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INVESTIGATION OF CYTOMEGALOVIRUS AMONG MALE INFERTILITY DISORDERS

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RASOOL

DECLARATION

Hereby, I declare that this thesis study is my own study, I had no unethical behaviors in all stages from planning of the thesis until writing there for, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list; and, had no behavior of breeching patent rights and copyright infringement during the study and writing of this thesis

Aras Othman Rasool Rasool

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LIST OF ABBREVIATIONS

CID	**Cytomegalic Inclusion Disease**
HHV-5:	**Human Herpesvirud-5**
DNA:	**Deoxy Ribonucleic Acid**
HHV	**Human Herpesvirus**
HSV-1:	**Herpes Simplex Virus Type 1**
HSV-2:	**Herpes Simplex Virus Type 2**
VZV	**Varicella Zoster Virus**
EBV	**Epstein–Barr Virus**
HHV-6	**Human Herpesvirus 6**
HHV-7	**Human Herpesvirus 7**
HHV-8	**Kaposi Sarcoma-Associated Herpes Virus**
HCMV	**Human Cytomegalovirus**
CMV	**Cytomegalovirus**
IE	**immediate early gene**
E	**Early (E) gene**
L	**late (L) gene**
pp	**Phosphoprotein**
kbp	**Kilo Bite Protein**
CNS	**Central Nerve System**
ORFs	**Open Reading Frames**
ER	**Eendplamic Reticulum**
Ab	**Antibody**
Ag	**Antigen**
IgM	**Immunoglobulin M**
IgG	**Immunoglobulin G**
HLA	**Human Leukocyte Antigen**
CD13	**Cluster Of Differentiation**
HIV	**Human Immunodeficiency Virus**

ASA	**Antisperm-Antibodies**
STD	**Sexually Transmitted Diseases**
CM	**Cervical Mucus**
T-Cell	**T Lymocytic Cell**
NK	**Natural Killer Cell**
gC	**glycoprotein C**
gB	**glycoprotein B**
gN	**glycoprotein N**
gM	**glycoprotein M**
gO	**glycoprotein O**
gL	**glycoprotein L**
gH	**glycoprotein H**
Fc	**Fragment Crystallization**
IL-1-β	**Interluken-1-Beta**
TNF-α	**Tumor Necrotic Factor-Alfa**
TLR	**Toll Like Receptor**
AGG	**Agglutination**
ROS	**Reaction Oxidative Stress**
PCR	**Polymer Chain Reaction**
FSH	**Follicle Stimulating Hormone**
BTB	**Blood Tests Barrier**
LCS	**Leukocytospermia**
WBC	**White Blood Cell**
RBC	**Red Blood Cell**
CPE	**Cytoplasmic Effect**
TORCH	**Toxoplasmosis Rubella Cytomegalovirus Herpes simplex**
dGTP	**Deoxyguanosine Triphosphate**
GCV	**Ganciclovir**
MAR	**Mixed Antiglobulin Reaction**
ESHRE	**European Society For Human Reproduction And Embriology**

WHO	**World Health Organization**
PH	**Potential Hydrogen**
CO2	**Carbon Dioxid**
IVF	**In Vitro Fertilization**
ICSI	**Intracytoplasmic Sperm Injection**
PMN	**Polymorphonuclear Leukocytes**
EP	**Epithelial Cell**
ELISA	**Enzime Linked Immunosorbent Assay**
SFA	**Seminal Fluid Analysis**
SPSS	**Statistical Package for the Social Sciences**
IGC	**immature germ cell**

ÖZET

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Erkek üreme sistemine sitomegalovirüsünün (CMV) varlığının, spermatozoa ve infertilite bozuklukları üzerinde potansiyel olarak etkisi olduğu düşünülmektedir. Infertilite problemi olan erkeklerin semen örneklerinde CMV sıklığını değerlendirmek için semen parametreleri ile yapılan çalışmalar bulunulmaktadır. Araştırmamızın çalışma grubunu spermlerinde anormal parametreler bulunan erkeklerden oluşmakta ve CMV enfeksiyonu prevalansı ve riskinin araştırılması hedeflenmştir. Ayrıca, CMV enfeksiyonunun prevalansının Irak-Erbil kentindeki erkekler arasında yüksek olması nedeni ile, CMV enfeksiyonunun değişen seminal sıvı parametrelerine neden olan herhangi bir etkisi olup olmadığının araştırılması hedeflenmiştir.

