Assessment of CD56 and CD14 by IHC in Placental tissues from women with miscarriage

Zainab A. Hamid* **BVM&S, PhD**
Jabbar R. Zangor* **BVM&S, PhD**
Ali H. Bayati** **MBChB, PhD**
Saad H. Mohammed Ali*** **MBChB ,PhD**

JFac Med Baghdad 2017; Vol.59, No .2
Receive Feb. 2017
Accepted June.2017

Abstract:

Background: Among many possible causes, CD14, CD 56, were implicated in immune mechanisms and might be involved in pregnancy loss. However the role of these Immunological factors has not been clearly elucidated. Some authors have shown that women with reproductive failure (such as spontaneous miscarriage) have increased CD14, uNK cells numbers; where as other authors reported no difference or even reduced numbers.

Objective: The aim of this study was to have an insight in a panel of the immunological factors shared in the placental microenvironment in an attempt to find a close relationships of these markers to the state of abortions.

Methods: Immunohistochemistry technique assay was used to detect CD14 and CD56 in 40 women with spontaneous miscarriage and in 40 healthy deliveries in Baghdad/Iraq.

Results: The CD56 protein detected as by IHC in 60.0% and 5.0% of miscarriage and healthy delivered women groups respectively which showed statistically Significant differences ($p<0.05$) . The CD14 – IHC reactions were found in 37.5% and 7.5% of miscarriage and control placental tissues, respectively with statistically significant differences ($p <0.05$).

Conclusion: The increased expression of CD14 marker in the miscarriage patients as well as significant correlations of CD56+NK cells with the state of spontaneous abortion introducing these markers as good immunological biomarkers for predicting possibility of abortions.

Key words: CD14; CD56, immunohistochemistry, miscarriage, pregnancy, placenta.

Introduction

The etiology of abortion is diverse, but may include maternal age, chromosomal abnormalities, endocrine disorders, thrombophilia, infection, immunological rejection of fetus, and environmental factors, however, the causes of abortions in many cases are still unknown (1). Microbial Infections represent a major cause of abortion, of which viruses appear to be the most frequently involved pathogens (2). Several events may occur at the maternal–fetal interface, including generation of maternal fetal tolerance, uterine smooth muscles, its glands and spiral arteries remodeling as well as placental construction. (3). among many possible causes of abortions, Immunological factors might be attributed to abortion, yet these factors has not been clearly elucidated. The CD14, CD 56 CD100, CD72, CD45, and HLA-G implicated in immune mechanisms and are involved in pregnancy loss which have been examined their placental expression with real-time PCR, immunohistochemistry (IHC) and western blotting techniques (4) Natural killer (NK) cells are the key immune cells that predominantly populating the uterus in normal pregnancy at the maternal-fetal interface, contrasting to their name, these cells are not killers, but rather providing a microenvironment in pregnancy that is compatible to support a healthy placentation. (5). CD14 is a" pattern recognition receptor (PRR)", enhances innate immune responses by sensitizing host cells to bacterial LPS, lipoproteins, lipoteichoic acid, and other microbial products .CD14 transfer these lipidated microbial products to various TLR such as (TLR 4 and MD-2) signaling complexes that induce intracellular pro inflammatory signaling cascades upon ligand binding, mediating the innate immune response by cytokine secretion and the inflammatory response (6). It has long been recognized that routine histological examination of the placenta has limitations, especially with regard to the diagnosis of infectious diseases and immune markers or that may cause severe in utero fetal damage. Immunohistochemical testing of the placenta in such situations can be very useful in terms of identifying the infectious agent as well as in

**Dept. of Microbiology, Baghdad Medical College, Baghdad, Iraq*
***Community Health Dept. Technical College of Health, Sulaimani Polytechnic University, Sulaimani city. E-mail zainabhamid96@gmail.com*
****Communicable Disease Research Unite, Baghdad Medical College, Baghdad, Iraq.*

demonstrating a marked change in immune marker expression (7). The study was done to obtain answers for the questions: the relevance of expression CD14, CD 56, in relation to spontaneous abortion . The detection of the CD14, CD56, will bring the attention of obstetricians and nearby specialties to look for these markers as possible targets to explain abortions of unknown etiology.

Method:

This retrospective study made the use of paraffin embedded placental tissues which were collected from histopathological archives of Teaching Laboratories at AL-Yarmouk Teaching Hospital /Iraq and belonging to (40) female patients with miscarriage as patients group, their ages were ranged between 19 to 43 years, and 40 placental tissues of normal delivery as a control group. Expose Mouse and Rabbit Specific HRP \DAB Detection IHC Kit ab80436 (2013)Abcam was used for detection of CD14 andCD56 specific primary antibodies . Statistical analysis**:** Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences- version 22).

