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EXPRESSION OF IMMUNOHISTOCHEMISTRY MARKERS (BCL 2 AND CD 34) IN THE PLACENTA OF IRON DEFICIENCY ANAEMIA.

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ABSTRACT
Background: The most common haematological disease that occurs during pregnancy is anaemia. Apoptosis, or programmed cell death, is crucial for the placenta's formation and for the regulation of its ageing throughout pregnancy. Bcl2 an antiapoptotic protein that is essential to cell survival and proliferation. Anaemic women's placentas may have different degrees of its expression, which might affect the viability and functionality of the cells. A marker for endothelial cells, which line blood arteries, is CD34. Examining the CD34 expression in the placenta may show changes in blood flow and oxygen delivery in the setting of anaemia, as well as insights into the formation and function of placental blood vessels. **Methods:** A prospective observational study was conducted in the Department of Obstetrics and Gynaecology, Subharti Medical College, Meerut, U.P. from 2022-2024 which included 100 study participants (50 tests and 50 controls). **Results:** Among the non-anaemic group mean age (58% women) were % women were of less than 20 years age, 78% women were between 21-30 years age group expression of IHC markers CD 34 was diffuse strong CD 34 positivity was seen 100% of very severe anaemia and 60% of severe anaemia and in 51 % of moderate anaemic women. Results indicate a higher incidence of diffuse strong BCL2 positivity was seen 100% of very severe anaemia and 60% of severe anaemia and in 51 % of moderate anaemic women **Conclusions:** Bcl-2 and CD34 could serve as valuable IHC markers for early detection of placental dysfunction associated with IDA. This could pave the way for timely interventions to improve pregnancy outcomes in women with anaemia.

KEYWORDS : placenta, anaemia, histomorphological changes, BCL2 and CD 34

INTRODUCTION

The most common haematological disease that occurs during pregnancy is anaemia. Approximately half of all pregnant women suffer from anaemia. The commonest cause is Iron insufficiency which may be due to nutritional insufficiency or increased demand of oxygen in gestation. It can be an indirect symptom of maternal malnutrition or a direct cause of the foetus' poor gestational growth because it limits oxygen flow to the placenta. Because the placenta frequently undergoes significant pathogenic alterations before the foetus gets affected, it is a developing area in current obstetrics research. As a result, placental abnormalities may function as an "early warning system" for foetal difficulties. Anaemia-related morphological defects have an influence on the placenta's exchange and hemodynamic functions.

Apoptosis, or programmed cell death, is crucial for the placenta's formation and for the regulation of its ageing throughout pregnancy. As an antiapoptotic protein that is essential to cell survival and proliferation, Bcl-2 belongs to the antiapoptotic category. Anaemic women's placentas may have different degrees of its expression, which might affect the viability and functionality of the cells. A marker for endothelial cells, which line blood arteries, is CD34. Examining the CD34 expression in the placenta may show changes in blood flow and oxygen delivery in the setting of anaemia, as well as insights into the formation and function of placental blood vessels.

Hence, we want to look at the immune histochemistry markers CD34 and BCL 2 to see whether there is a link between anaemia severity and histopathological changes in the placentas of anaemic mothers. By combining IHC and histopathological data, this study seeks to establish potential correlations between BCL-2 and CD34 expression levels and observed structural and functional abnormalities inside the placenta.

Methods

A prospective observational study was conducted in the Department of Obstetrics and Gynaecology, Subharti Medical College, Meerut, U.P. from 2022-2024 which included 100 study participants (50 tests and 50 controls).

Inclusion Criteria

All pregnant women visiting the Obstetrics and Gynaecology Department: Singleton pregnancy, Full term pregnancy (>37weeks), Cephalic presentation will be included in the study.

Exclusion Criteria:

Pregnant women presenting with: Ante partum Haemorrhage, Gestational Diabetes Mellitus, Hypertensive disorders in pregnancy or any medical illness other than anaemia which affects neonatal outcome will be excluded like breech presentation, IUD, RH isoimmunised pregnancy, Pregnancy with heart disease.