Çalışmamızda toplam 152 hasta (100 hasta grubu, 52 kontrol grubu) yer almaktadır. CMV IgG ve CMV IgM sonuçları ELISA yöntemi kullanılarak değerlendirilmiştir. Sperm örnekleri hem mikroskobik hem de makroskobik olarak değerlendirilmiştir. 25 hastanın CMV IgM pozitif ve 100 hastanın tamamı IgG pozitif idi. Kontrol grupları, istatistiksel analiz konsantrasyon, sayım, morfoloji, beyaz kan hücresi,epitel hücresi, aglütinasyon gibi semen parametresi arasında CMV IgM'nin P değeri hesaplanmıştır. CMV-IgG ile konsantrasyon, sayım, hareketlilik, morfoloji, kırmızı kan hücresi, beyaz kan hücresi ve Germ hücrelerinde istatistiksel olarak değerlendirilmiştir. Sonuç olarak çalışmamızdaki temel, infertil erkeklerde CMV enfeksiyonu prevalansının, kontrol gruplarına göre daha yüksek olduğunu ve CMV IgM ve CMV IgG türlerinde tip arasında az sayıda farklı belirti olduğunu göstermiştir. CMV enfeksiyonu öncesinde seminal sıvı parametresi erkek kısırlığının önemli bir parçası olarak düşünülebilir. Semende CMV enfeksiyonu ile infertilite arasındaki ilişki önceki çalışmalarda elde edilmiş ve çalışmamız verilerini desteklemektedir.

Anahtar Kelimeler: İnfertilite; Sitomegalovirüs; Sperm; ELISA

ABSTRACT

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The presence of cytomegalovirus (CMV) in male genital its influence with spermatozoa and the development of a potentially effect on male infertility disorders. And to evaluate frequency of CMV in the semen samples of men with infertility problems referring to the parameters of semen. In order to investigate the latest prevalence and risk of multiple CMV infection in men who suffer from abnormal parameters in their semen, as well as the types of maternal CMV infection associated with congenital CMV infection among their generations. The main object is investigation CMV infection prevalence is high among males in Iraq-Erbil city, following if there is any influence of CMV infection that leads to changing seminal fluid parameters. In this study ELISA method used to detect both type CMV-IgM and IgG, there was 152 samples involve in this study 100 of them was patient how suffering infertility history and the other group was control group use in this study to compare the patints result with. The results of the experiment show that there were from 100 samples 25 patients samples were positive for CMV IgM and 100 were positive for IgG with seminal fluid analysis .Also 100 patient have at least have 4 abnormality in semen sample analysis and comparing with 52 cases in control groups, the statistical analysis show P.value (< 0.05) of CMV-IgM among semen parameter in concentration, count, morphology, WBCs, EP cell, agglutination. Indifferences, with type of CMV-IgG were significamt found in volume, concentration, count, motility, morphology, RBCs, WBCs, Germ cell. Foundation in our study as a conclusion showed that the prevalence of CMV infection was higher in an infertile men compared to control groups and there was few different signification among type if CMV-IgM and CMV-IgG, but in both

cases there was visible affect CMV on seminal fluid parameter.Therefore CMV infection can be considered as an important part of male infertility. The relationship between CMV infection in semen and infertility was obtained in previous studies and was confirmed by our study.

