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Immunohistochemical expression of CD68 for Hofbauer cells in different size human placentae

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Abstract

The placenta supports intrauterine life through nutritional, immunologic, and endocrine functions. Hofbauer cells (HBCs) are a unique population of fetal-derived macrophages originating from fetal mesoderm that play crucial roles in regulating pregnancy. CD68 macrophages promote placental cell growth via angiogenesis induction and are predominantly located in the endosomal/lysosomal compartment within cells. To evaluate the histological and immunohistochemical expression of CD68 for HBCs and their density in placentae of different sizes. Human placental samples (n=50) were collected from Al-Qimma Surgical Hospital from normal vaginal deliveries of women aged 18-34 years. Specimens were classified into three groups based on diameter and weight: Group A (small: diameter <21cm, thickness <2.0cm, weight ≤460g), Group B (large: diameter >26cm, thickness >2.6cm, weight ≥500g), and Group C (control: diameter 22-25cm, thickness 2.0-2.5cm, weight 470-499g). Paraffin-embedded sections underwent H&E staining and immunohistochemical staining for CD68. Quantitative analysis was performed using Aperio ImageScope software with Positive Pixel Count algorithms. IHC expression of CD68 for HBCs was recorded as 63,690±31,306, 65,312±32,407, and 113,469±83,188 pixels per micron for small, large, and control group placentae, respectively. Higher expression was observed in control and large placentae compared to small placentae. Tertiary villi revealed more syncytial knots in small placentae than in large and normal-sized placentae. Low HBCs with abundant syncytial knots were more prevalent in intervillous spaces of small placentae. CD68 immunohistochemical expression serves as a specific marker for HBC detection. This study demonstrates that CD68(+) HBCs are involved in different placental structural states and are associated with developmental maturation of the placental unit. Higher CD68 expression in larger placentae indicates better placental health status.

Keywords: CD68, Hofbauer cells, Placenta, Immunohistochemistry, Placental development, Macrophages.

Introduction

The placenta is a vital organ that supports intrauterine life through its multifaceted functions including nutritional transport, immunological protection, and endocrine regulation. At term, the placenta typically measures approximately 22 cm in diameter, 2.0-2.5 cm in thickness, and weighs around 470 grams.

Hofbauer cells (HBCs), first discovered by Dr. Hofbauer in 1905, represent a unique population of fetal-derived macrophages originating from fetal mesoderm. These cells play crucial roles in pregnancy regulation and maintenance of gestational tolerance through semi-allogeneic fetal acceptance mechanisms and microenvironmental homeostasis preservation [1,2]. Despite maternal immunological activity, fetal rejection is circumvented through these specialized cellular mechanisms.

Placental macrophages demonstrate phenotypic heterogeneity and spatiotemporal functional adaptation throughout gestation, with critical involvement in implantation, placentation, and parturition [3]. HBCs derive from early gestation villous stromal mesenchymal precursors and later from yolk sac monocyte progenitors, while decidual macrophages originate from maternal hematopoietic stem cells [4].

The CD68 glycoprotein serves as a valuable marker for identifying various immune cells, with applications extending beyond simple macrophage detection. CD68 can be found on the cell surface but is predominantly located in the endosomal/lysosomal compartment within cells. CD68+ macrophages have been shown to promote placental cell growth via angiogenesis induction and demonstrate M1-like characteristics (attacking microbes and tumors) versus M2-like functions (supporting tissue repair, immune tolerance, and placental development) [5,13].

Understanding the relationship between placental size and HBC distribution is crucial for

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comprehending placental development and function. This study aims to evaluate the histological, histomorphometric, and immunohistochemical expression of CD68 for HBCs and their density in placentae of different sizes.

Materials and Methods

Study design and sample collection

A cross-sectional investigation of placental morphology was conducted between October 2024 and July 2025 within the Department of Human Anatomy laboratories at Al-Nahrain University College of Medicine. Human placental specimens (n=50) were procured from the obstetric units of Al-Qimma Surgical Hospital, Baghdad. Inclusion criteria mandated the absence of discernible gross morphological anomalies from normal vaginal deliveries of women aged 18-34 years.

