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Molecular and Immunological study of Human Cytomegalovirus, Epstein Barr virus and Human Herpes virus-6 among patients with fertility disorders

A Thesis

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1.1. Introduction

The decreasing birth rate is one of the most important social problems facing developed countries today, with the fact that the number of infertile couples in many countries is on the rise (Miyamoto *et al.,* 2012).

The inability to get pregnant after a year of unprotected sexual activity or infertility was inability to conceive after one year of regular intercourse under the age of 35, or after six months over the age of 35 in women (Vander Borght & Wyns, 2018).There are two types of infertility, primary for a couple with no children, or as secondary after having one child (Abdelhameed, A. *et al,* 2020). Depending on gender, male infertility means men can not fertilize women (Barratt *et al.,* 2017) , and Around 20-30% of infertility is male. Male infertile due to changing sperm concentration, motility, or morphology causes 40–50% of human infertility (Agarwal *et al.*, 2015; Silea *et al*., 2019). Female's infertility creates pregnancy problems; up to 70% of infertility instances are attributed to the female reproductive system's complexity, like polycystic ovary syndrome, abnormal hormones, premature congenital uterine defects, ovarian failure, endometriosis, vaginal infections, , fallopian tube obstruction, or other medical disorders (such as thyroid problems and diabetes) (Benksim *et al.*, 2018).

Idiopatic infertility is a type of infertility in which all of the results of routine tests, such as ovulation tests (serum progesterone level), hysterosalpingograms for tubal and uterine patency, and spermiograms for semen analysis, are normal (Kumari P. *et al.*,2017). The prevalence of idiopathic infertility ranges from 15 to 25% among infertile couples after their diagnostic procedures (Practice Committee of the American,2006). Pathological agents, mainly viruses, are important causes of idiopathic infertility, especially secondary infertility (Tsevat *et al*., 2017).

Herpesviruses are big DNA viruses grouped into the α-herpesviridae, β-herpesviridae, and γ-herpesviridae families (Bezold *et al*., 2007). The

Herpesviridae family is divided into three subfamilies and genera based on antigenic cross-reactivity, genome size, and structure(Burrell *et al.*, 2017).

Cytomegalovirus (CMV) and Human Herpes virus-6 (HHV-6) were related to Subfamily: *Betaherpesvirinae* (Tomtishen, 2013); (Salahuddin *et al.*, 1986), while Epstein Barr virus (EBV) was related to *Gamma herpesvirinae*(Alhakim, 2015). Earlier studies related CMV to male infertility because viral nucleic acid invades the neurons that generate pituitary-stimulating enzymes, destroying their molecular integrity. These viruses now affect pituitary hormones like FSH and LH (Yang *et al.,* 1995).In Females, Cytomegalovirus' ability to change the host's immune response leads to spontaneous abortions, fetal abnormalities, complete abortion, early delivery of infants with congenital defects, and infertility (Saraswathy *et al.,* 2011).

Also, Infertility has been linked to EBV and some other herpes viruses. EBV has recently been observed to present in seminal fluid. (Huerta *et al*., 2021) .Epstein-Barr virus is rarely linked to female infertility ,like Ovarian failure may be caused by Epstein-Barr virus infection in combination with severe infectious mononucleosis and autoimmune disease (Virant-Klun & Vogler, 2018). Primary unexplained infertility may be caused by HHV-6 A infection of the endometrial Natural Killer cells, epithelial cells, and maybe trophoblasts. This may prevent implantation, which is necessary for pregnancy. HHV-6A DNA was found in endometrial epithelial cells of approximately 43% of women with primary unexplained infertility (Komaroff *et al*., 2021).

Interleukin-8 (IL-8) IL-8, commonly known as CXCL8, is a cytokine produced by the *IL-8 gene* that belongs to the CXC chemokine family. IL-8 helps migrate certain types of cells to sites where there is tissue damage and inflammation (Rutz *et al*., 2014).Interleukin-23 (IL-23) is a novel member of IL-12 family (Oppmann *et al*., 2000). IL-23 may play a critical role in pro-

inflammatory factors, primarily acting on memory T cells and impacting immune function and anticancer and anti-infective effects (Qian *et al*., 2011).