Severity of anaemia was noted according to ICMR classification. Placenta were sent for HPE and expression of IHC markers (BCL2 and CD 34) in 10% formalin.

Data Analysis

Statistical analysis will be performed using SYSTAT 13.2. Chi-square test will be used for categorical data and one way analysis of variance (ANOVA) test for continuous data. Kruskal Wallis test will be used for non-parametric data like verbal descriptive scale and patient satisfaction score. P value < 0.05 will be considered significant and <0.001 will be considered highly significant.

RESULTS

Socio Demographic Data: Among the non-anaemic group mean age (58% women) were % women were of less than 20 years age, 78% women were between 21-30 years age group, in our study the two groups were compared based on the residence. In the anaemic group 30 % women belonged to the urban while 70% were from rural areas. In the non-anaemic group 20 % women belong to the rural area while 80% women were from urban area. In our study the 2 groups were compared based on the educational status of women. In the anaemic group women who received secondary education were highest in number that is 36% while in the non-anaemic

group 22% women were illiterate. In our study the anaemic group was compared on the basis of gravida status of women, In the anaemic group majority of women were multigravida (78%).

Histomorphological Changes:

Table 1: Comparison Of The Diameter Of The Placenta In Between The Groups

No Anaemia	Anaemia	f-value	p-Value
17.374±1.19	16.548±0.84	12.46	0.01*

The mean placenta diameter in the anaemic group was 16.5 cm. In the non-anaemic group women 17.3 cm. The comparison was statistically significant between both the groups

Table 2: Comparison Of The Weight Of The Placenta In Between The Groups

No Anaemia	Anaemia	f-value	p-Value
531.88±25.72	504.38±51.91	17.30	0.01*

The mean placenta weight in the anaemic group was 504 gm. In the non-anaemic group women 531gm.The comparison was statistically insignificant between both the groups

Table 3: Comparison Of Number Of Cotyledons In The Placenta In Between The Groups

No Anaemia	Anaemia	f-value	p-Value
17.50±2.17	25.38±51.91	15.20±2.65	0.01*

The mean number of cotyledons in the placenta of the anaemic group was 25. In the non-anaemic group women 17.

Table 4: Incidence Of Calcifications In The Placenta In Between The Groups

Calcification			
Non-Anaemic		Anaemic	
NIL	36	28	p-Value-0.04
+	6	1	
++	4	4	
+++	1	8	
++++	3	9	
TOTAL	50	50	

Of 50 women with anaemia, calcification was not present in 56% in the placenta. Of the 50 non anaemic women 72% had no calcification in the placenta. The calcification was 1+ in 2% of the anaemic mothers and 2+ in eight percent of the anaemic mothers. Among the non-anaemic mothers, 12% had 1+ calcification, eight percent had 2+ calcification and two percent had 3+ calcification. This difference in degree of calcification in placenta was statistically significant between the two groups (p= 0.04).

Table 5: Incidence Of Cytotrophoblast Proliferation In The Placenta In Between The Groups

incidence cytotrophoblast proliferation	Non-anaemic	anaemic	Chi-square (p-value)
NIL	28	23	0.94 p-value:0.04
+	6	2	
++	5	5	
+++	4	7	
++++	7	13	
TOTAL	50	50	

Of the 50 non- anemic women, the cytotrophoblast proliferation was1+ in 12%, 2+ in 10% and 3+ in 8% and 4+ in 14% of the non- anemic mothers. The syncytial knot 1+ was seen in 4% of the anemic women and 2+ in 10 percent, 3+ in 14 % and 4+ in 26 % of the anemic women. 46% percent of anemic women as compared to 56% of non-anemic women had no cytotrophoblast proliferation in the placental microscopy. This difference was statistically significant (p= 0.004).

