An Immunohistochemical Study of Human Cytomegalovirus Infection in Spontaneous Abortion in Egyptian Women

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Abstract: Background: Miscarriage, the most common complication of pregnancy, is the spontaneous loss of a pregnancy before the fetus has reached viability. Cytomegalovirus (HCMV) has been described in abortion tissues. The histopathologic changes of the placenta during viral infection show a wide spectrum, but seldom associated with inclusion bodies. However, the possible pathogenic role of this virus in abortion is under discussion. CMV is found throughout all geographic locations and socioeconomic groups but the incidence and frequency of HCMV infection can considerably vary among different study populations with definite correlations to low socioeconomic levels and bad hygienic measures, making it more prevalent in developing countries than developed ones. **Objective:** Determining the expression of HCMV associated antigen in spontaneous abortions to verify prevalence of HCMV in abortive tissue in Egyptian women and correlated it to certain pathologic criteria providing a clue of connection between HCMV and the described pathological criteria. **Material and methods**: This study was carried out on fifty four placentas of abortive specimens collected from aborted women during 8 to 13 week of gestational age. All were formalin fixed, routinely processed and paraffin embedded. Five micron thick serial sections were obtained from all the chosen specimens one was stained by H&E for histopathological evaluation and the others were mounted on positive charged slides for immunostaining using Avidin-Biotin techniquel method to detect HCMV antibodies. We collected the available clinical data regarding age of patients, gestational age and obstetric history from available request sheet. **Results and conclusion:** It was found that our studied histopathological criteria are related to HCMV infection and that there is a high prevalence of HCMV positive cases in our studied group (67%). This could be attributed to high incidence in our community and to selection of cases based on trying to exclude most commonly related causes and in choosing those with no obviously related abortion causes to any proposed etiology.
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1. Introduction:

Pregnancy loss has been attributed to several factors involved in human reproduction. Genetic and uterine abnormalities, endocrine and immunological dysfunctions, infectious agents, environmental pollutants, psychogenetic factors and endometriosis are most important causes (**Basim, 2014),** However there has been some evidence suggesting that intrauterine infections play a major role in the pathogenesis of spontaneous early pregnancy loss, but the implication and prevalence of pathogenic microorganisms in the etiology of spontaneous abortion during the first trimester of pregnancy has not yet been well established **(Zaki & Goda, 2007)**. Human cytomegalovirus (HCMV) one is the most common source of congenital malformation resulting from viral intrauterine infection in developed countries **(Jahromi *et al.*, 2010).** Some evidence has shown a relationship between human cytomegalovirus (CMV) infection and pregnancy loss **(Roya *et al.*, 2014)**. The incidence and frequency of HCMV infection can considerably vary among different study populations with definite correlations to low socioeconomic levels and bad hygienic measures, making it more prevalent in developing countries than developed ones **(Kenneson and Canon, 2007).** HCMV infection of abortive tissue have reported pathologic changes ranging from massive destruction of villi by severe necrotic inflammation to absence of lesions with or without fetal injuries (**Spano *et al.*, 2002).** However **Cunningham *et al.*(2001)** reported that on viral infections in pregnant women, there is not necessarily histological evidence of placental involvement, even if it was involved.

2. Material and methods

Fifty four archival cases representing abortion specimen cases of Egyptian female patients, retrieved

from the surgical files of the pathology department of Al-Zahraa hospital and forensic labs of medicolegal department of ministry of justice during the period from January 2005 to March 2009.

The cases were chosen based on gestational age ranging from 9 to 13 weeks gestation**.** Specimens were chosen with sufficient amount of placental tissue and decidua, cases with marked hemorrhagic areas, many blood clots, extensive fibrinoid and ghost villi and those with criteria of chromosomal anomalies proposed by **Kürman, 1994** including large mononuclear cells, resembling cytotrophoblasts, infiltrating the villous stroma, marked villous edema and associated trophoplastic invaginations, all were excluded. Cases with documented high risk pregnancy e.g diabetics and hypertensives were also excluded.

All were formaline fixed, routinely processed and paraffin embedded.