Sample classification

Each specimen was systematically classified into three cohorts based on diameter and weight:

• ***Group A (Small):*** Term delivery placentae with diameter <21 cm, thickness <2.0 cm, weight ≤460 grams

• ***Group B (Large):*** Term delivery placentae with diameter >26 cm, thickness >2.6 cm, weight ≥500 grams

• ***Group C (Control):*** Term delivery placentae with diameter 22-25 cm, thickness 2.0-2.5 cm, weight 470-499 grams

Histological processing

Hematoxylin and eosin staining

H&E staining was performed following established protocols for general histomorphological assessment. The staining sequence included:

1. Dewaxing in xylene (20-30 minutes)
2. Rehydration through graded ethanol series (100% → 90% → 70%), followed by distilled water immersion (3 minutes per step)
3. Nuclear staining with hematoxylin (4-5 minutes)
4. Differentiation via acid alcohol (1% HCl in 70% ethanol)
5. Bluing under running tap water (10 minutes)
6. Cytoplasmic counterstaining with alcoholic eosin (2-3 minutes)
7. Dehydration through ascending ethanol concentrations
8. Clearing in xylene and mounting

Immunohistochemical staining

HBCs were identified using rabbit monoclonal anti-CD68 IgG primary antibody (PathnSitu Biotechnologies). Chromogenic visualization employed a commercially available detection kit (Dako, Denmark) with HRP/DAB micro-polymer system.

IHC protocol

1. Overnight dewaxing at 58°C
2. Xylene clearing (2×10 minutes)
3. Rehydration through descending ethanol gradients
4. PBS wash and endogenous peroxidase quenching
5. Non-specific blocking with protein block reagent (10 minutes)
6. Primary antibody incubation (anti-CD68, 1:200 dilution, 90 minutes)
7. Secondary antibody incubation (20 minutes)
8. Streptavidin-peroxidase conjugation (20 minutes)
9. Chromogen development (DAB:substrate 1:35 dilution, 5-10 minutes)
10. Harris hematoxylin counterstain and mounting

Controls

• ***Positive control:*** CD68-immunolabeled placental sections exhibiting brown cytoplasmic staining

• ***Negative control:*** Placental sections processed identically except primary antibody substitution with PBS

Image analysis

Digital imaging was performed using LEICA DM750 microscopy with a 5-megapixel digital camera (MC500) at 10× and 40× magnifications. Quantitative evaluation of immunohistochemical reactions was performed using Aperio ImageScope software (v12.3.3.5048) with Positive Pixel Count algorithm

implementation.

The system employed predefined parameters for tripartite staining intensity classification:

- ***Strong positive:*** Brown
- ***Positive:*** Orange
- ***Weak positive:*** Yellow
- ***Negative:*** Blue

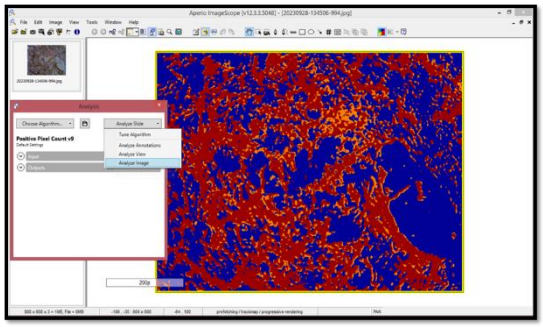


Figure 1. Choose analyze image from (analyze).