Table 6: Incidence Of Capillaries Per Villous In The Placenta In Between The Groups

capillaries			
	Non-Anaemic	Anaemic	
P value 0.04 Chi Square 113.56	+ (8-10)	22	28
	++ (10-15)	7	4
	+++ (15-20)	13	5
	++++ (>20)	11	13
	TOTAL	50	50

Of the 50 non- anemic women, the capillaries per villous was1+ in 44%, 2+ in 14% and 3+ in 26% and 4+ in 22% of the non- anemic mothers. The capillaries per villous 1+ was seen in 56% of the anemic women and 2+ in 8 percent, 3+ in 10 % and 4+ in 26 % of the anemic women. This difference was statistically significant (p= 0.004).

Table 7: Comparison Of IHC Markers BCL-2 In The Placenta In Between The Groups

EXPRESSION OF IHC - BCL-2	Non anaemic	anaemic	Chi-square (p-value)
+ (focal positive)	42	14	P value 0.04 Chi Square 113.56
++ (focal strong positive)	3	09	
+++ (diffuse strong positive)	5	27	
TOTAL	50	50	

Of the 50 non- anemic women, the BCL 2 positivity was focal positive (+) in 84 %, focal strong positive (++) in 6% and diffuse strong positive (3+) in 10% of the non- anemic mothers. The BCL 2 positivity focal positive (1+) was seen in 28% of the anemic women and focal strong positive (++) in 18 percent, diffuse strong positive (3+) in 46% of the anemic women.. This difference was statistically significant (p= 0.004).

Table 8: Incidence Of Comparison Of IHC Markers BCL-2 In The Placenta According To Severity Of Anemia

	Mild anaemic	Moderate anaemia	Severe anaemia	Very-severe anaemic	Chi-square (p-value)
+ (focal positive)	9	6	0	0	Chi Square 125.62 p-Value 0.03
++ (focal strong positive)	3	7	2	0	
+++ (diffuse strong positive)	5	14	3	01	
TOTAL=50	17	27	05	01	

Of the 50 anaemic women BCL2 was diffuse strong positive in 10% of mild anaemia and 28% of moderate anaemia and 6% of severe anaemia and 2% of very severe anaemia. It was focal positive in 18% of mild anaemia, 12% of moderate anaemia. This difference was statistically significant (p= 0.03).

Table 9 : Comparison Of IHC Markers CD 34 In The Placenta In Between The Groups

EXPRESSION OF IHC - CD 34	Non-anaemic	anaemic	Chi-square (p-value)
+ (focal positive)	22	28	p--0.04 Chi square 108.95
++ (focal strong positive)	7	4	
+++ (diffuse strong positive)	21	18	
TOTAL	50	50	

Of the 50 non- anaemic women, the mean incidence of IHC markers CD 34 in the placenta was 1+ in 44%, 2+ in 14% and 3+ in 26% and 4+ in 22% of the non- anaemic mothers. The CD 34 expression 1+ was seen in 56% of the anaemic women and 2+ in 8 percent, 3+ in 10 % and 4+ in 26 % of the anaemic

women. This difference was statistically significant (p= 0.04).

Table 10: Incidence Of Comparison Of IHC Markers CD 34 In The Placenta According To Severity Of Anemia

incidence of comparison of IHC markers CD 34	Mild anaemia	Moderate anaemia	Severe anaemia	Very-severe anaemia	Chi-square (p-value)
+ (focal positive)	9	6	0	0	Chi square 116.65 p-0.04
++ (focal strong positive)	3	7	2	0	
+++ (diffuse strong positive)	5	14	3	1	
TOTAL	17	27	05	01	50

The mean incidence of comparison of IHC markers CD 34 focal positivity was seen in the placenta of the mild anaemic group was 18%. In the moderate anaemic group women was 12%. the CD34 expression was seen as diffuse strong positivity was seen in the placenta of the mild anaemic group was 10%. In the moderate anaemic group women was 28% the severe anaemic group was 6%, very severe anaemic group was 2%. The comparison was statistically significant between both the groups