Five micron thick serial sections were obtained from all the chosen specimens one was stained by H&E for histopathological evaluation and the others for immunostaining for HCMV antibodies. We collected the available clinical data regarding age of patients, gestational age and obstetric history from available request sheet.

Immunohistochemical staining

Histological sections were immonohistochemically analyzed for expression of Anti-Human CMV antibodies (BioGenex, CA, USA) using avidin biotin complex methods (ABC) according to ***(Spano et al., 2002)***.The sections were deparaffinized in xylene, rehydrated in graded alcohol dilutions, washed in PBS. The slides were incubated with peroxidase-blocking reagent, followed by the primary antibody then

the visualization reagent (secondary goat-antimouse immunoglobulin and horseradish peroxidase linked to a dextran polymer backbone). After rinsing with distilled water, the slides were incubated with DAB (3, 3-diaminobenzidine) substrate–chromagen solution and a Mayer hematoxylin counter stain was applied before cover slipping.

Interpretation of the Anti-Human CMV immunostain

The positive results of Anti-Human CMV immunostain was recorded as brown granular cytoplasmic staining; the immunostain was evaluated at the following four sites: Vascular endothelial lining of maternal vessels, maternal glandular epithelium, decidual cells and/or chorionic villi.

Scoring methods

For HCMV ***positive*** immunostain, it was scorred as follows: Negative: No detected immunostaining. Positive: detected immunostaining. The positive immunostaining of the chorionic villi was further scored as: Moderate staining: with interrupted staining around the villous. Marked staining: with complete ring staining pattern around the circumference of villous.

For inflammatory cellular infiltrates; the H&E staining was scored according to **Redline *et al.*, 1999** as Negative: not evidently detected. Moderate: with occasional small foci of inflammatory cells ranging from 4-15 leukocytes / HPF and marked: as marked inflammatory infiltrate more than 15 leukocytes / HPF.

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Echo soft Corporation, USA. Qualitative data were expressed as frequency and percentage. Independent-samples t-test of significance was used when comparing between two means. Chi-square (X2) test of significance was used in order to compare proportions between two qualitative parameters.

3. Results:

The age of all patients ranged from 18 years to 40 years with mean age 27.5 $\pm$5 years. The gestational age ranged from 9 weeks gestation to 13 weeks gestation with mean gestational age 11.2 $\pm$1 weeks.

Histological results:

Thirty three cases showed one or more of the described histopathological criteria suggestive for HCMV infection.

Necrosis was detected in thirty cases. Twenty seven (50%) cases showed moderate necrosis and three were severely necrotic. The inflammation was observed in thirty (55.5 %) cases, twenty seven had moderate inflammation and three showed marked inflammation. The seen inflammatory infiltrates were not restricted to certain type of cells. Polymorphs, mononuclear cells and plasma cells were all seen; yet no eosinophils could be evidently detected. The inflammatory cells were mostly seen in the deciduas; intervillous and eroding the chorionic villi. The enlarged cells with or without vacuolation were seen in eighteen (33.3%) cases in cells of maternal glandular epithelium, and in trophoblastic covering of the chorionic villi and occasionally in vascular endothelial lining of maternal vasculature. The fibrin was seen in eighteen (33.3%) cases as intravascular plugs and intervillous. Twelve (22.2%) cases showed all criteria *(Fig. 1)*.

It was found that 67% of the studied cases (n=36 out of 54) expressed positive HCMV immunostaining.

The vascular endothelial lining of maternal vessels showed positive HCMV immunostaining in 12 cases (22.2%). The maternal glandular epithelium was positive in 24 cases (44.4%). The decidual cells were positive in 30 cases (55.6%). Eighteen cases (33.3%) showed marked staining of the chorionic villi with

complete ring staining pattern around the circumference of the villous and 12 cases (22.2%) showed Moderate staining: with interrupted HCMV immunostaining around the villous *(Fig. 2)*.

