Nat. Volatiles & Essent. Oils, 2021; 8(6): 2129-2142

NVEO
Natural Volatiles & Essential Oils

The Role Of Meconium-Stained Liquor In Histopathological And Immunohistochemical Changes In Term Maternal Placenta

Hanan Fawzi Salman , Sahar A. H. AL-Sharqi* and Alia Essam Mahmood Alubadi

Department of biology, College of Science, Mustansiriyah University, Baghdad/ Iraq

Abstract

The current research focuses on certain histopathological of the placenta related with Meconium stained liquor (MSL) and immunohistochemical technical in the study groups. The samples were taken (maternal placental tissue) from 30 women with MSL and 30 control women with clear liquor in labour. The placenta samples were collected and preserved in formalin (10%) for histological study and ethanol (70%) for the immunohistochemical study were obtained from AL-Liqa Hospital for Maternity and Al - Yarmouk Teaching Hospital. Samples were collected from January to June 2021. The mean age of cases with MSL were 26.1±6.44 years compared with the mean age of clear liquor stain as control group 21.8±4.88 year. Histopathological changes in the placenta showed highly significant differences between the two study groups (Intervillous space, intravillous fibrinoid, perivillous fibrinoid, calcification, fibrosis, syncytial knots, blood vessel changes). Immunohistochemical All result is significant at $p < 0.01$.

Key Words: Meconium stained liquor, CD 16, Immunohistochemical, maternal placenta

Introduction

The placenta is a short-term organ that is responsible for the successful completion of pregnancy (Burton and Fowden, 2015). It's made up of fetal membranes and maternal tissues that have fused (Kay et al., 2011). The placenta is composed of two parts: the fetus, which forms the same blastocyst that forms the fetus, and the other part is the maternal part that arises from the tissues of the mother's uterus, which is shed after the birth of the fetus (Roberts et al., 2016). Meconium-stained amniotic fluid (MSAF) is a condition that happens once the fetal muscles of the sphincter anal relax within the womb, making the amniotic fluid (AF) seems green, yellowish, or brownish. It's quite rare if the fetus is premature(less than 37weeks).The MSAF is considered uncommon before 37 weeks of pregnancy, 7 to 22 percent of term pregnancies (from 37 till 42 weeks gestation) are complicated globally by MSAF, in which minimal groups of babies suffer from respiratory distress needing adequate oxygen (Mundhra and Agarwal, 2013; Madhuri, 2013). The existence of meconium in the amniotic fluid increases the potential for neonatal morbidity and mortality (Sundaram and Murugesan,2017).

The meconium stained liquor (MSL) is either thick or thin, and compared to the clear liquid meconium thick is associated with higher morbidity and mortality (Biradar et al.,2018; Shrestha et al .,2018). Meconium is thought to cause placental and umbilical cord vasoconstriction along with cerebral and fetal hypoperfusion. These may be the causes for a major poor outcome in MSL (Hiersch et al ., 2016; Aplin et al.,2020). It is not clear that whether MSL is associated with demonstrable placental pathology which may give rise to fetal distress, either directly or indirectly, or whether placental pathology gives rise to meconium staining and in turn causes fetal distress. It is not discernible which is the primary event among these variables.

There are many questions regarding the cause, effect, clinical significance and pathologic expression of in-utero meconium passage which are, as yet, unresolved. In the majority of infants, meconium passage is a reflection of physiological immaturity, although in some, it appears to be associated with adverse stimuli, out of which fetal distress is a significant factor. There are many studies which describe meconium induced fetal distress. A few studies have described placental changes in MSL. However, there lack of studies describing histopathological and immunohistochemical changes in term maternal placenta with MSL.

This study has been undertaken to determine the association between MSL specific placental pathology and immunhistochemical in such cases. For this purpose, macroscopic and microscopic changes of placentae associated with MSL were compared with those in clear liquor in otherwise uncomplicated term pregnancy.

Materials and Methods

The study included (30) women with MSL with ages ranging from (14 - 39 years), and gestational age (37- 43 weeks). In addition to (30) women with clear liquor as a control group, who were obtained from AL-Liqa Hospital for Maternity and Al - Yarmouk Teaching Hospital, samples were collected from January to June 2021.

The tissue specimens (maternal side of placenta) were obtained after normal vaginal deliveries or caesarian sections from (30) cases with MSL as a case group and (30) clear liquor stain as a control group. The placenta samples were collected and preserved in formalin (10%) for histopathological study. Haematoxylin & Eosin stain, Ali zarien stain and masson trichrom stain were used according to the method of Suvaran et al (2013). The specimen was examined macroscopically according to the ( ) , for the following parameters ( ):

1- Intervillous space (IVS): It is graded as 0 (narrow IVS), 1(normal) and 2 (widened IVS).

2- Intravillous fibrinoid and perivillous fibrinoid: In this study, both are graded as 1 (normal), 2 (mild increase) and 3 (marked increase).

3- Villitis : It is graded as 0 (absent) and 1(present).

4- Syncytial knots (SK): It is increased at term as well as in hypoxic conditions. It is graded as 1 (up to 5 SK/villous in the majority of the villi), 2 (5- 10 SK/villous in majority of the villi) and 3 (>10 SK/ villous in the majority of the villi).

5- Calcification: Focal calcification is considered normal at term, but excessive calcification may have underlying pathology. In this study it is graded as 0 (no or focal calcification) and 1 (marked calcification).

6-Villous Fibrosis: Villous stromal fibrosis may occur in hypoxic conditions. It is graded as 0 (absent) and 1 (present).

7- Blood vessel changes: There may be thrombosis/ avascular villi/ atrophy of villi/ fetal thrombotic vasculopathy. In the present study, blood vessel changes are graded as follows: 0 (no change); 1 (mild changes (focal); 2 (marked changes (global).