Keywords: Infertility; Cytomegalovirus; Semen; ELISA

CHAPTER 1

1. INTRODUCTION

1.1. Cytomegalovirus (CMV)

1.1.1. History

Ribbert in 1881 wrote that in section of kidney unit he note there is swallowing cell in dead childbirth in addition he was unable to interpret children parotid gland later on he review some papers include Kiolemenoglou paper with Jesionek, They defined these swallowing cells alike as “protozoan like cells” in various type of organ such as (liver, lungs and kidney of children eight months of gestation. The unusual appearance of nuclei in side cell more huge than normal nuclei cells resemble like “central nuclear body”, when Lòwenstein works in the laboratory relate to Ribbert’s he discover that there is numerous of these huge cells in 30 infant when he working on their parotid glands, and this lead another scientist named Wrst explanations of cytomegalic cells with inclusions of intranuclear (Ho, M, 2008). Lipschutez discover some cells scratches with associated intra-nuclear insertion of patient diseased related to genitals herpes /or herpes zoster, following, Von Glahn and Pappenheimer they supposed such as unusual tissue/or cell that formed via virus, and they thought at that time it is interrelated with protozoa, later on, scientist name Wrst suggestion those abnormal cells appear like inclusion body (owl eye) cause by a group of family relate to viruses, nowadays recognized this group by herpesviridae family, there is some cases in 1932 of lethal congenial that they have some characterized as petechiae, hepatosplenomegaly, and intracerebral, all of them described intranuclear inclusions by calcification, after that named as “generalized cytomegalic inclusion disease (CID). After that Weller named the virus “cytomegalovirus” (Ho, M, 2008).

1.1.2. Classification

According to general feature such as biological properties, structure, size, and arrangement of their deoxynucleotide sequences, even though the morphology of the virion they classify to the family Herpesviridae (Roizman, B., 1982; Roizman, B., et al, 1981). Cytomegalovirus (CMV) are associated with lymphotropic pertaining disease (Salahuddin, S. Z., et al, 1986), the CMV also termed as human cytomegalovirus (HCMV) which is one of the members of the herpesviridae family recognized as Herpesviruses /or human herpesvirus-5 (HHV-5) (Mozaffar, M., et al 2018; Leung, A., et al, 2003), which related to individual of more complexes virus follower that relate to one of largest DNA virus (Nelson, C., et al, 1997; Frenkel, N., et al, 1990).There are currently eight membrane families are include: (1) herpes simplex virus type 1 which abbreviated as (HSV-1), (2) herpes simplex virus type 2 known as (HSV-2), (3) varicella zoster virus summary as (VZV), (4) Epstein–Barr virus shortened as (EBV), (5) human cytomegalovirus condensed as (HHV-5), (6) human herpesvirus 6 noun as (HHV-6), (7) human herpesvirus 7 summarized as (HHV-7) and (8) Kaposi sarcoma-associated herpes virus (HHV-8) (Koskinen, P., et al, 1999). Also there are more than one species relate to CMV have been identified which supply for different mammalians.

CMV is a beta-herpesvirus that causes lifelong infection in humans (Liu, X., et al, 2013). Also CMV is one of those viruses that spread in whole world wide, in normally person didn't possessing any symptoms whatever human harbor CMV and spread to end-organ especially in those individuals with weak immune system dysfunction, until know around 150 memberships relate to herpes virus type recognized as it is associate to humans complication (Ngai, J. J., et al, 2018).

1.1.3. Structure of Virus

1.1.3.1. Capsid

Genomic material of CMV consist of ds-DNA linear, kilobases size consist nearly 240 (150x106 daltons) with ability of isomerization (Nelson, C., et al, 1997), the sequenced of genome will be complete with express have non-overlapping open-reading frames approximately 200 potentially protein relate to immunologic (Numazaki, K., & Chiba, S., 1997), the 162 capsomeres create capsid icosahedral in shape and this capsid will surround genomic material of virus (Nelson, C., et al, 1997), regulated genomic sequence of CMV transcribed after infection, this processes undergo three way, the first viral gene products with protein involve immediate early gene (IE). Following, second viral gene noun as Early (E) gene products by virus and it has role in genomic material of virus during duplication. This group of gene have role in targeting antiviral drug, the last gene produce is late (L) gene these genes code for viral structural proteins (Numazaki, K., & Chiba, S., 1997).