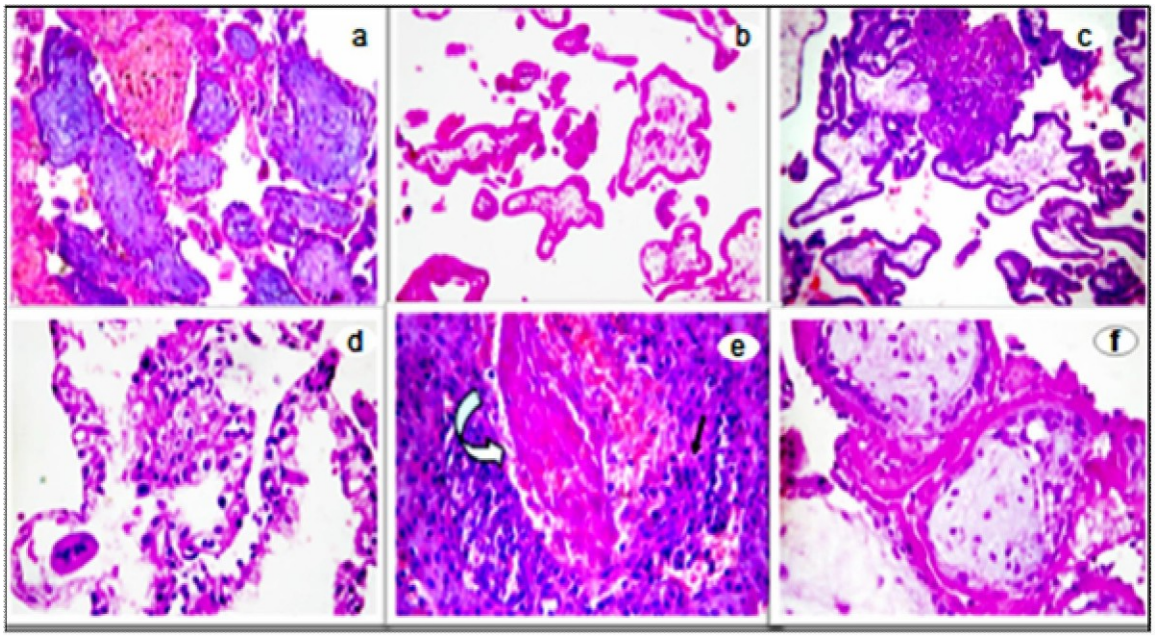

Figure (1) a) Villi showed marked necrosis with eroded border, obscured vascularity, stromal fibrosis, loss of double trophoblastic lining **(H&E X 125). b)** Moderately necrotic immature intermediate villi showing occasional stromal fibrosis with occasional loss of double trophoblastic layering **(H&E X 125). c)** Moderately necrotic chorionic villi impaired vascularity and intermediate trophoblastic cell column admixed with intervilous inflammatory cells **(H&E X 125). d)** Cellular enlargement plus vacuolations of maternal glandular epithelium **(H&E X 300). e)** Moderately necrotic chorionic villi with cellular enlargement plus vacuolation of trophoblastic layer; Intravascular fibrin plug (*white arrow*) inflammatory cells & karyorrhectic nuclei denoting decidual necrosis (*black arrow*) (H&E X 300). f) Intervillous fibrin with associated moderately necrotic chorionic villi, yet no evident inflammatory reaction (H&E X 500).