Results:

The score results of CD14 – IHC reaction in miscarriage placental tissues group show that (37.5%) were positive for this marker while the control group has showed 7.5% of the examined placental tissues. The highest percentages of CD14-IHC reactions (40%, 6 out of 15) has high score (score III). The results of CD14- IHC of the control placental tissues according to signal scoring showed that the highest score was found to have score I. Significant differences ($p < 0.05$) were found on comparing the results according to CD14-IHC scoring between placental tissues in the miscarriage and control groups. Regarding the signal intensity, the positive expression of CD14 protein as immune marker was detected as a brownish discoloration at cell surface localization (Figure 1, A) . Were predominated with moderate intensity (intensity II) , (constituting (46.7) . In the control placental tissues group (7.5%), among them 2 out of 3 have weak signal intensity and the remaining tissue revealed moderate signals intensity. Significant correlations ($p < 0.05$)were observed between miscarriage and control placental tissue groups for CD14 protein IHC- expression according to their signals intensity (Table 1) regarding the immunostaning of CD56 protein as cell surface marker was detected as a brownish discoloration at cell surface localization (Figure 1, B) . The percentage of CD56 protein was found in 60.0% (24 out of total 40 cases) in the placental miscarriage group , and in 5.0% (2 out of total 40 cases) of normal placental tissues from those healthy delivered women group. Significant differences ($p<0.05$) were observed among the results of CD56-IHC scoring of the studied groups. High percentage (41.7% %) of CD56 antigenic expression which have (score II) were found among placental tissues of miscarriage women group with predominated moderate intensity that constituted (75%), while in control placental tissues groups , all CD56-IHC expression of (2 out of 40; 100%) have score II . Significant differences ($p<0.05$) regarding scoring and intensity were statistically noticed among the study groups (Table 2).

Table (1): Stratification of signal scoring and signal Intensity of immunohistochemical reactions for detection of CD14 protein in the studied groups

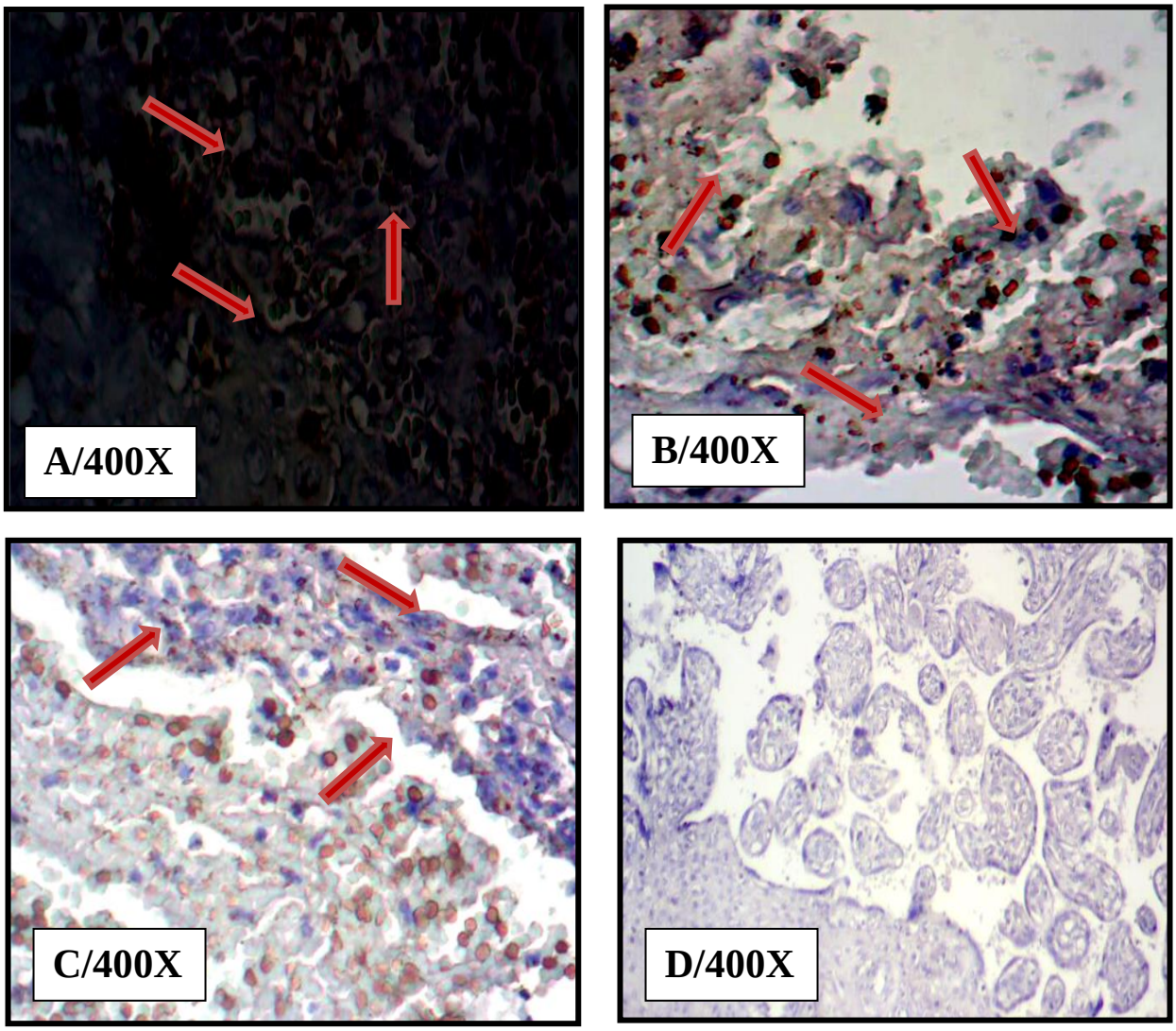
CD14 IHC Signal Score & Signal Intensity		Miscarriage placental tissue		Control placental tissue		P value
		No	%	No	%	
CD14 IHC Positive Score	Negative	25	62.5	37	92.5	0.001*
	Positive	15	37.5	3	7.5	
	Score I	5	33.3	2	66.7	0.376
	Score II	4	26.7	1	33.3	
	Score III	6	40.0	-	-	
CD14 IHC Positive Intensity	weak / I	3	20.0	2	66.7	0.217
	Moderate /II	7	46.7	1	33.3	
	Strong / III	5	33.3	-	-	

