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Characterization of lymphocyte infiltration in molar pregnancies

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Abstract
Background: In normal pregnancy around 30% of stromal cells in decidualised endometrium are leucocytes, lymphocyte subtypes may play a role in the pathogenesis of molar pregnancies. The aim of the study is to investigate the pattern of infiltrating lymphocytes in paraffin embedded tissue sections obtained from molar pregnancies.
Material and Methods: This study included thirty-five archived paraffin-embedded samples of molar pregnancies; divided into 16 incomplete and 19 complete hydatid mole. Then dual immunofluorescence staining was used for phenotyping of lymphocytic infiltrate (CD3- CD19) and (CD4-CD8). Independent sample t-test was used to compare the mean cell counts between different study groups.
Results: There is higher T cells (CD3) infiltrate in complete hydatid mole than normal placental tissue (p=0.003) and not significant with incomplete hydatid mole (p=0.089). Plasma cells (CD19) were higher in both complete hydatid mole CHM (p=0.012) and incomplete hydatid mole iCHM (p=0.013). Cytotoxic cells (CD8) were none significant in all groups while Helper cells (CD4) were significantly higher in CHM (p=0.017) and iCHM (p=0.025).
Conclusion: The higher tissue infiltration with plasma cells and helper cells in both CHM and iCHM highlight the importance of these cells in molar pregnancy pathogenesis.

Key words: Hydatid mole, lymphocytes infiltration, Complete hydatid mole, incomplete hydatid mole.

Introduction

Leukocytes comprise the majority of the developing placenta includes maternal blood and decidual cells (1). These cells giving different biological molecules, especially cytokines and growth factors, which play an important role to enhance the placental development and function. Study role of these cells during pregnancy critical to our comprehension of the achievement or disappointment of a pregnancy (2).

In an ordinary early pregnancy, the proportion of CD8+ and CD4+ T cells is around , 2.5-3:1 (3) . The quantities of T cells diminish in the early decidua contrasted with a non-pregnant endometrium (4).

It has been found that decidual CD8+T cells have cytolytic activity, don't bring out a prevalent local intrauterine, Th2 sort cytokine environment, and control trophoblast intrusion into the decidua. A bigger rate of decidual CD3+T cells expresses CD38, HLA-DR and VLA-1, than of peripheral blood CD3+T cells (5).

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Hydatidiform mole is a gestational trophoblastic disease characterized by abnormal proliferation of trophoblast cells (6) with no fetus (7). In contrast, partial hydatidiform moles usually result from the fertilization of a normal ovum by two spermatozoa, (8) and affect only part of the placenta. Similar to a typical early pregnancy, obtrusive additional villous trophoblast cells of complete and partial hydatidiform moles are reactive to the monomorphic determinant however not to polymorphic epitope of class I HLA antigens (9).

In hydatid form moles, positive CD3, CD8 lymphocytes and mast cell tryptase are expanded, macrophages are unchanged while NK cells are diminished (10). This proposes a dysfunction in the control of the trophoblast attack by decidual leukocytes, maybe through their creation of growth factors and cytokines in gestational trophoblastic diseases.

This study aimed to investigate the pattern of infiltrating lymphocytes in paraffin embedded tissue sections obtained from molar pregnancies.

Material and methods

Tissues

Formalin-fixed, paraffin-embedded archival decidual tissues from sixteen partial and nineteen complete hydatidiform moles evacuated during the first trimester were retrieved from the archive files of the Histopathology department, AL-Emamain Medical City, Baghdad.

Fifteen normal (first trimester) tissue interface were obtained with elective termination of pregnancy for a maternal indication under approved consent of gynecologists. Thin paraffin-embedded sections (4 μm thick) of tissue section were mounted on poly- lysin-couted (positively charged) slides for the immunocytological characterization in these tissue sections.

Immunoreagents and immunocytochemical procedures

Lymphocyte subpopulations were identified by two sets of dual staining monoclonal antibodies labeled with Florescen Isothiocyanate (FITC) and Rhodamin Phycorethin (RPE) as ordered as (anti-CD3-FITC and anti-CD19-RPE, SEROTEC, Cat. No. DC005) and anti-CD4-FITC, anti-CD8-RPE, SEROTEC, Cat. No. DC048) respectively.