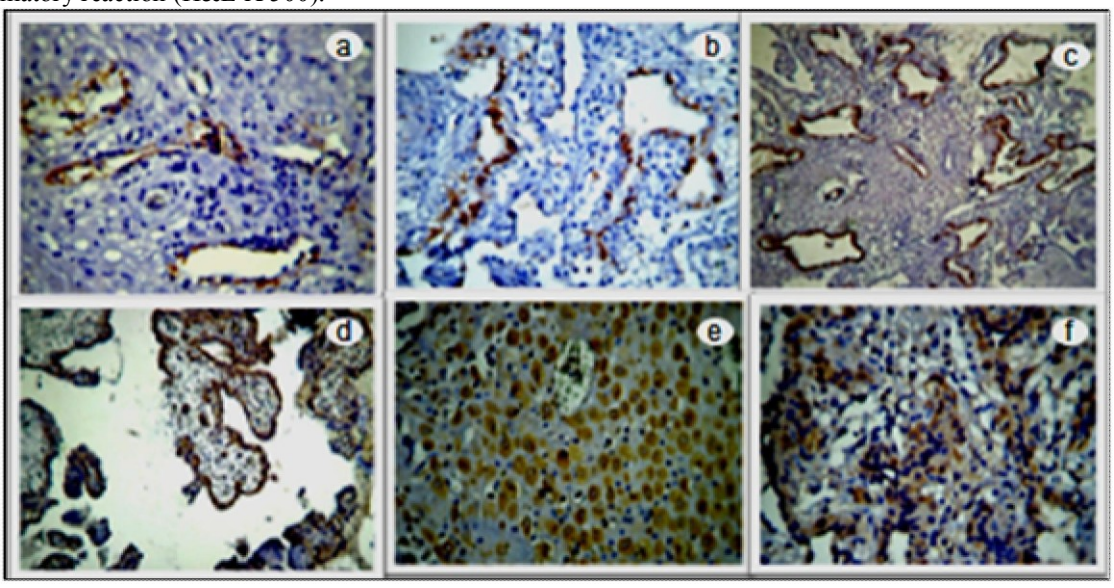

Figure (2): Positive HCMV immunostaining of:

a) Vascular endothelial lining **b)** glandular epithelium with foci of vascular endothelium positivity **c)** glandular epithelium lining **d)** Markedly positive of chorionic villi (ring staining around the chorionic villi) **e)** decidual cells.**f)** glandular epithelium, scattered endothelial lining & decidual cells positivity (a,b,c,d & f X 125, e, X 500)).

Correlation of histopathological results to HCMV immunostaining results:

There was significant statistical correlation between positive HCMV immunostaining of maternal endothelial lining, glandular epithelial, decidual cells and chorionic villi with necrosis involving chorionic villi and deciduas in the studied sections P value was 0.000, 0.012, 0.031 and 0.000 respectively, Correlation between necrosis and HCMV positive immunostaining are displayed in Table (1).

Table (1) Correlation between Necrosis and HCMV positive immunostaining

	Necrosis				
	Negative	Moderate	sever	Total	Value & **Pearson Chi-Square**
ENDOTHELIAL HCMV					
Negative	24 44.4%	18 33.3%	--------	42 77.8%	19.286 0,000
Positive	--------	9 16.7%	3 5.6%	12 22.2 %	
GLANDULAR HCMV					
Negative	**18 (33.3%)**	**12 (22.2%)**	**----**	**30 (55.6%**	8.775 .012
Positive	**6 (11.1%)**	**15 (27.8%)**	**3 (5.6%)**	**24 (44.4%)**	
DECIDUAL HCMV					
Negative	**15 27.8%**	**9 16.7%**	**----**	**24 44.4%**	6.919 **.031**
Positive	**9 16.7%**	**18 33.3%**	**3 5.6%**	**30 55.6%**	
CHORIONIC VILLI HCMV					
Negative	**18 33.3%**	**6 11.1%**	**-----**	**24 44.4%**	**26.125** **.000**
Moderate	**6 11.1%**	**6 11.1%**	**-----**	**12 22.2%**	
Marked	**-----**	**15 27.8%**	**3 5.6%**	**18 33.3%**	

There was significant statistical correlation between positive HCMV immunostaining of maternal endothelial lining, glandular epithelial, dedidual cells and chorionic villi with inflammation involving chorionic villi and deciduas in the studied sections- *P* value was 0.000, 0.00, 0.031 and 0.000 respectively, Correlation between necrosis and HCMV positive immunostaining are displayed in Table (2).

The HCMV immunostaining positivity of endothelial lining, glandular epithelial and chorionic villi were found to be of significant statistical correlation to cellular enlargement plus or minus vacuolation. *P*-value was 0.000, 0.02, 0,000 respectively. Among cases with no detected cellular enlargement (n=36) 50% had no HCMV positive immunostaining of decidual cells and 50% were positive, while among cases with detected cellular enlargement plus or minus vacuolation (n=18) 66.7% were positive for HCMV decidual immunostaining and 33.3% were negative; however the HCMV immunostaining positivity of decidual cells was found not to be statistically correlated to cellular enlargement plus or minus vacuolation. *P*-value was 0.245 i.e. insignificant, as seen in Table (3).

