CORRELATION OF CD44THE RECEPTOR OF HYALURONAN IN DECIDUAL STROMAL CELLS AND MISCARRIAGE

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Abstract To evaluatethe expression of CD44 proteins in women with incomplete first trimester miscarriage. The study included 172 women age 16-41 years were further classified into three categories: Group A- Recurrent spontaneous miscarriage (RSM): n= 65 women, with a mean age of (25.2±7.28) years. Group B- Non- recurrent spontaneous miscarriage (non-RSM): n= 36 women, with a mean age of (26.61 ±6.97)years.

Group C- Control (normal pregnancy): n=71 women, with a mean age of (26.17±7.01).years The expression of CD44 proteins were detected by Immunohistochemistry IHC .ANOVA test analysis revealed a highly significant difference ($p<0.001$) in the mean percentage of CD44 positive decidual stromal cells DSCs between group A and C (37.71±2.93; 65.35± 4.23, respectively). Also a highly significant difference ($p<0.001$) was found between the mean percentage of CD44 in group B and C and between group A and B. This study supports that higher level of HyaluronanCD44 promotes growth of decidual stromal cells in human first-trimester Pregnancy.

Index Terms : Hyaluronan CD44,,Decidual stromal cells DSCs, Miscarriage

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1 INTRODUCTION :

Endometrium is composed mainly of two types of cells, endometrial stromal cells (ESC) and endometrial glandular cells (EGC). The growth and differentiation of ESC are under the control of ovarian steroid hormones. After implantation of the fertilized egg into the endometrium, ESC transform into decidual cells (DC) (Imai,1992) .

Decidual stromal cells (DSCs) are important during pregnancy which constitutes 75% of decidual cells forming the main cellular part of maternal fetal tissues. DSCs are included in immune modulation, as antigen phagocytosis and cytokine production in addition to nutrition and embryo support (Olivares *et al.*,

1997 ; Ruiz *et al.*,1997 **;** Piao *et al.*, 2012**)** However their biological function in pregnancy remains unclear. DSCs are essential for embryo implantation and pregnancy development. Any defect in decidualization is ended in recurrent pregnancy loss **(** Ledee *et al.*,2011**;** Xu *et al.*, 2012 **)** . Hyaluronan (HA) and its receptor CD44 are expressed at the maternal-fetal interface .

Hyaluronan (HA) is a main compound of extracellular matrix (ECM), which lead to proliferation and migration of cell (Jiang *et al.*,2011). HA is formed by (3) various HA synthetase namely (HASs), (HAS1, HAS2, and HAS3). HAS2 is able to produce high-molecular-weight hyaluronan (HMW-HA) and is responsible for inflammation, cellular proliferation and differentiation (Jiang *et al.*,2005). CD44 is the distinct receptor for HA on cell surface. Binding of HA to CD44 has been implicated in lymphocyte homing, tumorgenesis, and monocyte activation (Powell *et al.*,2005). Both HA and CD44 are noticed in human conceptus and in Decidual stromal cells (DSCs) (Termeer*et al.*, 2002 ; Takahashi *et al.*,2005) . HA is also detected in the fetal vessel walls and connective tissue closely underlying the trophoblastic cover (Bhilocha*et al* ., 2011 ;Liang *et al.*, 2011) The possible function of HA to maintenance normal pregnancy by the expression of DSCs higher HAS2, reactivates PI3K/Akt and MAPK/ ERK1/2 signalings through fusing to CD44 in DSC membrane and then enhances cell proliferation and prevents cell apoptosis. (Zhu *et al* ., 2013a).

2 MATRERIAL AND METHODS

The study included 172 women, aged between16 – 41 years, who admitted to the Maternity and Children Hospital in Basrah because of incomplete first trimester miscarriage, in addition to women with normal pregnancy at time of delivery in the period from February

2012 to May 2014were then divided into 3 groups :

- Group –A included (36) women with recurrent spontaneous miscarriage (RSM) during the first trimester , with a mean age of (26.7±4.28) years .
- Group –B included(12) women with incomplete first trimester miscarriage and had at least three previous normal pregnancy (non-recurrent spontaneous miscarriage) with a mean age of (25.93 ±7.12) years .
- Group C- Control (normal pregnancy): n=26 women with a mean age of (26.09± 3.24) years .