Direct Dual-Immunofluorescence procedure:

1. Dewaxing and rehydration: paraffin embedded sections were placed inside a hot air oven at 65°C overnight, then dipped in xylene and ethanol containing jars in the following order: Xylene for 5 minutes, fresh xylene for 5 min., absolute ethanol for 5 min., ethanol (95%) for 5 min., ethanol (70%) for 5 min., ethanol (50%) for 5 min. and distilled water for 5 min.
2. For blocking the non-specific binding sites, 100 μl of a protein-blocking reagent was placed onto the section and incubated for 10 minutes in a humid chamber at room temperature. Then the slides were drained and blotted gently.
3. 50 μl of diluted primary antibody was placed onto the section and incubated for 1 hour at 37°C in a humid chamber. After incubation, the slides washed with washing buffer for 5 minutes, drained and blotted gently.
4. Slides were dehydrated by dipping in ascending concentration of ethanol and xylene containing jars in the following order: Ethanol (50%) for 5 min., ethanol (70%) for 5 min., ethanol (95%) for 5 min., absolute ethanol for 5 min., fresh xylene for 5 min.

Two drops of mounting media [(nine parts glycerol to one part of 0.2M carbonate buffer, pH=9 (Batty, 1986) to enhance fluorescence and retard fading on exposure to UV-light (Narin, 1968)) placed on each smear of slides. Then cover slips were lowered into place slowly to avoid bubbles; cover slips may be sealed around edges with clear nail polish. Slides were examined then with fluorescence microscope at 490 nm; positive cells give green-apple or red when stained were recorded at X400 magnification.

Statistical analysis

Numerical data were recorded as mean and standard deviation of mean. Independent sample t-test used to compare between study groups and p-value ≤ 0.05 was considered statistically significant.

Results

Patient's characteristics

A total of fifty pregnancies divided into three groups were studied. The group I consisted of 19 CHM, group II consisted of 16 iCHM and group III consisted of 15 normal pregnancies. Patient characteristics are presented in (Table 1).

All tissue biopsies were belonging to Iraqi pregnant ladies their mean age control group was 31.7±9.4 years, iCHM was 29.32±6.23 and CHM was 32.40±8.21 all groups were statistically none significant (p=0.732). There were no differences mean gestational age of all groups were statistically none significant (p=0.732).

Table 1. Description of study group characteristics.

	Control group n=5	iCHM group n=16	CHM group n=19	P value
Age (years)	31.7±9.4	29.32±6.2	32.40±8.2	0.732^{NS}
Gravidity	2-5	2-4	1-6	
Parity	1-4	1-4	0-5	
Gestational age (days)	85.3±14.2	74.32±19.1	81.92±17.21	0.085^{NS}

Lymphocytic constituents in molar pregnancy and control tissue:

All cases were investigated for CD3/CD19 and CD4/CD8 expression based on dual immunofluorescence staining technique. (Figure 1). Total number of T cells and subsequent cellular subsets (CD3+, CD4 +, CD8+, and CD19 cells). T cells were 36.68±10.92 infiltrated in iCHM and 42.62±9.12 in CHM compared with 27.80±7.21 among normal trophoblastic tissues. While, plasma cells were significantly higher in its pattern of infiltration in iCHM 7.08±1.12 and CHM 6.82±.91 than those in normal trophoblastic tissue (p=0.013 and 0.012 respectively).

Dual immunofluorescence labeling confirmed that the majority of cellular infiltrates were helper cell subtype (CD4) in iCHM 29.33±12.84 and CHM 34.02±16.23 than those of control 14.74±5.32 (p=0. 025 and 0.017 respectively). While, cytotoxic cells were not significantly different among study groups (Table 2).

Discussion

This is the first study describe the lymphocyte subsets infiltration inthe decidual tissuee of hydatid mole disease. The results demonstrate that most decidual T cells express CD4 T cell receptors whereas CD8 T cells comprise only a small proportion compared with normal trophoblastic tissue. These results had been argued by other researchers and they reported that these cells express perforin, granzyme A, granzyme B, granulysin and Fas ligand (FasL) (11-12). Therefore, these cells may protect the maternal-fetal unit from infections, control trophoblast invasion, and create a local immune tolerance toward the semi allogeneic conceptus by killing fetal reactive lymphocytes.