Immunohistochemical staining was carried out using after formalin had been fixed. Paraffin-embedded tissue blocks were cut at a 5 µm thick section. All sections deparaffinized in xylene, then decreasing grades of ethanol and incubated with phosphate-buffered saline. Antigen retrieval as required by the primary antibody. The steps of staining protocol with monoclonal antibodies toward CD16 from the commercially available kit (Abcam, Pathn Situ). The intensity of positive staining with the anti-CD16 of natural killer cell was graded as: (0) Negative of staining, (+) weak positive staining, (++) Moderate positive staining ,(+++) strong positive staining.

Statistical analysis

At the end of the study, all the data was compiled and tabulated. Analysis was done by using suitable statistical methods (Pearson Chi-square test) and appropriate software (SPSS v.20). Also Used fisher test for immunohistochemistry. Statistical calculations were carried out using MINI tab -13.

Results and Discussion

Examination of histopathological in term maternal placenta

Meconium is a germ-free, thick, black-green, odorless material which is first recognized in the fetal intestine around 12 weeks of gestation and stores in the fetal colon throughout gestation . Meconium is found in the small bowels of fetuses before they reach the third trimester, but it is not usually removed until after childbirth. The risk factors for(MSL) are both maternal and fetal. The maternal factors are gestational diabetes mellitus, hypertension, maternal chronic respiratory or cardiovascular diseases, post term pregnancy, eclampsia, preeclampsiaand. The fetal factors include oligohydramnios, intrauterine growth restriction, and poor biophysical profile. Meconium staining is an indicator of increased perinatal morbidity, though many cases may have normal outcome.

The mean age of the studied groups which found significant differences between the MSL group and control group were 26.1±6.44 and 21.8±4.88 years respectively. There is significant difference in the mean age between case and control population (by 2 tailed significance test the value is 0.13238 **)**. Our results showed that the mean age of women in the case group was higher than the mean age of women in the control group, and this result is consistent with the results of several studies (Naveen et al. 2006 ; Sharma et al ,. 2015 ; Addisu et al. 2018) . This result justified according to Addisu and his colleagues in 2018 explanation about the woman age when gets older could accrue a gradual loss of elasticity of the cardiovascular vessels that lead to stiffness of uterine blood vessels and arterial which may cause insufficient placental perfusion and in utero fetal hypoxia and finally leads to passage of meconium into the amniotic fluid.

The weight of the placenta in this study did not show a significant difference between the control group 534.15±176 g and the MSL group 557.83±180.75 g. Statistical analysis of this factor did not show a significant difference between the two study groups, as in the (Table -1). Our result does not match the results of the studies (Mondal et al.,2019; Tamayev et al.2021), where they found that the lowest mean placenta weight was for the MASF group and significantly lower in the continuous meconium exposure group as 160 compared with the acute meconium exposure group.

Where the average placenta diameter for the case group was 19±1.7 cm, while the average placental diameter for the control group was 19.7±1.8 cm as the (Table -1). The results of our study do not match the result of a study carried out by Mondal et al. (2019) where the researcher found that there are significant differences between the average diameters of the placenta.

The results of the microscopic examination of the tissue sections of the placenta on the mother's side with clear meconium liquor stain (control group) showing normal intervillous space(Figure -1A-B).while the histological sections of the placenta from the mothers side in the case of meconium showed the presence of histopathalogical changes and the presence of highly significant differences ($P<0.01$) of intervillous space. between the two study groups, where the highest percentage was for the case group (46.67 %). fibrin deposits were due to poor circulation in the intervillous space (Figure -1 C-D) ,statistically highly significant ($P<0.05$) of Intravillous fibrinoid between the two study groups, where the highest percentage was for the case group (40 %), (Figure -1 E-F), statistically highly significant ($p < 0.001$) of Perivillous fibrinoid between the two study groups, where the highest percentage was for the case group (60 %) (Figure -2 A-B) ,

statistically highly significant (p < 0.001) of Syncytial knots between the two study groups, where the highest percentage was for the case group (46.66 %) (Figure -2 C-D) , significant differences on the level of probability (p value 0.001) of villitis between the two study groups, where the highest percentage was for the case group (73.33 %). villitis are defined as an infiltration of large number of lymphocytes, neutrophils and macrophages in villous tissue. The villitis in our study is more present in the case group ,where inflammatory cells were observed in abundance with an oval shape , as in the (Figure -2 E-F) , significant differences on the level of probability (p value 0. 01) of Calcification between the two study groups, where the highest percentage was for the case group (60 %). Intra villous calcification in this study is related to the case group , and this is evident in the following (Figure-3 A-B) , significant differences on the level of probability (p value 0. 01) of Villous fibrosis between the two study groups, where the highest percentage was for the case group (63.33%). Villous fibrosis appears clearly as in the (Figure -3 C-D) ,significant differences on the level of probability (p value 0. 02) of Blood vessel change between the two study groups, where the highest percentage was for the case group(53.33 %). As in the (Figure -3 E-F).

Salient microscopical findings in this study as observed from different tables and statistical analysis were increase in inter-villous space ((46.67 %), prominence of intra- ((40 %),) and peri-villous fibrinoid (60 %) , exaggerated syncytial knots ((46.66 %) (Table -2) , obvious blood vessel changes (53.33 %), calcification (intra- and peri-villous as well as within vessel wall) (60 %), villitis (73.33 %) (Table -3) .

The results of the current study of placental tissues on the mother's side with meconium staining of liquor showed significant differences between the two study groups, and the presence of changes in intervillous space, intravenous fibrinoid, perivillous fibrinoid, syncytial knots The number of syncytial knots increases with increasing maternal ischemia , with high blood pressure and over weight of mother (Kurman et al., 2011 ; Ali and AL-Alalfe , 2021).

