Cd30 molecule expression in sera and on t cells of trophoblast tissue from women with recurrent spontanious abortion

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Abstract

Background: Immune responses during pregnancy show a distinct shift towards Th2-type reactions occurs, especially at the feto-maternal interface. CD30 has been described as being preferentially expressed, and released, by human T cells producing the Th2-type cytokines**.**

Objective: To determine the level of soluble CD30 (sCD30) in serum and in the trophoblasts of patients with recurrent spontaneous abortion (RSA).

Materials and methods*:* A total of sixty one women attending the Obstetrics department in al- kadhemia hospital, age range from (23.9 - 28.5years), were enrolled in the current study and were further classified into three categories: Group A: 35 women included cases with recurrent spontaneous abortion, group B: 16 women included non- recurrent spontaneous abortion (non-RSA): group C: 10 women was Control (successful pregnancy). From each subject blood sample and placental tissues were collected and serum was seperated for the estimation of soluble CD30 (sCD 30) levels using ELISA method and trophoblasts tissues (an image for the local microenvironment) were screened to determine the levels of CD30 using immunohistochemistry.

Results: Trophoblast expression of CD30 and sCD30, showed a highly significant increased values for both patients groups ($p<0.001$) when compared with control group.

Conclusion: It is likely that there may be an association between normal pregnancy and CD30 density on the cell surface.

Key words: recurrent spontaneous abortion, CD30, mmunohistochemistry, ELISA

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Introduction

Successful human pregnancy appears to be an immunological paradox, in that the fetus represents a semi-allograft developing in the potentially hostile environment of the maternal immune system [1-3] One important mechanism involves the down-regulation of the cellular immune response, which has been shown to be dependent upon the suppression of T-helper (Th)1 and T-cytotoxic (Tc)1cells,which produce interleukin(IL-2), interferon (IFN)-γ, and tumor necrosis factor (TNF)-β, and the up-regulation of Th2 and Tc2 cells, which produce IL-4, IL-6, IL-10 and IL-13[4-7].

Previous investigations of Th1/Th2 immune responses during pregnancy were able to show that a distinct shift towards Th2-type reactions occurs, especially at the feto-maternal interface [8-12]. CD30 has been described as being preferentially expressed, and released, by human T cells producing the Th2-type cytokines [13,14]. Surface CD30 is cleaved proteolytically, resulting in the release of the soluble form of the molecule (sCD30) by CD30-expressing cells [15].

Since CD30 has been reported to be associated with Th2-type reactivity, we measured soluble CD30 in the serum and the density of CD30 on the surface of T cells of normal pregnant women and in women undergoing abortion ; to clarify if there is any association between normal pregnancy

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and CD30 density on the cell surface we designed this study.

Subjects, materials and methods

Sixty one women attending the Obstetrics and Gynecology department of Al-Kadhemia Teaching Hospital in Baghdad between December 2004 and August 2005 were the subject of studying. They included 35 women with 3-6 consecutive aborti**n; group A** (RSA), 16 women with abortion for the first or second time; group B, 10 pregnant women who had at least two previous normal pregnancies;group C.

Trophoblastic tissue was collected from the evacuation of retained pieces during the procedure of curettage and placed in 10% formaldehyde. Two to three paraffin embedded blocks were prepared for each patient. Staining with haematoxyline and eosin was carried out to decide which block can be used in the study (only sections that contained trophoplastic tissues were included. These samples were subjected for immunohistochemical staining (IHC) protocols with the anti CD30 marker according to [16]. The expression of CD30 was measured by counting the number of positive trophoblastic cells that gave brown cytoplasmic staining under light microscope. The extent of the IHC signal in the villi was determined in 10 fields (100 X magnification). In each field the total number of villi were counted and the extent of cytoplasmic staining of the trophoblast cells in a given villous was determined as a percent. The total staining score was divided by the number of whole villi per field in 10 fields [17] so, the percentage of positively stained villi in the 10 fields was calculated for each case by taking the mean of the percentage of the positivity stained villi in the 10 fields. In each field the total number of villi was counted and the extent of staining of the cytotrophoblast and syncytiotrophoblast in a given villous was scored as: score 3 (75-100%); 2 (25-75%); or 1 (<25%).

Detection of soluble CD30 by ELISA:

Sample collection: Five ml of venous blood was collected from each subject group and serum was separated and stored at -20 ^{o}C until used.

The ELISA test was perfomed,using two anti- CD30 monoclonal antibobies (Primary and secondary)antibodies which were the product of DAKO.Cut –off value was calculated according to[18].

Statistical Analysis

The ANOVA analysis program, chi-square and the relationship between the indicators was measured qualitatively by using the correlation coefficient.

Results

Correlation between abortion and CD30:

In thirty five women with RSA (group A), a negative significant correlation (p<0.05) between abortion and CD30 in sera and trophoblast tissues (r=-0.651; r=-0.496, respectively), was found, (Table **1**). The data also showed a negative significant correlation(r=-0.529; $p\leq0.05$) between abortion and sCD30 in group (B).

Table 1: Correlation between abortion and CD30 in women involved in this study.