Table (2): Correlation between inflammation and HCMV positive immunostaining

	inflammation				Value & Pearson Chi-Square
	Negative	Moderate	sever	Total	
ENDOTHELIAL HCMV					
Negative	24 44.4%	18 33.3%	--------	42 77.8%	19.286 .000
Positive	--------	9 16.7%	3 5.6%	12 22.2 %	
GLANDULAR HCMV					
Negative	**21 38.9%**	**9 16.7%**	**----**	**30 (55.6%**	19.069 0.000
Positive	**3 5.6%**	**18 33.3%**	**3 (5.6%)**	**24 (44.4%)**	
DECIDUAL HCMV					
Negative	**15 27.8%**	**9 16.7%**	**----**	**24 44.4%**	6.919 **.031**
Positive	**9 16.7%**	**18 33.3%**	**3 5.6%**	**30 55.6%**	
CHORIONIC VILLI HCMV					
Negative	**18 33.3%**	**6 11.1%**	**-----**	**24 44.4%**	**26.125 .000**
Moderate	**6 11.1%**	**6 11.1%**	**-**	**12 22.2%**	
Marked	**-----**	**15 27.8%**	**3 5.6%**	**18 33.3%**	

Table (3): Correlation between cellular enlargement plus or minus vacuolation and HCMV positive immunostaining

	cellular enlargement plus or minus vacuolation			
	Not seen	seen	Total	Value & **Pearson Chi-Square**
ENDOTHELIAL HCMV				
Negative	**36 66.7%**	**6 11.1%**	42 77.8%	**30.857 .000**
Positive	--------	**12 22.2%**	12 22.2 %	
GLANDULAR HCMV				
Negative	**12 22.2%**	**12 22.2%**	**24 (44.4%)**	**5.400 .020**
Positive	**24 44.4%**	**6**	**30 55.6%**	
DECIDUAL HCMV				
Negative	**18 33.3%**	**6 11.1%**	**24 44.4%**	**1.350 .245**
Positive	**18 33.3%**	**12 22.2%**	**30 55.6%**	
CHORIONIC VILLI HCMV				
Negative	**3 5.6%**	**21 38.9%**	**24 44.4%**	**30.938 .000**
Moderate	**---**	**12 22.2%**	**12 22.2%**	
Marked	**15 27.8%**	**3 5.6%**	**18 33.3%**	

Eighteen cases show fibrin that was seen intervillous or as intravascular plugs, 33.3% of them were associated with positive HCMV immunostaining of endothelial linning, and in cases that did not show fibrin (n=36) 16.7% of them were associated with positive HCMV immunostaining of endothelial lining. However the endothelial positivity of HCMV immunostaining was found not statistically correlated to fibrin *p*-value=0.165. also there was significant correlation of glandular epithelium, decidual cells, chorionic villi positive HCMV immunostaining with fibrin deposition. Table (4).

Table (4) Correlation between fibrin and HCMV positive immunostaining

	Fibrin			Value & **Pearson Chi-Square**
	Not seen	seen	Total	
ENDOTHELIAL HCMV				
Negative	**30 55.6%**	**12 22.2%**	42 77.8%	**1.929 .165**
Positive	**6 11.1%**	**6 11.1%**	12 22.2 %	
GLANDULAR HCMV				
Negative	**21 38.9%**	**9 16.7%**	**30 (55.6%**	**.338**
Positive	**15 27.8%**	**9 16.7%**	**24 (44.4%)**	**.561**
DECIDUAL HCMV				
Negative	**18 33.3%**	**6 11.1%**	**24 44.4%**	**1.350 .245**
Positive	**18 33.3%**	**12 22.2%**	**30 55.6%**	
CHORIONIC VILLI HCMV				
Negative	**18 33.3%**	**6 11.1%**	**24 44.4%**	**3.375 .185**
Moderate	**9 16.7%**	**3 5.6%**	**12 22.2%**	
Marked	**9 16.7%**	**9 16.7%**	**18 33.3%**	