Villitis are defined as an infiltration of large number of lymphocytes, neutrophils and macrophages in villous tissue. The villitis in our study is more present in the case group ,where inflammatory cells were observed in abundance with an oval shape , as in the (Figure-6) ,and this result does not match the result of (Mondal et al.,2019; Kariniemi et al.,1990). Calcification Intra villous calcification in this study is related to the case group , the cause of calcification can be explained as end result of villous atrophy and fibrosis ,which leads to maternal and fetal hypoperfusion.

Our result is in agreement with the result of conducted by Mondal et al.,(2019) to study histopathological changes of the placenta with meconium stained liquor where they found changes in the structure of the villous and an increase in intervillous space, intravillous fibrinoid, perivillous fibrinoid, calcification, fibrosis, syncytial knots, blood vessel changes .

The results of the current study showed the presence of features of placenta inflammation in the case of MSL, which did not appear in the control group, and our result is in agreement with) Mohammed, 2019; Pakniat ,2016 ; and Kim et al. 2017) , where they found that inflammation within the amniotic fluid in the case of MSAF occurs more frequently than it is in the amniotic fluid clear , in addition to that they suggested that Meconium has a role in stimulating local inflammation with tissue degeneration in the membranes of the fetus. Jaiman et al .,(2020) explained Lesions demonstrating histologic patterns suggestive of hypoxia included nucleated red blood cells, hypercapillarized villi, intravillous hemorrhage, massive perivillous fibrinoid deposition, and laminar necrosis of the decidua capsularis. The frequency of these lesions was seven times higher in the cases than the normal pregnancy controls.

Ali and Al- Allaf, (2021) They made a histological study of the part of the placenta on the mother's side and on the fetus's side for women who suffer from overweight, where they noticed different histological changes in two groups in comparison with control group ,These changes include syncytial knotting ,villous hypovascularity, villous fibrinoid necrosis, thickening of trophoblastic basement membrane, cytotrophoblastic hyperplasia, perivillous fibrin deposition ,increase in number of nucleated red blood cells (NRBC), stromal fibrosis, chorangiosis ,paucity of vasculosyncytial membrane (VSM), villous edema, features of deciduitis, villitis, and increase the thickening of tunica media of the placental blood vessels, Also Brouwers et al. (2019) they found specific placental pathology (ie, chronic villitis and difference in villous maturation) was associated with a higher body mass index (BMI) .

Many of studies also suggests, that vascular changes in villous structures are possibly present due to placental insufficiency, causal factor for fetal distress and meconium passage, either due to maternal or due to fetal hypoperfusion, and not the effect of long standing meconium exposure. (Redline et al., 2000 ; Sienko and Altshuler ,1999 ; Altshuler et al., 1992).

Table -1: The differences in the maternal age , placental weight and diameter among the MSL and control group.

Parameters	Groups	Mean ±S.D
Maternal age (Year)	MSL	26.1 ±6.66
	Control	21.8 ±4.88
P- value	0.13 significant at P<0 .05	
Placental weight (g)	MSL	557.83±180.7
	Control	534.15±176.11
P- value	0. 64 NS at P< 0.05	
Placental diameter (cm)	MSL	19±1.7
	Control	19.7±1.8
P- value	0.17 NS at p< 0.5	
MSL: meconium stained liquor SD: Stander division, NS : not significant		

Table -2 : Histological grading of maternal placental tissues (intervilllous space, intervilllous fibrinoid, perivillous fibrinoid, and syncytial knots) of MSL and control group.

Histological finding / Grade	Control group NO. =30 (%)	MSL group NO. =30 (%)	P-Value
1. Intervillous space			
Narrow (0)	0 (0 %)	4 (13.33 %)	
Normal (1)	29 (96.67 %)	14 (46.67 %)	**0.01***
Widened (2)	1 (3.33 %)	12 (40 %)	
Total (%)	30 (100 %)	30 (100 %)	
2. Intravillous fibrinoid			
Normal (1)	30 (100 %)	11 (36.67 %)	**0.05***

Mild increased (2)	0 (0 %)	12 (40 %)	
Marked increased (3)	0 (0 %)	7 (23.33 %)	
Total (%)	30 (100 %)	30 (100 %)	
3. Perivillous fibrinoid			
Normal (1)	30 (100 %)	4 (13.33 %)	
Mild increased (2)	0 (0 %)	18 (60 %)	**0.001***
Marked increased (3)	0 (0 %)	8 (26.67 %)	
Total (%)	30 (100 %)	30 (100 %)	
4. Syncytial knots			
Up to 5/ villous (1)	28 (93.33 %)	14 (46.66 %)	
5-10/ villous (2)	2 (6.67 %)	11 (36.67 %)	**0.001***
> 10/ villous (3)	0 (0 %)	5 (16.67 %)	
Total (%)	30 (100 %)	30 (100 %)	
*Statistically significant (Pearson's Chi- square test). MSL: meconium stained liquor			

Table -3: Histopathological grading of maternal placental tissues (Villitis, Calcification, Villous fibrosis, and Blood vessel change) of MSL and control group.

Histopathological finding	Control group N=30 (%)	MSL group N=30 (%)	P-Value
1.Villitis			
Absent (0)	30 (100 %)	8 (26.67 %)	**0.001***
Present (1)	0 (0 %)	22 (73.33 %)	
Total (%)	30 (100%)	30 (100%)	
2. Calcification			
No/ focal (0)	30 (100 %)	18 (60 %)	**0.01***
Marked increased (1)	0 (0 %)	12 (40 %)	
Total (%)	30 (100%)	30 (100%)	
3. Villous fibrosis			
Absent (0)	30 (100 %)	11 (36.67 %)	**0.01***
Present (1)	0 (0 %)	19 (63.33 %)	
Total (%)	30 (100%)	30 (100%)	
4. Blood vessel change			
No	29 (96.67 %)	1 (3.33 %)	
Mild	1 (3.33 %)	16 (53.33 %)	**0.02***
Marked	0 (0 %)	13 (43.34 %)	
Total (%)	30 (100 %)	30 (100 %)	
*Statistically significant (Pearson's Chi- square test). MSL: meconium stained liquor			

Mild changes in blood vessels were focal narrowing of lumen, while marked changes as observed in this study were obliteration of lumen in wide areas. Villous fibrosis and blood vessel changes are statistically significant applying.