A trophoblastic tissue placenta sample from each curettage patient and control subject was collected and placed in 10% formaldehyde. then embedded in paraffin and confirmed by a pathologist, and then subjected for immunohistochemistry technique using DAKO cytomation detection kit (USA). the IHC procedure was performed according to the manufacturer instructions .

The work has been approached by the ethical committee of the College of Medicine ,University of Basrah.

2.1 Evaluation of the Immunostaining : It was done with the aid of a histopathologist.

The expression of CD 44 was measured by counting the number of the positive cells with brown (DAB) cell surface membrane staining in one microscopic field under light microscopy (X400) and then divided by the total cells on the same field multiply by 100(Al-Thamery,2009) as explained in the equation below:

CD44 (%) =number of cells positive for CD44in one field / total cells (positive and negative) in the same field X 100.

2.2 Statistical analysis : Data were analyzed by using computer facility of SPSS (Statistical Package for Social Science) The results were expressed as numbers, percentage and mean ± S.D (standard division of the mean). ANOVA was used to assess the significance of differences between groups, taking $p \leq 0.05$ lowest limit of significance Boxplot was used to compare between expression of CD_{44} in three study groups.

3 Results:

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The expression of CD44 in decidual stromal cells in different groups of study subjects were estimated . The result revealed that a highly significant difference (p<0.001) in the mean percentage of CD_{44} positive DSCs between group A and C (37.71±2.93; 65.35± 4.23, respectively), also a highly significant difference (p<0.001) was found between the mean percentage of CD_{44} in group B and C and between group A and B as illustrated by (Table 1 , Figure 1).

Table (1) Expression of CD_{44} in DSCs by IHC :

Variable	Group	n=74	Mean± S.D	*P* value	Sig. between groups	
					groups	*P* value
CD44 (IHC)	A	36	37.71±2.93	<0.01	A/B	0.000**
	B	12	50.3±5.73		C/ A	0.000**
	C	26	65.35± 4.23		C/ B	0.000**