*Significant difference between proportions using Pearson Chi-square test at 0.05 level

Table (2): Frequency distribution of Immunohistochemistry results for CD56 protein according to signal scoring and Signal Intensity

CD56 IHC Signal Score		Miscarriage placental tissue		Control placental tissue		P value
		No	%	No	%	
CD56 IHC Positive Score	Negative	16	40.0	38	95.0	0.0001*
	Positive	24	60.0	2	5.0	
	Score I	6	25.0	-	-	-
	Score II	10	41.7	2	100	
	Score III	8	33.3	-	-	
CD56 IHC Positive Intensity	weak / I	-	-	1	50.0	0.002*
	Moderate / II	18	75.0	1	50.0	
	strong / III	6	25.0	-	-	

*Significant difference between proportions using Pearson Chi-square test at 0.05 level



Figure(1):Microphotograph of IHC staining of CD14&CD56 positive within the inflammatory cells of trophoblastic placental tissues from miscarriage patients (red arrow) : A- showed score 3 and strong intensity of CD14.
B- showed score 3 and strong intensity of CD56. C- showed score2 and strong intensity for CD56 . D- negative IHC-signal staining for IHC .

Discussion:

The current study has demonstrated a highly immunohistochemical detection of CD14 in the miscarriage placental tissues group (37.5%) than CD14 antigen expression in those tissues in the control placental tissues group (7.5%). Significant differences ($p < 0.05$) were found on comparing the results according to CD14-IHC scoring between the two groups. The obtained results are in agreement with the findings of a study done by Quenby in (8) where he found that there was an elevated expression in $CD4^+$, $CD14^+$, $CD16^+$, $CD56^+$and MHC class II^+ cellular surface markers in tissues obtained from placental women with recurrent miscarriage as compared to their controls . However, there are few reports about the role of CD14 during miscarriage cases. In this respect, Karhukorpi and his colleagues in 2002 have observed that CD14 was up regulated on monocytes during pregnancy(9) . The explanations for the higher rates of CD14 expression in the current results might be related to the presence of mild inflammation in miscarriage pregnancy which might result in activation of monocytes due to inflammatory response to low levels of fetal antigens derived from fetal tissues or placenta. This inflammation is mediated by innate immune mechanisms of which active monocyte (increased intracellular reactive oxygen species, and expression of surface CD14, CD11b, CD64 receptors) are one of them, and as observed and