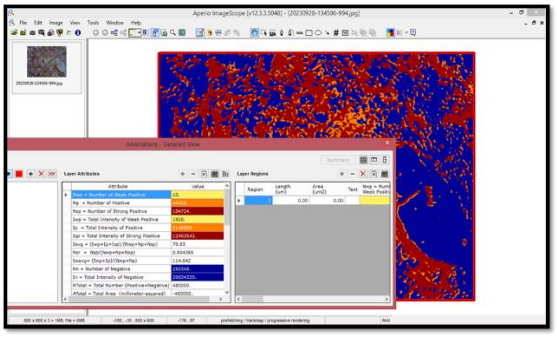
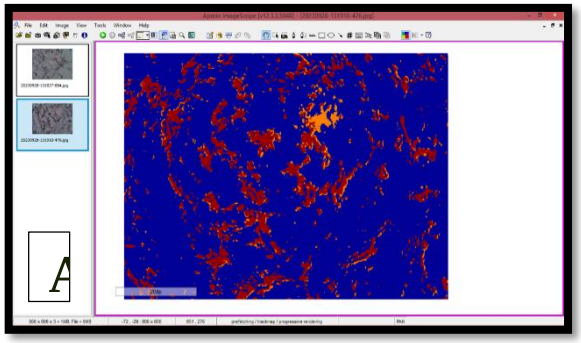


Figure 2. The strong positive pixel count algorithm per micron



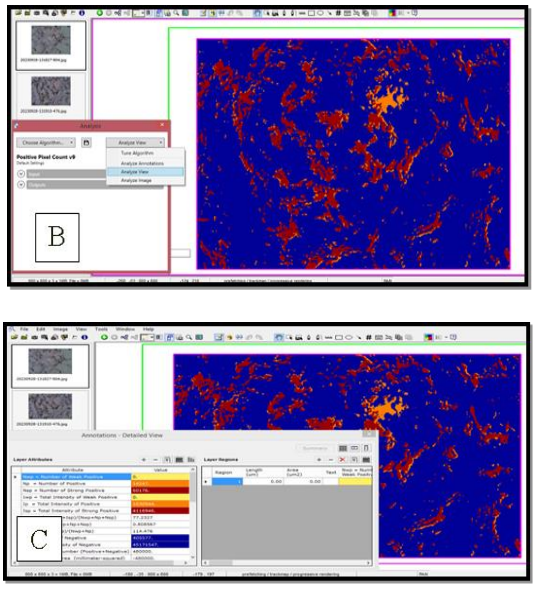


Figure 3. A. Applying Aperio positive pixel count algorithms program.B. Show selected the analyze slide and C. Show the annotation results.

For comparative analysis, only strong positive pixel counts representing anti-CD68 immunoreactivity were statistically evaluated.

Statistical analysis

Statistical analysis was performed using SPSS software version 24. Data are expressed as mean ± standard deviation. Significance thresholds were established as $p \leq 0.09$ for statistical significance and $p > 0.09$ for non-significance. Multiple comparison tests were used to measure significant differences in mean positive pixels of CD68 immunohistochemical reactivity between study groups.

Results

Histological features

H&E stained sections revealed distinct histological features across different placental size groups. The histological features of tertiary villi showed fewer syncytial knots in large placentae compared to small placentae. Abundant syncytial knots were observed more frequently in the intervillous spaces of small placentae compared to large and control placentae.

Immunohistochemical expression of CD68

The IHC expression of CD68 for HBCs in different

sizes of placental tissue was recorded as follows:

• ***Small placentae (Group A):*** 63,690±31,306 pixels per micron

• ***Large placentae (Group B):*** 65,312±32,407 pixels per micron

• ***Control placentae (Group C):*** 113,469±83,188 pixels per micron

The IHC of CD68 expression of the HBCs in different sizes of placental tissue. They recorded (65312+32407, 63690+31306 and 113469+83188 pixels per micron for large , small and control group size placenta, respectively) that had been evaluated by aprio scope image software program at magnification 40 X (Fig. 4).