**highly significant p<0.001

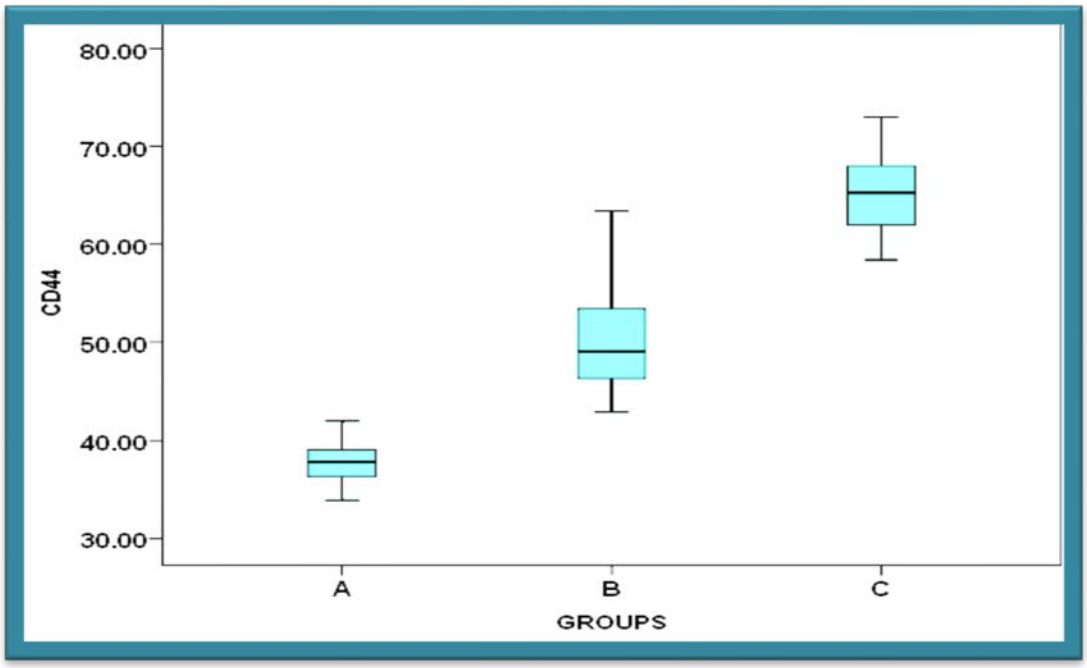

Figure (1) Mean concentration of CD44 in the three groups of cases (A, B and,C).

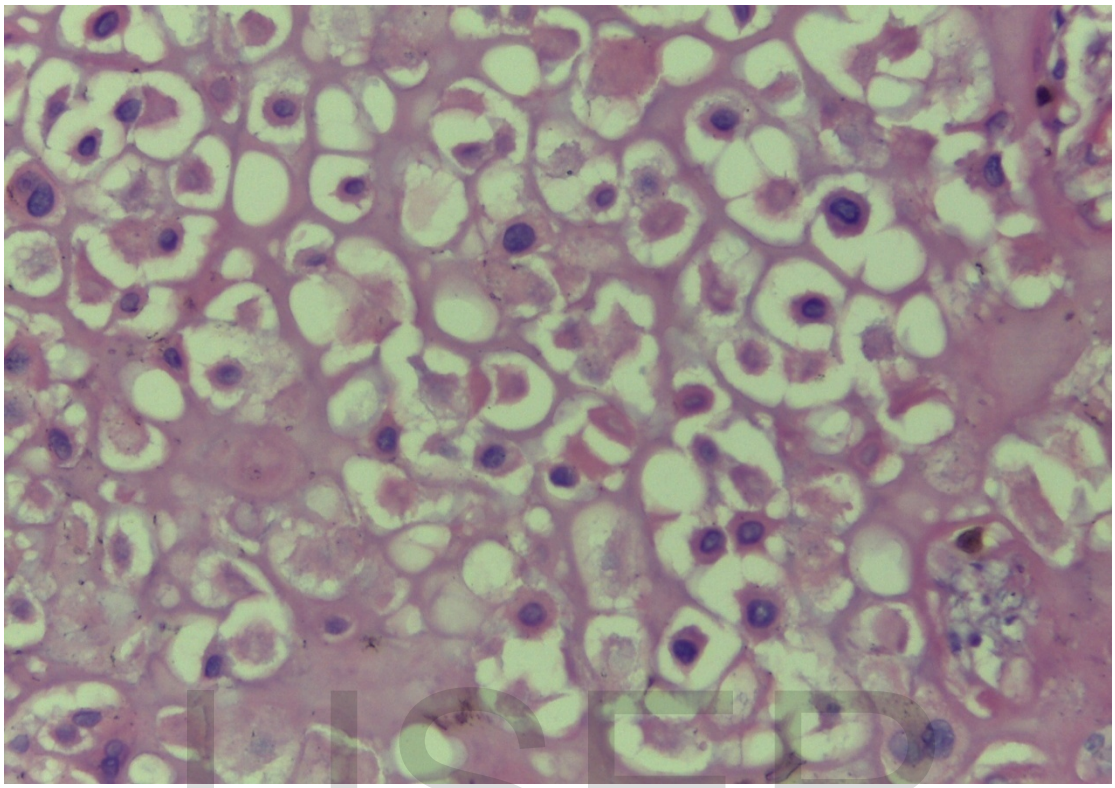

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Figure (2) H&E staining of DSCs