Variables	Groups	n	Correlation Coefficient r =	*P* value≤
Abortion– sCD30(ELISA)	**A**	**35**	**-0.651**	**0.05**
	B	**16**	**-0.529**	**0.05**
	C	**10**	0.510	N.S.
Abortion– CD30(IHC)	**A**	**35**	**-0.496**	**0.05**
	B	**16**	0.297	N.S.
	C	**10**	0.430	N.S.

N.S. =not significant

Table 2: Number and percentage of CD30 in trophoblasts of studied groups.

Variable	Score*	Groups			Chi-Square *P* value
		A	B	C	
		n=35 (%)	n=16 (%)	n=10 (%)	
CD30 (IHC)	1	16(45.7)	1(6.3)	0	0.001**
	2	19(54.3)	15(93.7)	5(50)	
	3	0	0	5(50)	

*In each field the total number of villi were counted and the extent of staining of the cytotrophoblast and syncytiotro-phoblast in a given villous was graded as 3 (75-100%); 2 (25-75%); or 1 (<25%), **= highly significant difference ($p<0.01$)

Expression of CD30 in villous trophoblasts detected by IHC:

The results showed that percentage of CD30 expression was moderate in 54.3% (19/35) and 93.7 %(15/16) of women in group A and group B, respectively.The corresponding figure in control group was 50%. These differences were highly significant (P≤ 0.001), (Table 2).

In (Table 3), The mean percentage of CD30 expression in the trophoplast was significantly declined ($p<0.001$) in group A as compared with group C (23. 7± 1.1vs 76 ± 3.3) respectively.The decline was also found in group B as compared with group C (39.6± 2.5 vs 76 ± 3.3) respectively.

Table 3 : Comparison between the mean percentages of CD30 expression in the trophoblasts of the studied groups.

Variable	Group	No=61	Mean± SE	F test *P* value	P values between groups	
					Groups	*P* ≤
CD30 (IHC)	A	35	23.7.±1.1	<0.01	A–B	0.001
	B	16	39.6±2.5		A –C	0.001
	C	10	76± 3.3		B –C	0.001

Soluble CD30 (sCD30) in sera detected by ELISA:

Table 4 shows that the mean value of serum levels of sCD30 was significantly higher (p<0.001) in group A (0.125±0.01) and B (0.127±0.01) as compared with group C (0.61± 0.06).

Table 4: Comparison between the mean values of sCD30 in sera of women involved in the study.

Variable	Group	No.=61	Mean±SE	F test *P* value	Pvalues between groups	
					groups	*P* ≤
CD30 (ELISA)	A	35	0.125±0.01	<0.01	A–B	NS.
	B	16	0.127±0.01		A –C	0.001
	C	10	0.61± 0.06		B –C	0.001

NS. =not significant

Discussion

The current study, showed highly significant increase in expression of CD30 (local and systemic) (p<0.001) in control group (successful pregnancy) compared with first and second trimester abortion. In addition, a significant difference in expression of CD30 (p<0.05) between first trimester and second trimester abortion was found. These results might be explained by the presence of association between normal pregnancy and CD30 density on the cell surface in trophoblast and circulation.

Results of group A, found a negative significant correlation between gestational age and CD30 in circulation and trophoblasts tissue(r=-.651; r=-.496, p<0.05, respectively), and gave a negative significant correlation (r=-.529; p<0.05) between gestational age and sCD30 in women in group B. This result indicated that in women with RSA the expression of CD30 decreasing with increasing gestational age. This might be explained by other studies showing that high concentrations of sCD30 have been found in a variety of disorders that are clearly Th2-mediated or Th2-dominated [13]. In addition, the current study, showed highly significant

difference (p<0.001) between CD30 expression in trophoblasts tissue in three groups of investigated women. However, previous reports showed that CD30 expression was associated with differentiation and activation pathway of human T cells producing Th2 –type cytokine [14, 19].

Previous reports showed that CD30, was a member of tumor necrosis factor receptor superfamily TNFR [19] and can give signals through the activation of the nuclear factor-κB (NFκB), which is an important transcriptional factor, regulating the pro-inflammatory cytokines, which were shown to be down-regulating during pregnancy, a mechanism that is essential for the maintenance of the Type2 cytokine profile required for pregnancy success [20]. High concentrations of sCD30 have been found in a variety of disorders that are clearly Th2-mediated or Th2-dominated. Since pregnancy appears to be a Th2-biased condition it is likely that the skew towards Th2-bias seen in peripheral blood cells may be reflected by increased concentrations of sCD30 in the blood [13, 19].

Moreover, the expression of CD30 in trophoblatic tissue was highly significant increased (p<0.001) in women with non-RSA (group B) compared with RSA (group A), but no significant difference (p>0.05) was found in the levels of sCD30between the mentioned groups. This result might be associated with level of cytokine and CD30 within local microenviro-nment and the peripheral circulation. The variation of expression suggests a possible role for hormones, preferably progesterone, in the regulation of CD30 expression , This would be a novel mechanism of CD30 induction [21] , progesterone produce an immunomodulatory protein known as progesterone –induced blocking factor(PIBF) which induces increased production of Th2 cytokines [22], therefore apart from the systemic changes in the maternal immune response, local immunomodulation at the feto-maternal interface via wide array of hormones and cytokines, and immune effector cells also play a critical role in maintaining the balance of the desirable immune response [23]

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