Discussion :

Miscarriage is a common condition, and like many disorders, the correct diagnosis is essential for proper management (**Sharifa, 2014**). Among all factors implicated as an etiological factor of abortion; the only undisputed causes of spontaneous pregnancy loss are genetic, anatomic or immunologic factors **(Speroff and Fritz, 2005)**. However Viruses appear to be the most frequently involved pathogens, since some of them can produce chronic or recurrent maternal infection. In particular, cytomegalovirus during pregnancy that can reach the placenta by viremia, following both primary and recurrent infection, or by ascending route from the cervix **(Gioanni *et al.*, 2011)**. Although HCMV is considered the most common agent involved in congenital infection, the few reports on the presence of nucleic acids or viral antigens in abortion tissues and association with abortion did not point out a potential role for pregnancy loss **(Spano *et al.*, 2007).**

The cases were chosen of gestational age ranging from 9-13 weeks gestation based on data described by **Kürman, 1994**, who stated that most chromosomal anomalies related abortions occur before 8 weeks gestation, while immunological factors,and maternal anatomic factors are the most related factors to abortion after 12 weeks gestation, leaving the era in between of the most obscured etiology.

Also, this result was in line with **Maria *et al.*, 2015** who reported primary CMV infection acquired in the first trimester of gestation were detected when they were first tested at 11–12 weeks of gestation.

In the current study the mean age of studied abortion cases was 27.5 ± 5.2 years. This was in accord with **Coluganti *et al.*,** and **2007 Osama and Sara, 2013** who stated that the average age of HCMV infection was 28.5, 28.6 years respectively. They also stated that the force of infection was significantly higher in the low household income group than in the middle and upper household income groups.

This was not correlated with **Gambaratto *et al.*, 1997** results who reported that 47.9%of women attending for antenatal care in their region were HCMV seropositive and that seropositivity is

associated with increased parity and older age (>35 years), yet **Mustakangas *et al.*, 2000, Spano *et al.*, 2002 and Zaki & Goda, 2007** stated that there is no correlation of HCMV infection to age or parity.

In our study four pathologic morphologic features were studied in the collected cases for anticipating the unevidently detected HCMV infection. It was reported by **Spano *et al.*, 2002** that HCMV infection of abortive tissue have reported pathologic changes ranging from massive destruction of villi by severe necrotic inflammation to absence of lesions with or without fetal injuries. They also reported that, no HCMV inclusion bodies could be detected in their studied abortive cases although were proven to be positive for viral antigens and had evidence of complete viral replication of HCMV in trophoblastic cells of first trimester abortion.

Cunningham *et al.*, 2001 reported that on viral infections in pregnant women, there is not necessarily histological evidence of placental involvement, even if it was involved. Also, **Osman and Sara, 2013,** reported that histopathological results showed 46%.While 54% showed no changes.

The studied pathological features were necrosis, inflammatory cellular infiltrates, cellular enlargement plus or minus vacuolation and fibrin; intervillous and intravascular plugs.

Studying and correlating these features to HCMV infection of abortive tissue were based on studies made and data gained from **Spano *et al.*, 2002,, Mc Galli *et al.*, 2004, Becfort *et al.*, 2004,** and **Geneva foundation for medical education & research, 2008**.

In this study necrosis, was described to assess the state of corruption of the abortive tissue in relation to positivity of HCMV immunostaining, hence, infection. This is in accord with **Spano *et al.*,** studies in year, **2002** who described necrotic lesions in their positively stained HCMV abortive specimens.

Inflammatory cellular infiltrates at all levels of the abortive tissue were also studied in the current study. The results were also correlated to positivity of HCMV immunostaining, hence, infection. This was also described by **Spano *et al.*, 2002**, who correlated inflammatory lesions in abortive tissue to their positively stained HCMV abortive specimens.