1.1.3.2. Tagument

CMV capsid has been surrounded by the layer called the tegument and this tegument itself also surrounded by a loose envelope having embedded viral glycoprotein complexes. The structure resemble as tegument is composed of 20 proteins which include 9 Phosphoprotein 65 (pp65), pp150 etc., with pp65 are the majority representative, the immunogenicity turn to these proteins because they have ability to decontrolling the cellular cycle of the host cell and important protein that use in diagnosis is pp65, also it can be seen in nuclei of polymorphonuclear granulocytes during infection, for endothelial cell infection these granulocytes are acquired, it is transport to nucleus fibroblasts directly after fusion in virion during in-vitro infection. These structures create mature particle of complete viral and it is around 200 n in diameter (Nelson, C., et al, 1997; Ayensu, F., 2014).

1.1.4. Viral Genome

CMV are very hard study genetically because of some special property of virus such as slow replication, large size of the genome, limited host cell range in culture and for experimental on animal there is no model animal for CMV. CMV genome is a double DNA particle consist near 235 kbp it is higher than other human herpesviruses possessing and more complex, and genome of virus strain consist of two region which are UL and US, these are two areas that CMV consist the UL=174 kbp and it is introduced among reversed recurrences of 10-6 kbp and Us equal to 35.6 kbp and it is bordered in small upturned recurrences 2.4 kbp, they are concerned with one or the other way which are result in genomic pre-mutation of four isomer relate to virus and it is happen in equal magnitudes, By using method cross-blot hybridizations with extra conformist plotting trials the DNA map cleaving from CMV made with a number of limited endo-nucleases, in addition when DNA fragments were molecularly cloned in plasmids and cosmids physical mapping was perform. In some paper experimental clarify regions of virus-cell homology and determines their map positions of the genome within the viral and compared together (Bankier, A., et al, 1991; Rüger, R., et al, 1984).

1.1.5. Replication

CMV is one of the fewest viruses that have the characteristic of slow replicating basis of time to appearance of cytopathic effect in cell culture than other Herpesviridae membrane when make comparison (Emery, V., et al, 1999). In many of experimental study event on animals mention that it is difficult to understand viral replication and it is the mechanism when experimental performs in animal models but also there is many mechanism of virus were under stud such as determination of viral particles with it is action associated with immune system in both acute and chronic state of infection,

which are clarified in some of experimental study on rhesus macaque CMV and rodent infection (Britt, W., 2008).

There is variability in HCMV infects and replicates according to tissue/or cells such as (glands associate with epithelial cells, macrophages, muscle cells type of smooth, hepatocytes, vascular endothelial cells, dendritic cells, , mucosal tissue, fibroblasts), because of the large cell tropism viral spread in all human body, following infection CMV lead to suffering latency and this latency cause a life-long infection to patient with irregular repetition, and the one of the dangerous feature of CMV are in normal healthy person didn't poses any symptoms relate to CMV but in patient that have low immune defense for example patients with surgical operation of organ transplant recipients or infect with HIV may appear symptoms. In many study mention around 99% of population suffering CMV the main cause turned to vertical transmission during pregnancy thought placenta and it is cause of congenital disease in childs and fetus (Jean Beltran, P. M., & Cristea, I. M., 2014).

To encode functional protein capacity, the HCMV genome was assessed and it is about 192 open reading frames (ORFs) expressive the largest genome of the herpesviruses and prolonged potential coding revealing great level of genome complication this code protein express in infectious virion particles or in any stage of infection, these dynamic virus-host interactions are necessary attractive variation of cellular functions and these are necessary in order successful viral replication take place then spread. (Jean Beltran, P. M., & Cristea, I. M., 2014).