DISCUSSION

- In present study, total of 100 patients included in the study after obtaining the informed consent. Among the study participants, 6% were less than 20yrs of age, 78% were in 21-30 yrs of age and 14% in 31-40 years of age in anaemic group.
- In present study there is no significant difference in the socio-economic status.
- On assessment of the educational status, lower educational status was seen in anaemic mothers compared to non-anaemic mothers.
- There is no significant difference in incidence of anaemic in rural residence compared to the urban area.
- There is no significant difference in incidence of anaemia in the parity between the groups.
- Of the 50 women with anaemia, 67% of the women with anaemia had complications of the labour such as PPH, PPROM, CHF, Sepsis ICU admission, DIC, Prolonged second stage of labour. 24 % of the anaemic women had post-partum haemorrhage, 16 percent had lactational failure. In present study, ten percent anaemic women were found to be suffering from congestive heart failure. 6 percent were having DIC, 16 % were having sepsis and 10 % of anaemic patients were admitted in the ICU.
- The placentas of anaemic mothers weighed an average of 504.38 grams.
- The mean diameter of the placenta in anaemic mothers was 16 CMS.
- The placentas of anaemic mothers had 20 cotyledons on average.
- Seventy percent of the placentas from 50 non anaemic women had no evidence of calcification.
- The calcification was positive in 44% of the anaemic mothers, and ++++ in 18% of them.
- Among the 50 anaemic women, the syncytial knot was positive in 58 %, +++ in 18%, and ++++ in 22% of the anaemic mothers.
- The Cytotrophoblast proliferation in the placenta was found to be positive in 54% of anaemic mothers, +++ in 14% of anaemic mothers, and ++++ in 26% of anaemic women out of 50.
- Out of 50 anaemic pregnant women, 56 % had 1+ (8-10)

capillary per villous, whereas 8% had 2++ (10-15), 10 % had 3+++(15-20), and 26 % had 4 ++++. (>20)

- Expression of IHC markers CD 34 was focal positive in 70% mild anaemic women compared to 61% moderate anaemic women, 40% in severe anaemia. Conversely, diffuse strong CD 34 positivity was seen 100% of very severe anaemia and 60% of severe anaemia and in 51 % of moderate anaemic women.
- Results indicate a higher incidence of focal BCL2 positivity in 70% mild anaemic women compared to 48.02% moderate anaemic women, 40.12% in severe anaemia. Conversely, diffuse strong BCL2 positivity was seen 100% of very severe anaemia and 60% of severe anaemia and in 51 % of moderate anaemic women.

CONCLUSION

1: Based on the numerous morphometric alterations identified in this study, it is determined that placentae derived from anaemic women show a considerable decrease in placental size and weight.

2: Based on histological investigation, Maternal anaemia was associated with a decrease in the number of cotyledons, an increase in placental infarctions and syncytial knot hyperplasia, an increase in signs of ischemic damage to tissues, as well as maldeveloped terminal villi at low maternal haemoglobin concentration, displaying adaptive alterations.

3: Our investigation into the expression of Bcl-2 and CD34 in placentas of women with iron deficiency anaemia suggests a potential link between altered protein expression and placental health. Anaemic women exhibited a higher frequency of focal and diffuse positive staining for both markers, with the intensity of staining increasing with the severity of anaemia. These alterations were associated with a higher risk of adverse pregnancy outcomes.

4: Bcl-2 and CD34 could serve as valuable IHC markers for early detection of placental dysfunction associated with IDA. This could pave the way for timely interventions to improve pregnancy outcomes in women with anaemia. However, Further research is warranted to validate these findings and explore the functional significance of these markers.

5: The influence of maternal iron supplementation on these findings is still uncertain.

DECLARATIONS
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