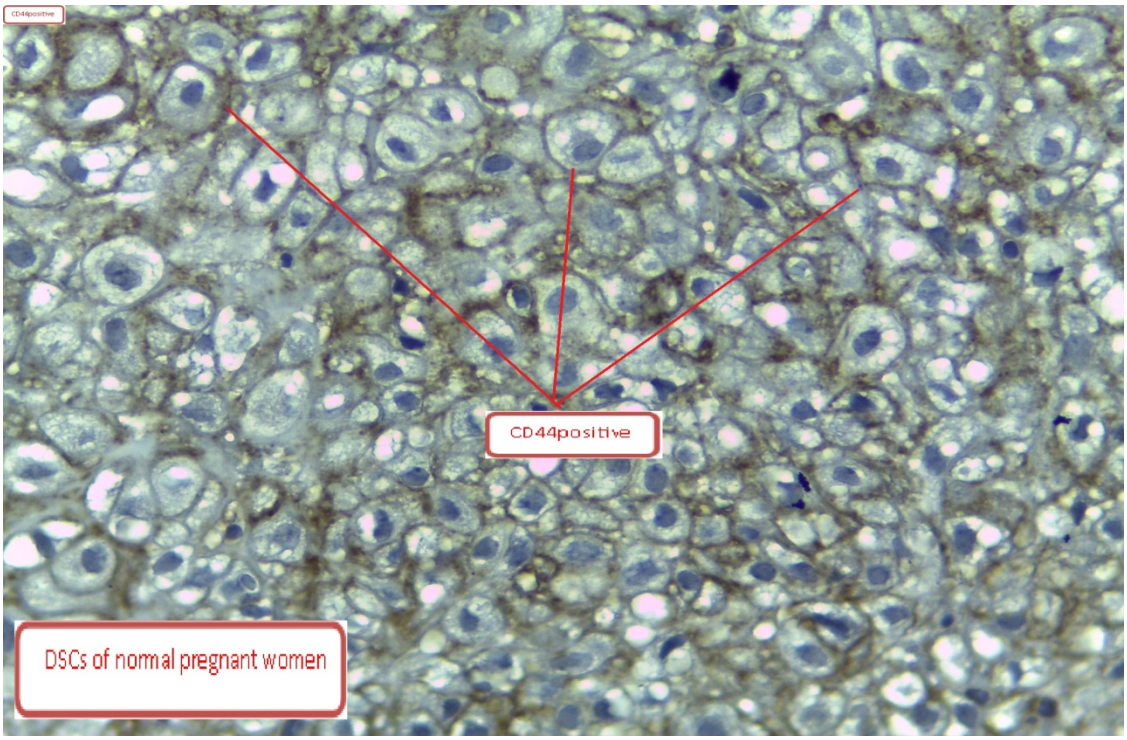

Figure (3) Expreation ofCD44 in DSCs of normal pregnancy by IHC technique .

4 DISCUSSION

The effects of CD44 from decidual stromal cells on the success or failure of pregnancy are interesting and have been reported in this study which illustrated that CD44 in feto-placental from the normal pregnancy was significantly higher (65.35± 4.23) than that of RSM women (37.71±2.93) and non- RSM women (50.3±5.73) . These result, are in agreement with Zhu *et al.*,(2013) which show high expression of CD44 in DSCs from early human pregnancy . In contrast, previous report about enhanced hyaluronan expression and abnormal localization at the feto maternal interface might be associated with murine abortion(Cordo-Russo *et al.*,2009) .Result of current study can be explained by Hyaluronan secreted from DSCs in normal pregnancy were mainly higher molecular mass while DSCs derived from miscarriage produced LMW-HA. Furthermore, the level of HAS2, not HAS1 or HAS3, was much higher in DSCs from normal pregnancy than that from miscarriage, similar with the fact that HAS2 is responsible for the synthesis of HMW-HA. Our results suggested that higher level of HMW-HA at the maternal-fetal interface maintained successful pregnancy, relative lower molecular weight of HA might contribute to pregnancy wastage (Cordo-Russo *et al.*,2009).

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