The structure of envelope cover outside virion and it are during entrance of the virus interact to host receptor the mechanism of endocytosis of virion occur toward the cell and the tegument are liked to capsid, it is supposed interact with microtubule of the host in order to transport capsids in to nucleus, when transcription of the virus take places the genome replication encapsidation arises following tegument proteins sit in infected cell with other virion directed in to various tissue, or organs in order to escape from immune and arrange gene expression. Later of the capsid assembled in the nucleus and release to cytoplasm then producing virion are followed and it is transport via cellular trafficking pathways, after that cellular parts as Golgi apparatus, endoplasmic

reticulum (ER), and endosomal machinery will controlled and formation of cytoplasm artist, within the AC the tegument layer and envelop in intracellular vesicles acquire by capsid following steps the next step is released into the extracellular space (Jean Beltran, P. M., & Cristea, I. M., 2014).

1.1.6. Epidemiology and Transmission

If you get infection with CMV for a while ago may resulting viral shed in to body secretion and urine and it is major reasoned for spreading viruses, there is chance to get CMV from the infected person, close contact, vertical transmission from mother to fetuses, children contact with secretion like saliva or kissing, blood recipient, body organ transplantation as (Bone marrow, liver, heart, kidney,…et), these are mane way to get infection and if you got infection from first time known as primary infection, the second time infection resulting from recurrence infection know as latent infection or reinfection (Salahuddin, S. Z., et al, 1986).

CMV infection establishes a long-lasting persistence in salivary glands and shed the virus in saliva for several months before termination of productive infection and establishment of latency (salivary gland virus) and isolated in humans in the 1950s, can establishes latency (lungs, liver, spleen, suprarenal glands, salivary glands and kidney) (Krmpotic, A., et al, 1003). HCMV has a prevalence of 55-99% within the human population, depending on different socioeconomic and geographical factors, but they can be life-threatening in an immunocompromised patient (Kim, J., & Lee, S., 2018). The course of infection in healthy individuals is mostly asymptomatic, but CMV is a major cause of morbidity and mortality in immunocompromised individuals. The spectrum of disease expression is broad, with CMV receptivity of nearly all organs (Revello, M. G., & Gerna, G., 2002; Demmler, G. J., 1994), for example, in normal hosts there is development of a mononucleosis syndrome with persistent fever, myalgia, and cervical adenopathy and complications such as hepatitis, vasculitis, involvement of the respiratory tract (e.g., interstitial pneumonia) and the heart (pericarditis, dilatative myocarditis), the gastrointestinal tract (esophagitis, gastritis, ulcerative colitis, pancreatitis), or the central nervous system (CNS, aseptic meningitis, encephalitis,

polyradiculitis), including severe ophthalmologic complications (uveitis, necrotising chorioretinitis) (Grundy, J., et al, 1998; Craigen, J., et al, 1997).

Endocrine organs such as the adrenal gland are frequently involved, and dysfunction may contribute to the ‘‘unspecific constitutional symptoms’’ often observed in CMV-infected individuals, the severity of CMV infection correlates with the grade of immunosuppression, that is, with a complex virus-host interaction involving humoral and cellular defense systems, promoting and protective factors, virus-associated immune disregulation and the immunosuppressive properties of the CMV infection itself (Grundy, J., et al, 1998; Craigen, J., et al, 1997).

Congenital CMV infection can cause morbidity, it is leading infectious cause of congenital abnormalities in the western world, affecting 1-2.5% of all live births and even death. After infection, CMV often remains latent (indicated by CMV specific IgG seropositive result), but it can reactivate at any time. (Mozaffar, M., et al 2018; Leung, A., et al, 2003). the presence of actively replicating CMV during pregnancy, whether from primary infection, reactivation from latency or reinfection, can result in congenital transmission to the fetus (Wilson, C. B., et al, 2015). Congenital CMV is a leading infectious cause of deafness, learning disabilities, and intellectual disability (Kim, J., & Lee, S., 2018), reactivation occurs when CMV is isolated in a woman known to have CMV IgG route of transmission, Infection with CMV can also occur via (i) Sexual contact (ii) Blood transfusions (iii) Vertical transmission from mother to fetus infection precautions (Rawlinson, W., & Scott, G., 2003; Buzzolini, T., 2015).