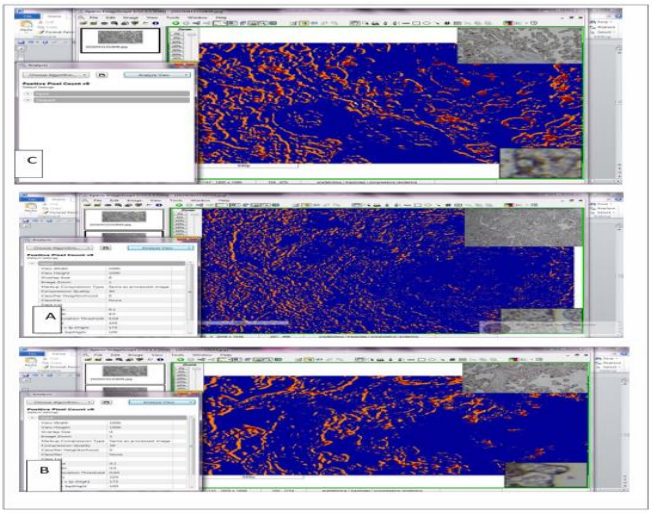


Figure 4. reveal the aprio software program analysis of IHC of CD68 expression of the HBCs in different sizes of placental tissue. They recorded (65312+32407, 63690+31306 and 70672.4+11771.21 pixels per micron for large , small and control group size placenta, respectively) that had been evaluated by aprio scope image software program at magnification 40 X.

The present study reveals that IHC of CD68 expression of the HBCs were seen in the main stem or even anchoring villi of placentae mainly in small sizes placentae in comparison to control and large sizes placentae and this indicate that mature placentae had more HBCs than immature small sizes placentae (fig 5).

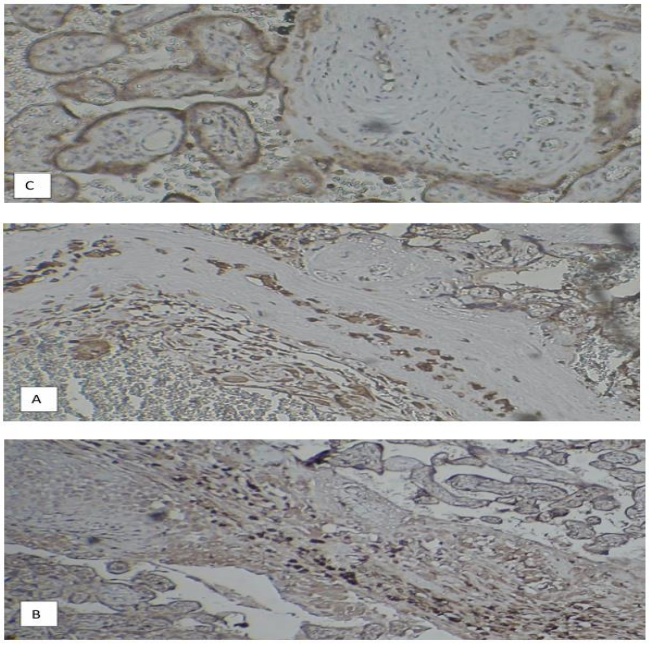


Figure 5. The present study reveals that IHC of CD68 expression of the HBCs were seen in the main stem or even anchoring villi of placentae mainly in small sizes placentae in comparison to control and large sizes placentae and this indicate that mature placentae had more HBCs than immature small sizes placentae. IHC at 10 X.

Statistical analysis revealed non-significant differences between groups:

• ***Large vs. Small:*** p=0.991 (NS)

• ***Large vs. Control:*** p=0.906 (NS)

• ***Small vs. Control:*** p=0.846 (NS)

However, descriptive analysis showed higher CD68 expression in control and large placentae compared to small placentae, indicating greater HBC prevalence in healthy larger placentae.

Distribution patterns

CD68 expression of HBCs was predominantly observed in the main stem and anchoring villi of placentae, with notable differences between groups. Small placentae showed lower HBC distribution compared to control and large placentae, suggesting that mature placentae have more HBCs than smaller placentae.

The IHC analysis of terminal villi revealed increased syncytial knots in small placentae versus large and normal specimens. An inverse correlation existed between reduced HBCs and abundant syncytial knots, predominantly observed within intervillous spaces of small placentae.