Kürman, 1994 stated that infections that involve the chorionic villi can be presented without inflammatory cells, but with stromal fibroblastic proliferation and that villous stromal fibrosis on advancement of infection contributes to fetal death and pregnancy termination.

The cellular enlargement ± vacuolation studied in our current study was also described and interpreted to positivity of HCMV immunostaining, hence, infection according to **Mc Galli *et al.*, 2004**.

As regards fibrin it was present intevillous and as intravascular plugs in our study. Intervillous fibrin implication was described by **Becroft *et al.*, 2004** who connected its presence to placental pathologies that may include immune-complex deposition in placental infections among other pathological contributors. Intravascular fibrin plugs was described by **McGalli *et al.*, 2004** who stated that intravascular fibrin thrombi of small capillary sized blood vessels is probably secondary to HCMV infection of endothelial cells. They also stated that they were the first to notice that intravascular fibrin plugs is correlated to positivity of HCMV immunostaining, hence, infection.

HCMV immunostaining positivity was correlated to certain pathologic criteria providing a clue of connection between HCMV and the described pathological criteria.

In this study HCMV immunostaining positivity was described and interpreted in the following sites vascular endothelial lining of maternal vessels, maternal glandular epithelium, decidual cells and chorionic villi.

This was also described by **Periera & Maidji (2008)**, They demonstrated that virus spreads from infected uterine vessels, represented by endothelial positivity, amplified and replicated in the decidual cells represented by decidual HCMV immunostaining positivity and disseminating to the placenta in immune complexes represented by chorionic villi positivity.

Also, the glandular epithelium lining was encountered in our study as it was reported by **McGalli *et al.*, 2004** that the glandular epithelium lining of the female genital tract is a reservoir site of HCMV and by **Penta and Luckic, 2003** as site of HCMV dissemination to the fetus.

In our study HCMV immunostaining positivity of vascular endothelial lining was seen in 22.2%, it was found that it has significant statistical correlations to all described pathological features except for presence of fibrin. We also found HCMV immunostaining positivity of glandular epithelium in 44.4%; it was of significant statistical correlations to all described pathological features except for presence of fibrin. HCMV immunostaining positivity of decidual cells was seen in 55.6%, it was found of significant statistical correlations to necrosis and to inflammatory cellular infiltrates but no significant statistical correlations were found for cellular enlargement plus or minus vacuolation or for presence of fibrin.

We demonstrated in our study that chorionic villi HCMV immunostaining positivity was seen in 55.6%, (22.2%) showed moderate positivity and 33.3% showed marked positivity. It was of significant statistical correlations to necrosis, to inflammatory cellular infiltrates and to cellular enlargement plus or minus vacuolation but not for presence of fibrin.

Our results were in accord with **McGalli *et al.*,**

2004 and Periera and Maidji, 2008, who reported that the described histopathological features are suggestive for presence of HCMV infection, yet **McGalli *et al.*, 2004**, postulated fibrin to be an associated histopathological finding of HCMV infection. In our study we could not find significant statistical correlation of fibrin, either intravascular or intervillous to HCMV immunostaining positivity. This could be attributed to that fibrin presence is probably related to hypoxia &acidosis as demonstrated by **Becroft *et al.*, 2004** which may be evident in later termination of pregnancy than our studied group.

Conclusion

It was found that our studied histopathological criteria are related to HCMV infection and that there is a high prevalence of HCMV positive cases in our studied group (67%). This could be attributed to high incidence in our community and to selection of cases based on trying to exclude most commonly related causes and in choosing those with no obviously related abortion causes to any proposed etiology.