A specific concern of CMV infection is the vertical transmission with severe sequelae in infected newborns (e.g., hepatosplenomegalies with hemolytic anaemia, thrompocytopenia purpura and icterus, and in particular complications of the CNS, e.g., microcephaly, intracerebral calcifications, mental retardation, chorioretinitis, and atrophy of the nervus opthicus). Congenital CMV is the leading infectious cause of CNS maldevelopment in children and is diagnosed in about 0.2%–2.2% of newborns (Murphsu, J. R., et al, 1998). Longterm sequelae also have to be considered (hearing, speaking, and motoric defects). These complications are usually the sequelae of the

primary infection of the mother and rarely occur after reactivation of persistent infection (Revello, M. G., & Gerna, G., 2002; Spano, L., et al, 2004).

In children and adults, both primary CMV infections and reactivations are typically asymptomatic as a result, many people are unaware that they have been infected. Since clinical symptoms are usually absent, the only reliable way to estimate the prevalence of infection in a population is through laboratory testing. The right way to investigation CMV virus seropositivity in laboratory by measure of previous infection (in other word both IgM and IgG should be tested). (Lasry, S., et al, 1996; Pass, R. F., et al, 2009; Ross, D. S., et al, 2008), in this broadsheet, we reviewed the literature to identify patterns of CMV seropositivity in different populations in order to learn about CMV transmission and risk factors for infection. Establishing CMV seroprevalences by age and other demographic factors is also important for identifying populations of women who are at increased risk of primary CMV infection during pregnancy. These findings can inform behavioral interventions aimed at preventing infection in pregnant women (Bate, S. L., & Cannon, M. J., 2011; Vauloup-Fellous, C., et al, 2009), and can help identify target populations for future CMV vaccines (Ross, D. S., et al, 2008; Zhong, J., & Khanna, R., 2007). Infections with human herpesviruses CMV may lead to serious complications in solid organ transplant recipients and CMV, in particular, is associated with significant morbidity and mortality. Although CMV infection or reactivation is common in the post-transplant phase, only some of these patients develop symptomatic disease. While the degree of immunosuppression and donor seropositivity are associated with increased risk of progression to CMV disease, it is not clear that the pathogenesis of CMV diseases is explained exclusively by these two factors. There have been preliminary reports that infections with HHV cause illness in transplant recipients similar to that caused by CMV (Osman, H. K. E., et al, 1996). CMV infection in transplant recipients may be due to transmission of the virus with the donor heart, but more commonly it results from reactivation/reinfection (Söderberg-Nauclér, C., et al, 1997), and it is a major cause

of morbidity and mortality among the intrathoracic organ allograft recipients (Fishman, J. A., & Rubin, R. H., 1998; Dummer, J. S., et al, 1985).

Conversely CMV infection may induce a depressed cell mediated immune response, leading to increased susceptibility to opportunistic infections as fungal opportunistic infection (Dummer, J. S., et al, 1985; Oill, P. A., et al, 1977), CMV is a member of the Herpesviridae family of DNA viruses, which share structural similarities as well as the biological properties of latency and reactivation. CMV characteristically produces cell enlargement with intra nuclear inclusions, which led to the early designation of the term ‘‘cytomegalic inclusion disease.’’ Infected cells were originally described as ‘‘protozoon-like’’ or similar to owl eyes (Goodpasture, E. W., & Talbot, F. B., 1921; Farber, S., & Wolbach, S. B., 1932).