Discussion

Correlation between placental size and syncytial knots

The current analysis indicates a reduced prevalence of syncytial knots within larger placentae relative to smaller counterparts. This observation aligns with studies proposing that maternal thrombotic conditions can manifest in placental pathology characterized by diminished placental mass, infarctions, and elevated syncytial knot frequency [6]. Such pathological deviations correlate with pregnancies complicated by hypoxic conditions or thrombotic events, associating with increased incidence of preeclampsia and small-for-gestational-age infants.

CD68 expression and placental health

The elevated CD68 expression in control and large placentae versus small specimens signifies greater HBC prevalence in healthy large placentae. This aligns with studies identifying HBCs as chorionic villi-resident macrophages essential for placental morphogenesis and immune regulation [7,8]. HBC disruption associates with infection, inflammation, and impaired placental development.

Research examining fetal macrophage (CD68+) distribution in various placental conditions has identified significantly different CD68+ cell counts between healthy and pathological placentas [9]. CD68+ cells are typically observed near fetal vessels within placental villous stroma, indicating their role in anti-inflammatory protective responses.

Functional significance of HBCs

HBCs exhibit specific morphology featuring granular, vacuolated cytoplasm, distinctly visible under high-power microscopy. These cells differentiate from mesenchymal progenitors by conception day 18, persisting throughout gestation within placental villous stroma, amnion, and chorionic laeve [10].

The functional significance of HBCs includes:

- Antimicrobial and antitumoral activities
- Infection prevention and tissue repair
- Angiogenesis promotion
- Immunomodulation
- Maintenance of maternal-fetal tolerance

Contemporary research positions these cells as pivotal for placental development and homeostasis, suggesting HBCs as promising therapeutic targets due to their functional plasticity [11].

Clinical implications

The association between CD68 immunohistochemical expression and placental size has important clinical implications. Smaller placentae with reduced HBC populations may indicate compromised placental function, potentially correlating with adverse pregnancy outcomes. The inverse relationship between HBC density and syncytial knot abundance in small placentae suggests altered placental maturation patterns.

Studies have shown that HBC numbers substantially diminish in pathological conditions such as chorioamnionitis compared to normal pregnancies [12]. This reduction, coupled with increased inflammatory markers, indicates the critical role of HBCs in maintaining placental health and preventing complications.

Study limitations

This study has several limitations including the cross-sectional design, which prevents assessment of temporal changes in HBC distribution. Additionally, the sample size may limit the generalizability of findings, and functional studies of HBC activity were not performed.

Conclusion

1. The immunohistochemical expression of CD68 serves as a specific and reliable marker for detecting

HBCs in placental tissue.

2. CD68 expression for HBCs was higher in control and large placentae compared to small placentae, indicating that these cells are more prevalent in healthy, well-developed placentae.

3. Small placentae demonstrated more syncytial knots in tertiary villi compared to large and normal-sized placentae, with an inverse correlation between HBC density and syncytial knot abundance.

4. CD68(+) HBCs are involved in different placental structural states and are associated with the developmental maturation of the placental unit, suggesting their crucial role in maintaining placental health.

5. The findings support the hypothesis that placental size correlates with HBC distribution and may serve as an indicator of placental functional capacity.

Recommendations for future research

1. Investigate the correlation between delivery method (normal vaginal delivery vs. cesarean section) and CD68(+) HBC distribution in placentae.

2. Examine the relationship between maternal age (>30 years) and CD68(+) HBC expression patterns.

3. Conduct ultrastructural studies of HBCs using electron microscopy in placentae from women with various maternal diseases.

4. Perform longitudinal studies to assess temporal changes in HBC distribution throughout pregnancy.

5. Investigate the functional significance of different HBC phenotypes in healthy and pathological pregnancies.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Statement

This study was conducted in accordance with ethical principles and received appropriate institutional approval. All procedures followed institutional guidelines for human tissue research.

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