References

1. Basim M H. 2014. Study the Prevalence of ACL, APL, CMV, HSV, Rubella and *Toxoplasma Gondii* in Aborted Women in Baghdad. Medical Journal of Babylon 10 :2.
2. Becroft D.M, Thompson J.M., Mitchell E.A., *et al.,* 2004. Placental infarcts, intervillous fibrin plaques intervillous thromi, incidence, coocurances an epidemiological associations. Hum. Pathol. 35:729-733.
3. Colugnati F.A., Staras S.A., Dollard S.C., and Cannon M.J. 2007.Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. BMC. Infect. Dis. 7: 71.
4. Cunningham F., Gant N., Leveno K., Gilstrap L., Haut J., and Wenstrom K. 2001. The placenta and fetal membranes. In Williams Obestetrics. 21st edition, Prentice –hall International. 5:85-108.
5. Geneva Foundation For Medical Education & Research. 2008. CMV & Abortion. Available at WWW.gfmer/selectedimages.
6. Gioannin N, Manuela M, Elisabetta M, Gian C D,, Gaspare C & Maurizio M. A:2011. Role of the infections in recurrent spontaneous abortion. The Journal of Maternal-Fetal and Neonatal Medicine, volum 24, 983-989 Jahromi AS, Makiani MJ, Farjam MR, Madani A, Amirian M, Eftekhri TE, Hamidipour S.2010. Cytomegalovirus Immunity in Pregnancy in South of Iran. Am. J. Infect. Dis., 6(1): 8-12.
7. Kenneson A., and Cannon M. J. 2007. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev. Med. Virol 17:253–276. 32.
8. Kürman R.J. (editor). 1994. Blaustein's Pathology of the female genital tract. 4th ed. Springer-Verlag New York. Inc.
9. Maria G R, Cecilia T, Giulia M, Valentina F, Alessandra S and Arsenio .2015. Prevention of Primary Cytomegalovirus Infection in Pregnancy. Bio Medicine. Sep; 2(9): 1205–1210.
10. Mc Galie C. E., McBride H.A., and McCluggage W.G. 2004. Cytomegalovirus infection of the cervix: morphological observations in five cases of a possibly under-recognised condition. J. Clin. Pathol. 57:691-694.
11. Mustakangas P., Sarna S., Ämmälä P. Muttilainen M., Koskela P. and Koskiniemi M. 2000. Human cytomegalovirus seroprevalence in three socioeconomically different urban areas during the first trimester: a population –based cohort study. Int. J. Epidemiol. 29.3.587-591.
12. Osman and Sara A A. 2013. Detection of Cytomegalovirus in Product of Conception Using Histopathological Immunohistochemical, and Serological. College of Medical laboratory science 11-Jun.
13. Penta M. and Luckic A. 2003. Infectious agents in tissues from spontaneous abortion in the first trimester of pregnancy, New Microbiology. 26.329-337.
14. Pereira L. and Maidji E. 2008. Cytomegalovirus infection in the human placenta: maternal immunity and developmentally regulated receptors on trophoblasts converge. Curr. Top. Microbiol. Immunol. 325:383-95.
15. Redline R.W., Zarogoza M. and Hassold T. 1999. prevalence of developmental and inflammatory lesions in non molar first-trimester spontaneous abortions. Hum. Pathol. 30:1.93-100.
16. Roya S, Mohsen M, Hossein Z, Abbas R, and Ali G.2014. Seropositivity of cytomegalovirus in patients with recurrent pregnancy loss. J Res Med Sci. Mar; 19(Suppl 1): S22–S25.
17. Sharifa A A.2014. Value of Histopathologic Examination of Uterine Products after First-Trimester Miscarriage. Bio Med Research International Volume 2014 (2014), Article ID 863482, 5 pages.
18. Spano C., Pereira L., Basso N., and Mercon-de-Vargas P.R. 2002. Human cytomegalovirus infection and abortion: an immunohistochemical study. Med. Sci. Monit. 8(6):Br 230-5.
19. Speroff L., and Fritz M. 2005. Recurrent early pregnancy loss. In: Speroff Clinical Gynecologic Endocrinology and Infertility, 7th edition, Williams and Wilkins, Baltimore, MD, USA, Part III, pp.1068-1101 and Fritz Colugnati F.A., Staras S.A., Dollard S.C., and Cannon M.J. 2007.Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. BMC. Infect. Dis. 7: 71.
20. Zaki M. and Goda H. 2007. Relevance of Parovirus 19, Herpes Simplex virus 2, and Cytomegalovirus virologic markers in Maternal Serum for Diagnosis of Unexplained Recurrent Abortions. Arch. Pathol. Lab. Med. 131:956-960.

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