1.1.7. Pathogenicity

CMV can establish a latent infection, for example a type of persistent infection in which viral genome is present but only partially expressed, and infectious virus is not produced except during intermittent episodes of reactivation (Bruggeman, C. A., & Van Dam, J. G., 1998), the site of latent CMV infection is not clear, although the virus has been detected in epithelial cells in a variety of tissues and lymphocytes (Bruggeman, C. A., & Van Dam, J. G., 1998; Schrier, R. D., et al, 1985), as well as the following sequence of events occurs in the entry of CMV into a target cell (Compton, T., et al, 1992), (1) CMV attaches to cell surface heparan sulfate proteoglycans. (2) virion-heparan sulfate proteoglycan attachment is rapidly followed by high affinity, heparin-resistant attachment of the virion to the cell ((Compton, T., et al, 1993), (3) alterations in viral envelope, or cell membrane structures, permit interaction of viral glycoproteins with additional cell membrane components, and are followed by fusion of the cell membrane and virus envelope, leading to the release of CMV nucleocapsid into the cytoplasm (Compton, T., et al, 1992), other host cell proteins hypothesized to be involved in the initiation of CMV infection in permissive cells are class I HLA molecules (Grundy, J. E., et al, 1987), and CD13 (Söderberg, C., et al, 1993).

The genome of CMV can remain in cells in either a quiescent, persistent, or productive state (Bruggeman, C. A., & Van Dam, J. G. (1998), the viral genome is sequentially expressed, and the expression can be divided into three broad categories immediate early (6 hours after infection), early (24 hours & above), and late genes. The first genes transcribed after infection of a permissive cell are immediate early genes. The immediate early genes are probably transcribed by host cell RNA polymerase II, and these gene products allow the transcription of mRNA for subsequent viral proteins and possibly stimulate host cell gene expression (Davis, M. G., et al, 1987; Hermiston, T. W., et al, 1990).

1.1.8. Immunology

Antibodies of HHV5 can be identified in approximately 95 percentage of the adult in world population, much of the infection most probably occurring during pregnancy and early infantile (Clark, D. A., et al, 1993), all herpes viruses especially CMV consist of the largest number of serotypes dedicated to avoiding both innate and also adaptive protection inside the human body. CMV characterizes by a lifelong load of antigenic T-cell surveillance and immune dysfunction. (Kim, J., & Lee, S., 2018). Once you infected with HH5 human body following to produce both type of immunoglobuline IgM and IgG and it is lead to promote cytotoxic T lymphocyte, natural killer cells, Ab dependent killer cells and IgM produce after primary infection between 3 to 4 month later however it is again produce in reactive infection later on IgG will produce and it is remain for life long which indicate past infection, When immune-competent the cellular and humoral immune responses will combines responses against the virus thereby preventing severe CMV disease, Remaining virus in side body enters a stage of latency and possibility of reactive may occur in any time of immunosuppress or infection due to second strain of virus (Rook, A. H., 1988; Ayensu, F., 2014). Severe infection due to cytomegalovirus are connected in order setback of regular degree of human immune called helper T to make T cells suppression

, with complete reductions of helper T cells in addition consistently ricing T cells with suppressing it (Carney, W. P., et al, 1981).

The CMV can stimulate polyclonal immunoglobulin to produce as an in vitro (Hutt-Fletcher, L., et al, 1983), this may resulting in secretion more levels of an immunoglobulin in the blood serum termed as (Hypergammaglobulinemia), also lead to condition in which the blood covers large volumes of pathological cold sensitive antibodies called (Cryoglobulinemia) in addition to producing Ab against it is own body protein known as (Autoantibody) (Kantor, G. L., et al, 1970). There is many research experimental study conduct with clarifying that CMV have role with pro-inflammatory infection in addition to appearing IgG Fc receptors on the surface of cells (Keller, R., et al, 1976), in this case most likelihood increasing level of granulocytes take place with simple squamous epithelial tissue lining the blood vessels (MacGregor, R. R., et al, 1980).