stated by JE LIM (10) .Thus, this study have revealed an increase in the expression of CD14 which is among many possible mechanisms which might be associated with miscarriage. Two possibilities can explain the increment in CD 14 expression: first one is that abortion will lead to inflammation and this will increase CD14+ monocytes whereas the second is that due to the immunological process these CD 14 + monocytes will mediate the secretion of cytokines like TNF and others which are in turn would have role and then lead to the abortion (11). This may reflect the possibility of an elevated of CD 14 + monocytes among the miscarriage placental tissues in the current study.This finding did not rule out the possibility that endotoxin might also be an etiologic factor in spontaneous miscarriage. The possibility that endotoxin might be another etiologic factor in increase CD 14 + monocytes as a possible immune mechanism against bacterial infection as a causative agent for spontaneous miscarriage in humans (12). In this study we used IHC assay for surface marker antigen and we suggest that it would be of interest for further research to study the polymorphisms of genes coding for these molecules that are involved in endotoxin signaling. We need to highlight the environmental lipopolysaccharide triggers along with CD14 monocyte and other intrinsic mediators of lipopolysaccharide signaling to solve the enigmatic role of lipopolysaccharide in spontaneous miscarriage in humans. In the current study, the immunostaining of CD56 protein as a brownish discoloration was represented at cellular - surface localization. The IHC technique has showed that 60% of placental tissues in miscarriage group were positive for CD 56 protein , while only 5.0% of normal placental tissues of those healthy delivered women were positive for this marker and reach to the statistical significant level ($p<0.05$) among the studies groups . Compatible with our results , Theodora and their colleagues and by using IHC methods also found significantly increased CD56 cell expression in decidua parietalis of sections of miscarriage placental tissues ,which have showed higher significant percentage in both deciduas basalis and deciduas perietalis (13) .These differences may be due to the involvement of specific subsets of NK cells in human peripheral blood , deciduas and endometerium in cases of repeated implantation failure, or due to the use of CD56 antigen as the solely marker in detection(14). The current results are supporting many evidences which cited for the occurrence of functional changes in NK cells in patients with spontaneous abortion in humans which in turn might be linked to the states of hormonal dysregulation, impaired antigen education, or related microbial infection and inflammation . The present finding is in agreement with the Akhlaq and co-workers (2012) who revealed that decidual NK cells were present at term but that their numbers are maximal during the period of trophoblast invasion . The observations of the current study could indicate that the ability of NK cell-mediated immune regulation could possibly be lost in our series of cases (15). The reasons for these discrepancies are not entirely clear, one of the explanation of such increase numbers of CD56 cells could be related to the imbalance of TH1/TH2 immunity which might be lead to poor placentation as well as spontaneous abortion, since the NK cells were found to mediate changes in systemic type 1 and type 2 immunities, of the women with spontaneous abortions indicating a role for altered expression of dNK cell receptors in the development of miscarriage (16). Our results disagree with a study done by Srividya and Sesh (2014) in UK who showed that there was no difference in the percentage of uNK cells in women with RM compared with control group (17) . The present findings could be explained also in that natural killer cell activity in women with recurrent miscarriage can be mediated by high levels of stress as well as autoimmune disease The maternal-fetal interface is known to protect the fetus from destruction by the immune system of its mother (18). These findings support the hypothesis that endometrial NK cells are most important for the establishment of successful pregnancy while alterations in the endometrial NK cell population are associated with early pregnancy loss. Tang *et al*. (2011) who addressed their research on the role of NK cells in relation to pregnancy outcomes in women with spontaneous miscarriage and they concluded that abnormal uNK cells did not predict adverse pregnancy outcomes of miscarriage or implantation failure in women with spontaneous miscarriage (19) .

Conclusion:

Although significant correlation was observed between an increase rates of CD56+NK cells and CD14 with the state of spontaneous abortion studied in this research work ,we conclude that complexity of immune system as well as the use of only two variables , could not possible to predict in virtue the outcome in spontaneous miscarriage since NK cell and CD14 activity is probably only two measure among the overall immune system .Significant correlations of CD56+NK cells with the state of spontaneous abortion introducing these markers as a good immunological biomarkers for predicting possibility of abortions. The increased expression of CD14 marker in the miscarriage patients could indicate either an inflammatory influx of CD14 associated with results from abortion or an immunological process via CD 14 +

monocytes which mediate the secretion of cytokines like TNF and others that in turn would have role leading to these abortions.