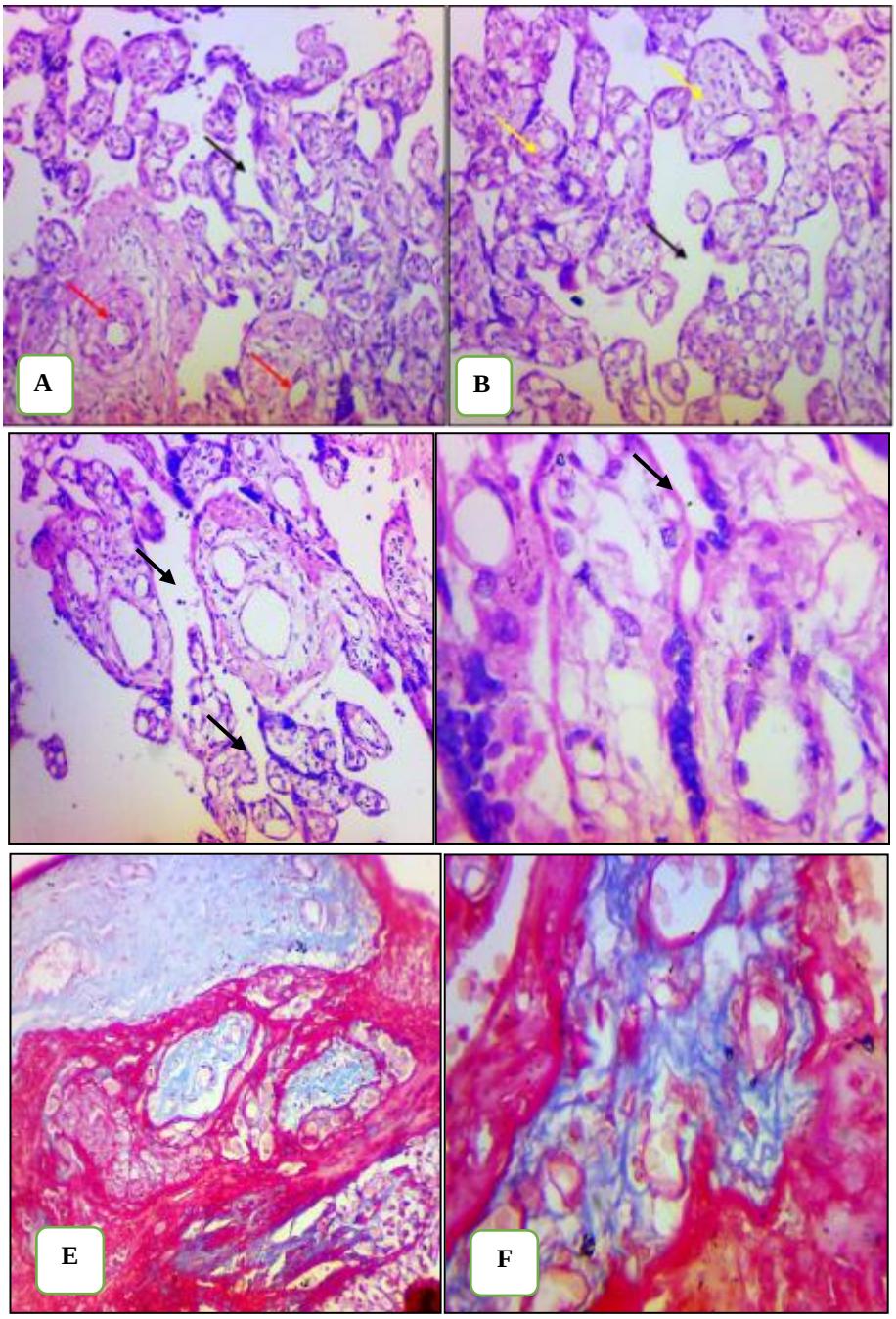

Figure -1: Cross section in maternal placenta tissue with clear MSL (control group) showing normal intervillous space (black arrows), villi (yellow arrows), and blood vessels (red arrows) (H&E stain, A& B: X10,Cross section in maternal placenta tissue with MSL showing intervillous space (arrows) (H & E stain, [C] X10, [D] X40), Cross section in maternal placenta tissue with MSL showing perivillous fibrinoid (red color) (Masson Trichrome stain, [E] X10, [F] X40).