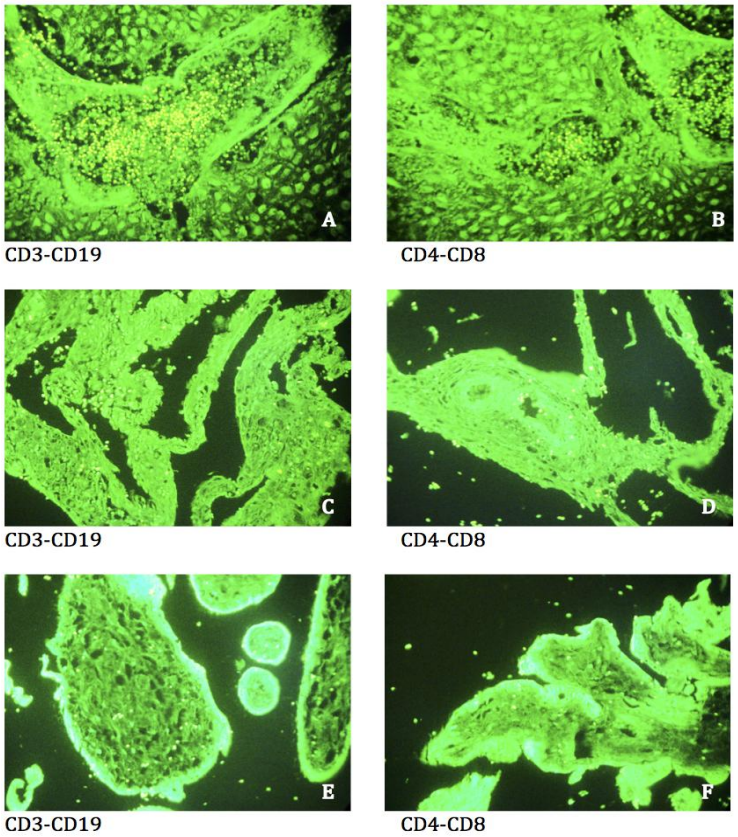

Figure 1. Dual immunofluorescence (A and B) CHM, (C and D) iCHM and (E and F) normal tissue. A, C and E stained with Anti (CD3-CD19) antibodies, stained cells can be seen several T-cells (CD3+ve) green color and fewer plasma cells (CD19+ve) red color. B, D and F stained with Anti (CD4, CD8) T-helper (CD4+ve) green color extensively infiltrated (B and D) and fewer T- cytotoxic (CD8+ve) red color infiltration seen.

Table 2. Result of comparison for total T cells and different subsets of T cells among different study groups.

	Normal N=5	Incomplete H mole N=16	Complete H mole N=19
CD3	27.80±7.21	36.68±10.92 (0.107NS)	42.62±9.12 (0.003*, 0.089NS)
CD19	5.22±1.92	7.08±1.12 (0.013*)	6.82±.91 (0.012*, 0.454NS)
CD8	16±3.11	12.9±8.22 (0.426NS)	14.29±7.03 (0.605NS, 0.593NS)
CD4	14.74±5.32	29.33±12.84 (0.025*)	34.02±16.23 (0.017*, 0.357NS)
CD3:CD19	5.6:1	5.3:1	6.3:1
CD8:CD4	1.2:1	0.44:1	0.41:1

Data presented as mean± Standard deviation, NS= none statistical significance (p>0.05). * = statistical significant difference (p≤0.05).

Decidual CD4+ T cell numbers are increased in complete and partial hydatidiform moles compared to a normal early pregnancy, and this result was accordance with those in most previous studies by Sanguansermsri and Pongcharoen (7); Dickson et al. (13) these findings suggest an altered maternal immune response against the molar trophoblast compared to a normal pregnancy. The decrease in CD8+ T cells in hydatidiform mole noted in the present study suggests that CD8+ T cells may not be primarily responsible for immune responses against the molar trophoblast and do not undergo apoptosis following activation (14).

The low number of CD8+ T cell possibly due to their down-regulation by two cytokines normally present in the fetoplacental tissue which is interleukin (IL)-15 and transforming growth factor (TGF)-β (14- 15). In last years, some in vitro studies have demonstrated that TGF-β (16) and IL-15 (17) up-regulate the de novo expression of CD94/NKG2A heterodimer killer inhibitory receptor (KIR) complex on CD8+ cytotoxic T cells. This, in turn, results in the inhibition of T-cell receptor-mediated cytotoxicity and cytokine production by CD8+ T cells.

Increasing consideration is being paid to the role of mucosal, instead of circulating, lymphocyte subpopulations in the pathogenesis of gestational diseases such as molar pregnancy (18,19). Investigation of tissue sections enables analysis of cell populations and their connection in situ, and also examination of the local microenvironment, will expand our comprehension to the role of the Decidual T lymphocyte activation in hydatidiform mole and normal pregnancy (20).

Conclusion

In conclusion the study of immunopathology of hydatidiform molar pregnancy may enhance our understanding of the maternal immune response in normal pregnancy, and the importance of plasma cells and helper cells in molar pregnancy pathogenesis.

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