The immunoregulatory cytokine interleukin-10 (IL-10) is essential for controlling inflammation. IL-10 has inhibitory action on pro-inflammatory cytokine production(Minshawi *et al*., 2020), Interleukin-10 could be an anti-inflammatory and inflammation-controlling cytokine. (Commins *et al.*, 2010; Marron *et al.*, 2018).Interleukin-9 (IL-9) was a pleiotropic Th2 type cytokin , as a member of a growth factor of cytokines that had pleiotropic functions in the immune system(Goswami & Kaplan, 2011). Pro-inflammatory cytokine IL-9 plays a role in the pathogenesis of chronic inflammatory disorders like rheumatoid arthritis, (Gounni *et al*., 2004).

The complement system is composed of more than 30 different proteins and. The complement system (C) is an enzyme cascade of proteins that is an essential component of the innate immune system(Mahdi *et al*., 2010). Complement protein C3, which is a critical protein in all complement pathways, is the complement protein that is found in the highest concentration in blood.Complement C4 is a key molecule in the complement system by helps the body quickly recognize and kill microbes trying to invade it(Helmy *et al*., 2006).

Beta2 macroglobulin levels in the blood and plasma have been identified as markers for cellular immune system activation and a tumour marker in certain hematologic malignancies (Range, 2020).

1.2. The aim of the study

1. Identifing the aetiology and viral effect of (CMV, EBV, and HHV-6) on unexplain infertility.
2. Detrmine the gentic variation of DNA sequences of CMV, EBV, and HHV-6 genomes,and determine the relationship between the viruses via a phylogenetic tree.

فحصها (S3و S4 و S6) والتي كانت مناسبة في المنطقة المجاورة مباشرة لسلالتين أوروبيتين من نفس تسلسل .فيما يتعلق بعينة EBV ، عينةS1 ، أشارت نتائجنا إلى عدم وجود أي تغاير للحمض النووي في هذه العينة حيث شوهد تماثل كامل مع التسلسلات المرجعية لـ EBV تم الاستدلال عليه من الشجرة التي كانت سلالة أمريكية لان لها نفس التسلسلات الفيروسية ، وقريبة للعديد من السلالات الصينية. فيما يتعلق بعينات HHV-6وS10 – S7 ، أشارت نتائجنا إلى عدم وجود أي تغاير للحمض النووي في هذه العينة حيث شوهد تماثل كامل مع التسلسل الخاص به من HHV6 تم الاستدلال من الشجرة على أن عيناتنا التي تم فحصها كانت من نوع HHV-6A ، وكانت بالقرب من سلالة أفريقية لها نفس التسلسلات الفيروسية.

فيما يتعلق بالسيتوكينات للكشف المصلي ، أوضحت الدراسة الحالية أن التركيز العالي للبين الابيضاضي -8(156.9 ± 72 بيكو مول / مل) بين الذكور المصابين بالعقم بالمقارنة بالذكور الذين لديهم خصوبة ، مع اختلاف معنوي .(P = 0.001) بالإضافة إلى ذلك ، كان IL-8 أعلى في الإناث المصابات بالعقم مجهول السبب (200.3 ± 80) مقارنة بالإناث الخصبات (47.6 ± 13) ، مع اختلاف معنوي (P = 0.001) .أظهرت النتائج الحالية أن البين الابيضاضي-23 كان له اختلاف في الذكور الأصحاء (645.2 ± 37) لصالح الذكور المصابين بالعقم (805.7 ± 172) وأظهرت فرقا معنويا (P=0.001). في الإناث الخصبات كان المستوى المصلي للبين الابيضاضي -23 (28 ± 610.9) بينما كان مستوى IL-23 للإناث المصابات بالعقم (716.3 ± 70). ، مع ظهور نتائج معنوية(P = 0.001) .

بينت هذه الدراسة أن البين الابيضاضي 10 (بيكو مول\مل) في مجموعة الذكور المخصبين كان (75.1 ± 9) والذكور المصابين بالعقم (114.2 ± 45) مع وجود فارق معنوي (P = 0.002) ، بينماالإناث الخصبات كان لديهن مستوى البين الابيضاضي-10 (15 ± (64.74) والإناث المصابات بالعقم كان

3. Serological detection of the serum level of intrlukins(IL-8, IL-23, IL-10 , IL-9) that have a role in increasing the disease complication for idiopathic infertile for both males and females ,and detection of the serum level of complements (C3 and C4) for infertile males and females.