Author's Contribution:

Dr. Zainab A. Hamid: substantial contribution to conception and design, acquisition of data, or analysis and interpretation of data.

Dr. Ali Hattem Bayati , Dr. Saad H. Mohammed Ali : drafing the article or revision it critically for important intellectual content .

Dr. Jabbar Resen Zangor: final approval of the version to be publication

References

1. *Oliver A, Overton C. (2014). Diagnosis and management of miscarriage. Practitioner. 258(1771):25-8, 3.*
2. *Khameneh ZR, Hanifian H, Barzegari R, Sepehrvand N.(2014) Human parvovirus B19 in Iranian pregnant women: a serologic survey. Indian J Pathol Microbiol. 57(3):442-4.*
3. *Jari Karhukorpi ,(2005). The search for links between immunogenetic factors and recurrent miscarriage Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium of Kastelli Research Center.*
4. *Barakonyi A, Kovacs KT, Miko E, Szereday L, Varga P, Szekeres-Bartho J.(2002) Recognition of nonclassical HLA class I antigens by gamma delta T cells during pregnancy. J Immunol. 168(6):2683-8.*
5. *Sharma S (2014)Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol. ;58(2-4):219-29.*
6. ***Stacy L.** Kelley, Tiit Lukk, Satish K. Nair, and Richard I. (2013). Tapping molecular and structural immunology ,The Crystal Structure of Human Soluble CD14 Reveals a Bent Solenoid with a Hydrophobic Amino-Terminal Pocket , J Immunol 190:1304-1311.*
7. *Turowski G, Rollag H, Roald B. (2014) Viral infection in placenta relevant cells - a morphological and immunohistochemical cell culture study. APMIS. Sep 22.*
8. *Quenby S. et al. (1999) . Preimplantation endometrial leukocytes in women with recurrent miscarriage. Hum Reprod.;14(9):2386–2391.*
9. *Karhukorpi J, Yan Y, Niemelä S, Valtonen J, Koistinen P, Joensuu T, Saikku P & Karttunen R (2002) Effect of CD14 promoter polymorphism and H. pylori infection and its clinical outcomes on circulating CD14. Clin Exp Immunol 128:326-332.*
10. *Je Lim, T Kim, NW Lee, MJ Oh, HJ Seol,JY Mini andHJ(2006). CD14 Receptor Gene Promer Polymorphism:Awakening Mternal Immunity during pregnancy. The Journal of International Medical Research;34: 176-182.*
11. *Kitchens RL. Chem.(2007). Role of CD14 in cellular recognition of bacterial lipopolysaccharides . Immunol.2000;74:61-82.*
12. *Jari Karhukorpi,(2003).The search for links between immunogenetic factors and recurrent miscarriage. American Journal of Reproductive Immunology Volume 50, Issue 4, pages 346–350.*
13. *Theodora Papamitsou , Alexandros Toskas, Kyriaki Papadopoulou,Antonia Sioga , Sotirios Lakis , Maria Chatzistamatiou , Zinon Economou and Luisa Adriopoulou1(2014) . Immunohistochemical study of immunological markers: HLAG, CD16, CD25, CD56 and CD68 in placenta tissues in recurrent pregnancy loss Histol Histopathol 29: 1047-1055.*
14. *Hedele Pitman .(2012). Investigation of the role of intrauterine interleukin 6 and interleukin 8 in normal early pregnancy and sporadic miscarriage. Thesis submitted for the Degree of Doctor of Philosophy, Newcastle University.*
15. *Cerdeiera AS, Rajakuar A, Royle CM, LO A, Huain Z, Thadhani RI, Sukhame VP, Karumanchi SA, and Kopcow HD (2013). Conversion of peripheral blood NK cells to a decidual NK –like phenotype by a cocktail of defined factors. J Immunol 190: 3939-3948.*
16. *Koopman LA, Kopcow HD, Rybalov B , Boyson JE , Orange JS, Schatz F, Masch , Lockwood CJ , Schachter AD , Park PJ, Strominger JL 2003 Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. J Exp Med 198 :1201–1212*
17. *Srividya Seshadri and Sesh Kamal Sunkara . (2014). Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis Human Reproduction Update, Vol.20, No.3 pp. 429–438.*
18. *Loustau, M. Wiendl, H. Ferrone, S. and Carosella, E. D. (2013). HLA-G 2012 conference: the 15-year milestone update. Tissue Antigens, Vol. 81, (3), pp. 127-136.*
19. *Tang AW, Alfirevic Z, Quenby S. (2011) Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. Hum Reprod; 26:1971– 1980.*