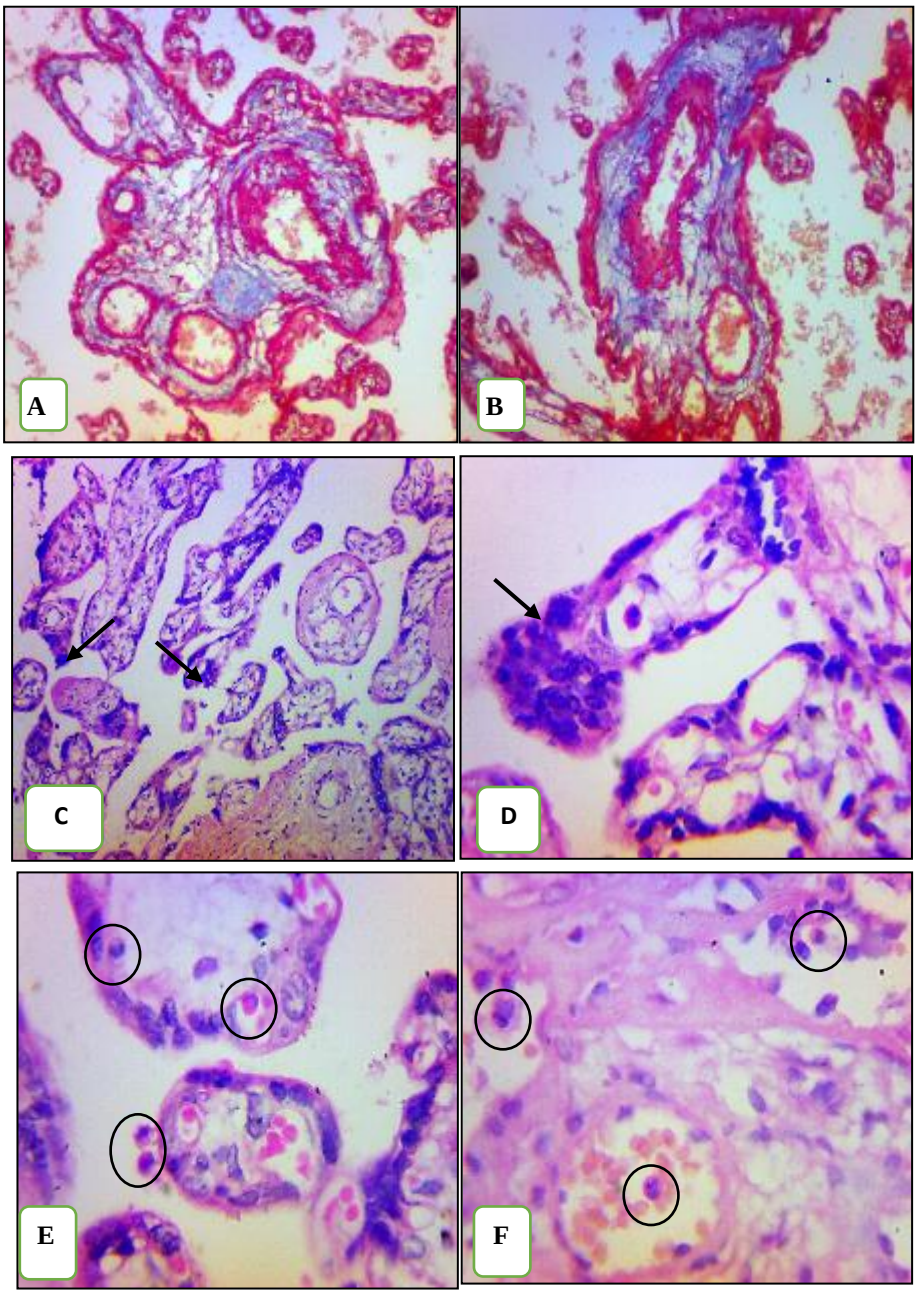

Figure -2 : Cross section in maternal placenta tissue with meconium liquor stain showing intervillous and perivillous fibrinoid (red color) (Masson Trichrome stain, A& B, Cross section in maternal placenta tissue with MSL showing syncytial knots (arrows) (H & E stain, [C] X10, [D] X40). Cross section in maternal placenta tissue with meconium liquor stain showing inflammatory cells infiltration (Villitis) (oval shapes) (H & E stain, E & F: X40).

