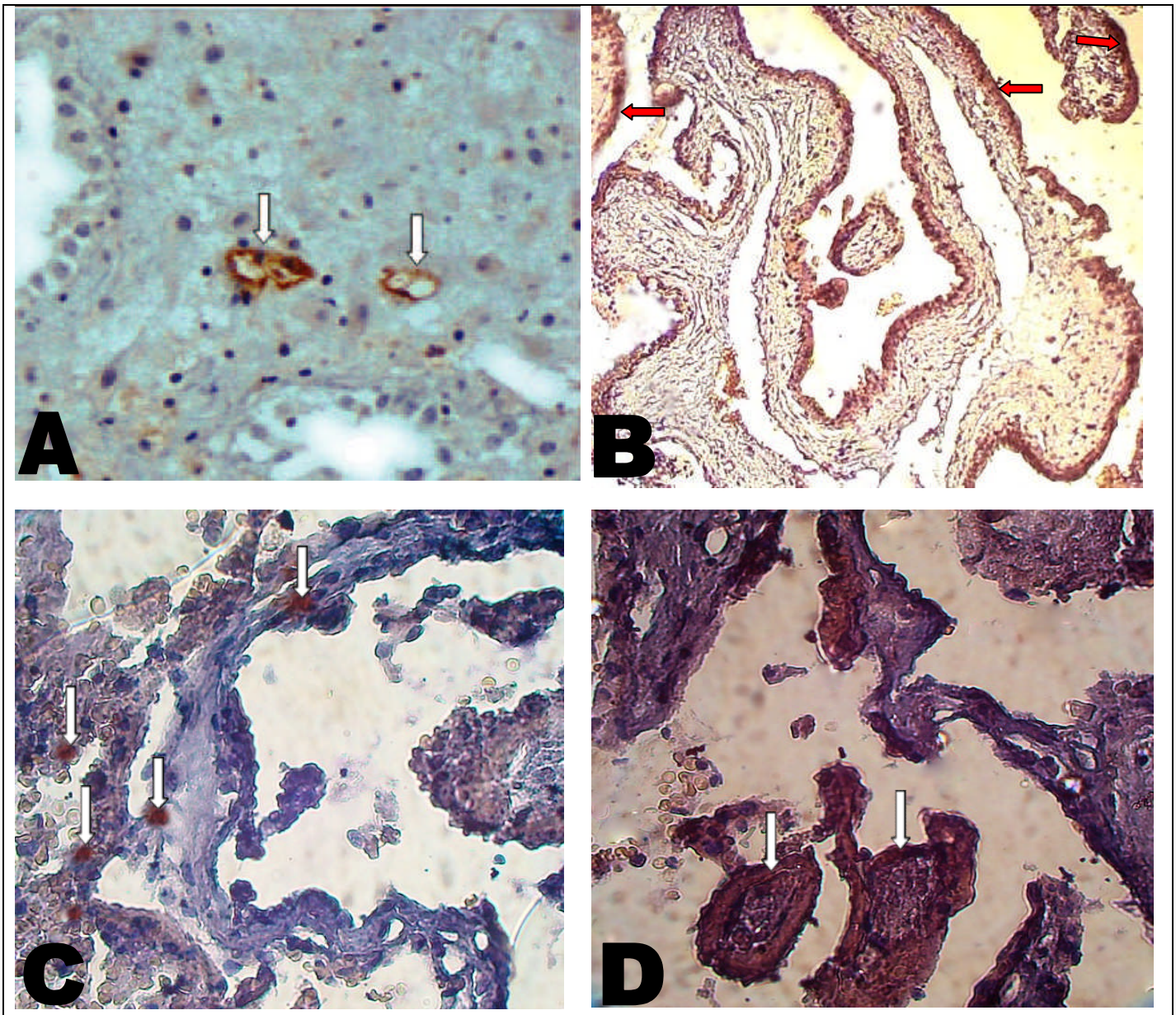

Figure 3:Immunostaining of ICAM-1 and ICAM-3 in women with pregnancy loss.

ICAM-1 was identified on decidual endothelial cells (**A**) (white arrows), and trophoblasts (**B**) (red arrows). While ICAM-3 was identified on tissue infiltrating leukocytes (**C**) (white arrows), and trophoblasts (**D**) (white arrows). Magnification power of A, C and D (X400), B (X100).

Discussion

Spontaneous abortion (resorption) in mice is thought to represent a rejection of the semi-allogeneic feto-placental unit by activated NK cells and activated macrophages [13, 16]. These cells infiltrate maternal mesometrial decidua at the site of implantation and the frequency of implantation sites with such an infiltrate is proportional to the percentage of embryos that resorb.[13] Murine resorptions are characterized by focal necrosis at the junction of the fetal trophoblasts and the decidua, an infiltrate with polymorphonuclear leukocytes at sites of necrosis and along the walls of large vessels in the decidua, and by thrombosis and hemorrhage [17, 18].

There are two main sources of this polymorphonuclear cell (PMN) infiltration; firstly, when thrombin is generated, it will activate IL-8 secretion by endothelial cells, and as a consequences IL-8 recruits PMNs[19,20] .Secondly, pro-inflammatory cytokines like IFN-γ and TNF-α induce endothelial adhesion molecules and increase the transendothelial migration of the recruited leukocytes[2,7,21].

All these studies support the present, which showed increase in the expression of endothelial ICAM-1 and ICAM-3 on tissue infiltrating leukocytes, making these CAMs good indicatos and participating in the pathology of pregnancy loss. Furthermore, recent studies showed that enhanced decidual IL-8 expression interacts with constitutively expressed ICAM-1 in decidual endothelium to modulate neutrophil trafficking into hemorrhagic and inflammatory first trimester deciduas [20]. In addition, other studies showed increase in ICAM-1 surface expression on endothelial cells of preeclamptic women in comparison with pregnant normotensive and non-pregnant women [22] .Therefore, midgestation measurements of circulating ICAM-1 and VCAM-1 (above the cutoff) have a high predictive value and may identify up to 55% of pregnant women who will later develop a severe pregnancy-related complication[23].

Mast cell- and macrophage-derived cytokines engage with their receptors on endothelial cells. This will ultimately leads to activation of nuclear transcription factors that modulate the biosynthesis of endothelial CAMs that mediate leukocyte rolling (E-selectin) and adherence (ICAM-1, VCAM-1)[2]. Which is in line with our previous study on the same groups of women showing significant increase in the transcriptional factor (NF-κB) and the pro-inflammatory cytokine (IFN-γ) in the recurrent loss group as compared with other two groups [24],and significantly higher surface expression of endothelial VCAM-1 in the same group also (unpublished data).

This study also showed that ICAM-1 and ICAM-3 expressed on the trophoblasts in some cases indicating that these CAMs might really have a role in the adherence, implantation and vascular invasion as mentioned in other studies [14, 25].

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