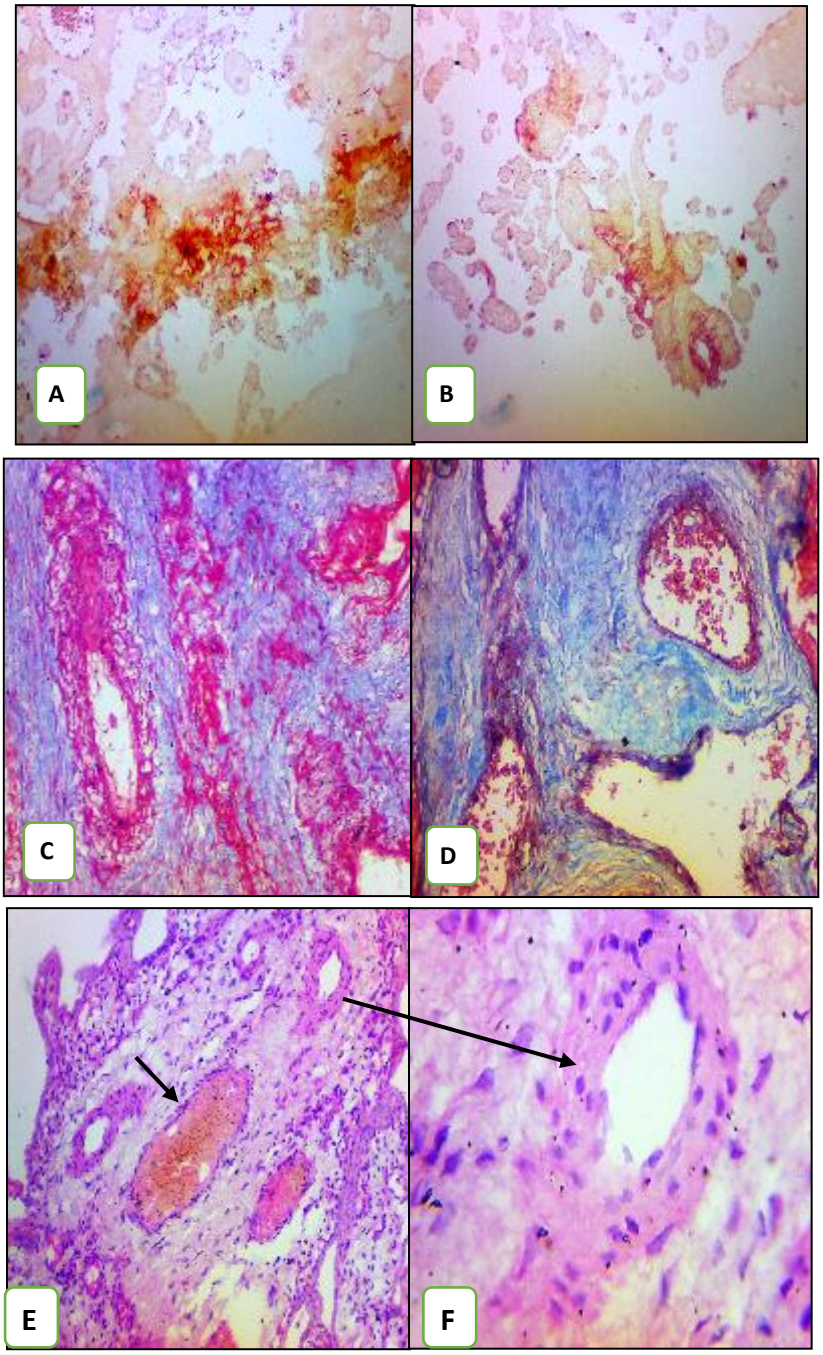

Figure -3: Cross section in maternal placenta tissue with MSL showing intra-villous calcification (orange-red color) Alizarin red S stain, A & B: X4). Cross section in maternal placenta tissue with meconium liquor stain showing villous fibrosis (blue color) (Masson Trichrome stain, C& D: X10).Cross section in maternal placenta tissue with MSL showing blood vessel changes in villous (arrows) (H & E stain, [E] X10, [F] X40).

Immunohistochemical study

CD16 (Natural Killer cells) in maternal placenta tissue

The results obtained by studying the immunohistochemical method is used to determine a cluster of differentiation molecule found on the surface of natural killer cells (CD16 Natural cell) in placenta tissues stained with meconium liquor and clear placenta tissue. The results of the microscopic examination showed

that the number of natural killer cells was) 0 (negative in the total control of part of the placenta on the mothers' side, as shown in the following (Figure -4).

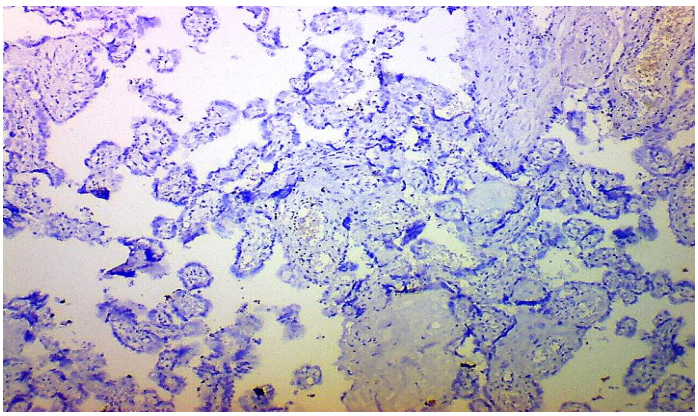

Figure -4: Immunohistochemical staining method detection of CD16 NK cells in maternal placenta tissue with clear MSL showing (CD16 Negative control of maternal placenta tissue)

The intensity of (CD16) NK cell expression was negative in 2 biopsies (6.66%) which had scored zero . While, 12 biopsies (40 %) showed weak positive that scored +1. Eight biopsies (26.67 %) showed moderate positive expression that scored +2, and 8 biopsies (26.67 %) showed strong positive expression that scored +3 (Table -4 , Figure- 5).

Table -4 : Intensity of CD16 NK cells expression in fetal placenta tissue with meconium liquor stain

Grade / (Intensity of CD16 NK cells)	**Control group (No.)**	**Control group (%)**	**Cases of MSL (No.)**	**Cases of MSL (%)**	**The fisher exact test statistic value**
Negative (-)	30	100	2	6.66	<0.00001
Weak (+)	-	-	12	40	0.0001
Moderate (++)	-	-	8	26.67	0.0046
Strong (+++)	-	-	8	26.67	0.0046
Total	30	100	30	100	All result is significant at $p < 0.01$
MSL: meconium stained liquor					

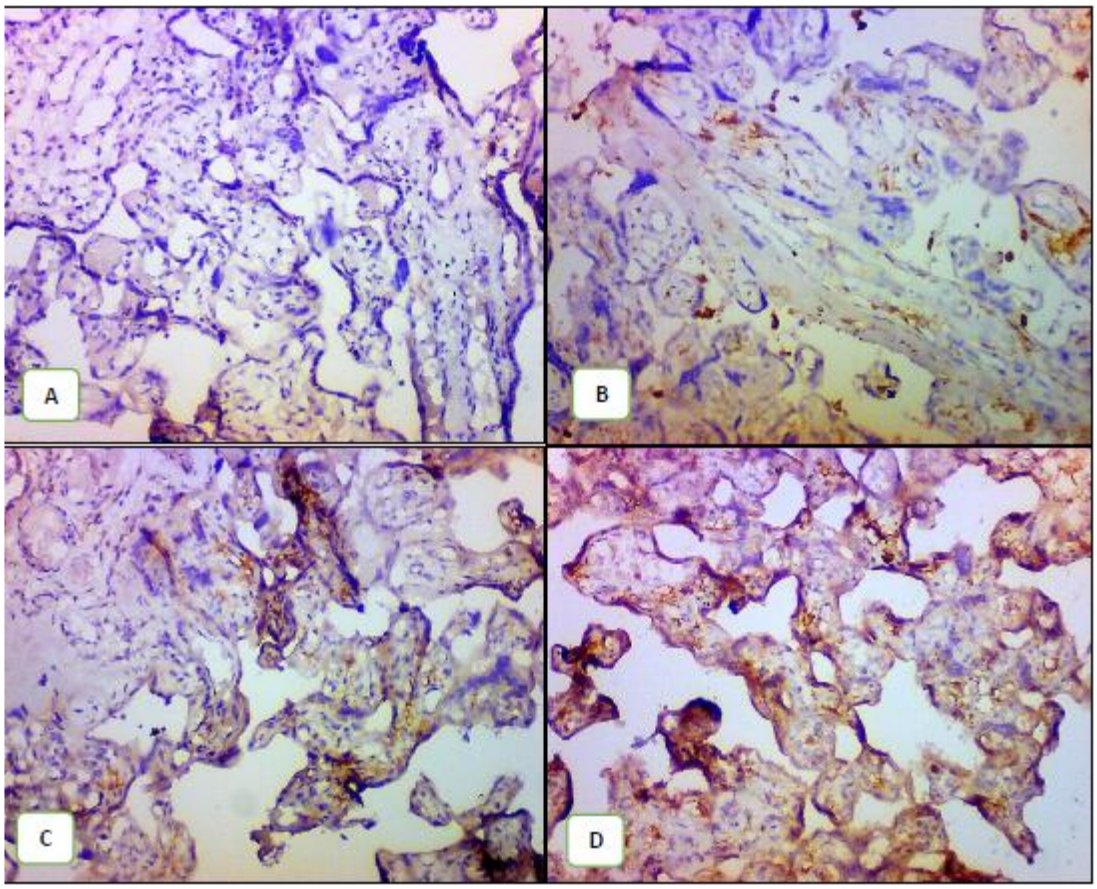

Figure -5: Immunohistochemical staining method detection of CD16 NK cells in maternal placenta tissue with meconium liquor stain showing: **(A)** Negative (0) expression **(B)** Weak (+1) positive cytoplasmic expression **(C)** Moderate (+2) positive cytoplasmic expression **(D)** Strong (+3) positive cytoplasmic expression. (X10).

. In the present study, we characterized the NK cell showing their differences as a function of meconium status.

NK cells normally found 5–15% of peripheral blood lymphocytes, and uterus, and lung, as well as to a lesser extent in the thymus, secondary lymphoid tissues and mucosal-associated lymphoid tissues (Yu et al. 2013).

Placental cells are derived from both maternal and fetal which express molecules that play a fundamental role in maternal-fetal tolerance (Guleria and Sayegh,2007). The present study showed an increase in the subsets of cells expressing the CD16+ marker in delivered mothers who have stained amniotic fluid with meconium, and an interesting result was that cells expressing CD16+ were more frequent in maternal placenta tissue with meconium liquor stains .

In the present study, the increase in CD16 suggest an intensification of the innate immune response in the meconium state, NK cells are one of the innate immune system soldiers, and the presence of meconium may stimulate the production of cytokines that stimulate NK cells.

Cox et al., (2015) indicate that umbilical cord blood plasma contains soluble NKG2D ligands that alter the NK function and act as a mechanism of fetal-maternal tolerance in human pregnancy and this explains the lack of NK cells in the placenta of the control group.

We did not find previous studies similar to the idea of this study, but there were many studies on natural killer cells in pregnant women who suffer from various diseases. For example, Papamitsou et al ., (2014) they

observed increased expression of CD16cell and CD68 cells in the placentas of women with recurrent miscarriages, while Senthilnayagam et al .(2016) found an increase in CD56+ NK cells in the placentas of women undergoing early miscarriage, finally, (Mohammed , 2019) found an increase in CD68 macrophage cells and CD117 mast cells in the placenta of women with meconium pigmentation

Acknowledgements

We gratitude to the Department of Biology, College of Science, Mustansiriyah University (http:// uomustansiriyah.edu.iq/), Baghdad for advice and support.

References

Burton, G. J. and Fowden, A. L. (2015). The placenta: a multifaceted, transient organ. Philosophical Transactions of the Royal Society B: Biological Sciences, 370(1663): 1-8.

Kay, H., Nelson, M. and Wang, Y. (2011). The Placenta: From Development to Disease , 1st Ed. Blackwell Publishing Ltd, 1-6.

Roberts, R. M., Green, J. A., & Schulz, L. C. (2016). The evolution of the placenta. Reproduction (Cambridge, England), 152(5): 179- 189.

Madhuri, S. (2013). Maternal risk factors and the perinatal outcome in meconium stained amniotic fluid (Doctoral dissertation). Department of Obstetrics and Gynecology, Vydehi Institute of Medical Sciences and Research Centre.

Mundhra, R. and Agarwal, M. (2013). Fetal outcome in meconium stained deliveries. Journal of clinical and diagnostic research. Journal of Clinical and diagnostic Research for doctors, 7(12): 2874- 2876.

Sundaram, R. and Murugesan, A. (2017). Risk factors for meconium stained amniotic fluid and its implications. International Journal of Reproduction, Contraception, Obstetrics and Gynecology , 5(8): 2503-2506.

Begum N, Mahmood S, Munmun SA, Haque M, Nahar K, Chowdhury S. **(2015).**Perinatal outcome associated with meconium stained amniotic fluid in pregnancy. Journal of Paediatric Surgeons of Bangladesh.;4(2):44–9 .

Qadir S, Jan S, Chachoo JA, Parveen S. Perinatal and neonatal outcome in meconium stained amniotic fluid. Int J Reprod Contracept Obstet Gynecol. 2017;5(5):1400–5.

Shrestha A , Singh SD , Tamrakar D . Associated Factors and Outcome of Babies Born Through Meconium Stained Amniotic Fluid. Kathmandu Univ Med J (KUMJ). 2018 Jan.-Mar;16(61):65-68. PMID: 30631020.

Hiersch, L., Krispin, E., Aviram, A., Wiznitzer, A., Yogev, Y., & Ashwal, E. (2016). Effect of meconium-stained amniotic fluid on perinatal complications in low-risk pregnancies at term. American journal of perinatology, 33(04), 378-384.

Aplin, J. D., Myers, J. E., Timms, K., & Westwood, M. (2020). Tracking placental development in health and disease. Nature Reviews Endocrinology, 16(9), 479-494.

Naveen, S., Kumar, S. V., Ritu, S., & Kushia, P. (2006). Predictors of meconium stained amniotic fluid: a possible strategy to reduce neonatal morbidity and mortality. J Obstet Gynecol India, 56(6), 514-7.

Sharma, U., Garg, S., Tiwari, K., Hans, P.S. and Kumar, B. (2015) 'Perinatal outcome in meconium stained amniotic fluid', Journal of Evolution of Medical and Dental Sciences, 4(48), 8319+.

Addisu, D., Asres, A., Gedefaw, G., & Asmer, S. (2018). Prevalence of meconium stained amniotic fluid and its associated factors among women who gave birth at term in Felege Hiwot comprehensive specialized referral hospital, North West Ethiopia: a facility based cross-sectional study. BMC pregnancy and childbirth, 18(1), 1-7.

Mondal ,T.R. , Bandyopadhyay ,G. , Mukhopadhyay, S. G. and ganguly, D. (2018) . Histopathological Changes of Placenta in Meconium Stained Liquor and Its Relevance in Fetal Distress: A Case Control Study . Turkish Journal of Pathology, 1(1): 1-12.

Tamayev, L., Mor, L., Herman, H.G., Schreiber, L., Kovo, M., Bar, J. and Weiner, E., 2021. Placental histology of acute versus continuous meconium exposure-Association with obstetric and neonatal outcomes. Placenta, 103, pp.214-219.

Al-Ali, H., 2021. Evaluation of the histology and the oxidative stress status in the placentas of obese mothers. Annals of the College of Medicine,Mosul, 43(1),pp.60-69.
Kurman, R. J., Ellenson, L. H., & Ronnett, B. M. (Eds.). (2011). Blaustein's pathology of the female genital tract (Vol. 1246). New York: Springer.

Kariniemi V, Harrela M. Significance of meconium staining of the amniotic fluid. J Perinat Med. 1990; 18:345-9.

Mohammed S,H . (**2019**).Histopathological changes in placenta and umbilical cord associated with meconium liquor stain in women.theMaster of Science. Mustansiriyah University . College of Science. Biology/Zoology.,40-45.

Pakniat, H., Mohammadi, F. and Ranjkesh, F. (2016). Meconium Amniotic Fluid is Associated with Endomyometritis. The Journal of Obstetrics and Gynecology of India, 66(1): 136-140.

Kim, B., Oh, S. Y. and Kim, J. S. (2017). Placental lesions in meconium aspiration syndrome. Journal of pathology and translational medicine, 51(5): 488- 498.

Jaiman, S., Romero, R., Pacora, P., Jung, E., Bhatti, G., Yeo, L., Kim, Y., Kim, B., Kim, C., Kim, J., Qureshi, F., Jacques, S., Erez, O., Gomez-Lopez, N. and Hsu, C. (2020). Disorders of placental villous maturation in fetal death. Journal of Perinatal Medicine, Vol. 48 (Issue 4), pp. 345-368.

Brouwers, L., Franx, A., Vogelvang, T.E., Houben, M.L., van Rijn, B.B. and Nikkels, P.G., 2019. Association of maternal prepregnancy body mass index with placental histopathological characteristics in uncomplicated term pregnancies. Pediatric and Developmental Pathology, 22(1), pp.45-52.

Redline RW, O' Riordan MA. (2000). Placental lesions associated with cerebral palsy and neurologic impairment following term birth. Arch Pathol Lab Med.; 124:1785-91.

Sienko A, Altshuler G. Meconium-induced umbilical vascular necrosis in abortuses and fetuses: A histopathologic study for cytokines. Obstet Gynecol. 1999; 94:415-20.

Altshuler G, Arizawa M, Molnar-Nadasdy G. Meconium-induced umbilical cord vascular necrosis and ulceration: A potential link between the placenta and poor pregnancy outcome. Obstet Gynecol. 1992;79:760-6.

Yu, J., Freud, A. G., & Caligiuri, M. A. (2013). Location and cellular stages of natural killer cell development. Trends in immunology, 34(12), 573–582.

Guleria and M. H. Sayegh, "Maternal acceptance of the fetus: true human tolerance," Journal of Immunology, vol. 178, no. 6, pp. 3345–3351, 2007.

Cox, S. T., Laza-Briviesca, R., Pearson, H., Soria, B., Gibson, D., Gomez, S., ... & Saudemont, A. (2015). Umbilical cord blood plasma contains soluble NKG2D ligands that mediate loss of natural killer cell function and cytotoxicity. European journal of immunology, 45(8), 2324-2334.

Papamitsou T, Toskas A, Papadopoulou K, Sioga A, Lakis S, Chatzistamatiou M, Economou Z, Adriopoulou L. Immunohistochemical study of immunological markers: HLAG, CD16, CD25, CD56 and CD68 in placenta tissues in recurrent pregnancy loss. Histol Histopathol. 2014 Aug;29(8):1047-55.

Balamurugan Senthilnayagam , Sridhar Karthikeyan , Jayapriya Sukumaran , ANOOP SRIVALSAN4 , Ramesh Rao , Vasantha Subbiah . Decidual CD56+ Natural Killer Cells in Spontaneous Early Pregnancy Loss- An Immunohistochemical Study . Journal of Clinical and Diagnostic Research. 2016 Oct, Vol-10(10): EC27-EC29.

Suvarna, S. K. and Layton, C. and Bancroft J. D. (2013). Bancroft's Theory and Practice of Histological Techniques.7th Ed. Elsevier. 173-186