CMV have mechanisms for the regulation and evading host immune responding including cellular cytokines production in addition coding interleukin-10 and upregulate IL-1β gene expression resulting in production more production of IL-1, CMV fixes to the cellular IL-10 receptor also result in immune suppression (Iwamoto, G. K., et al, 1990), Conversely, opposite reports exist (Reddehase, M. J., et al, 1984), there is also some other study mention of the immediate-early enhancer and it is promoting controlled by TNF-α this is lead to adaptable the balance in both state including latency and reactivation (Fietze, E. L., et al, 1994), following like other viruses belong to family of herpesviridae the virus will stay in an inactive state within body for life (Latency) and it is cause re infection in any state relate to suppressed of human immunity (Alford, C., et al, 1990; Tabatabaee, M., & Tayyebi, D., 2009).

1.1.9. Clinical Findings

Recent work has mainly focused on CMV infection in immunocompromised individuals, for example, transplant recipients, infected mother or patients positive for the human immunodeficiency virus (HIV), and CMV consider as an opportunistic pathogen cause many of disease tend to significant morbidity and mortality, most CMV infection didn't show any sign or symptom there is few of the patient that symptom are appeared there is only scarce information concerning its relevance for fertility, which is the subject of the present investigation. In general, the impact of viruses in the genital tract on male and female reproductive health has been a neglected field of interest (Dejucq, N., & Jégou, B., 2001; Detels, R.,et al, 1994; Ayensu, F. 2014), this is striking because a well-known example for virus-induced changes in the male genital tract is the mumps virus, which may directly attack the testes, destroying the testicular parenchyma, with loss of germinal epithelium and a deleterious effect on Leydig cell function and a potential impact on androgen production (Aiman, J., et al, 1980; Hedger, M. P., & Meinhardt, A., 2003).

There are several routes by which viruses might influence fertility such as the direct influence on spermatogenesis resulting in defective sperm function; inflammatory changes in the composition of genital secretions with impact on sperm-cervical mucus interaction and further sperm passage; cytokine-mediated changes in the local genital compartment as well as systemic effects in patients (and potential signal function to partners); induction of an immune response with production of antisperm-antibodies(ASA) and subsequent immunologic infertility; endocrine changes via influence on testicular Leydig cells and/or the hypothalamic-pituitary gonadal and/or -adrenal axis, decrease in libido, sexual dysfunction, behavioral changes and general morbidity of the individual, and oncogenic effects, for example, induction of the germ cell or adnexal gland tumors (Grundy, J. E., et al, 1987; Tabatabaee, M., & Tayyebi, D., 2009; Dejucq, N., & Jégou, B., 2001).

Viral infection with CMV and other herpesviridae may also increase the risk of acquiring other genital tract infections and sexually transmitted diseases (STDs) with known pathology including HIV (Wallach, E. E., & Alexander, N. J., 1990), close or even intimate person-to-person contact is believed to be required for the horizontal spread of these viruses (Revello, M. G., & Gerna, G., 2002; Demmler, G. J., 1994), primary infection is possible via the blood transfusion or organ transplantation. CMV is excreted in nearly all secretions of the human body, such as blood, urine, feces, tears, saliva, breast milk, cervical mucus (CM), and semen. CMV survives in frozen and thawed semen (Hammitt, D. G., et al, 1988). Viruses can attach to the surface of spermatozoa and sexual transmission is considered as a major route of infection with CMV, although this assumption was mainly based on serologic studies (Handsfield, H. H., et al, 1985; Chandler, S. H., et al, 1985).

1.1.10. Genotypes

The largest coding capacity of glycoprotein relate to HCMV which is containing 57 glycoproteins and they arranged by AD169 there also clinically 15 were isolates, there is several glycoproteins categorized of HCMV envelope most of them are complex with great molecular mass excluding of gpUL33-GCR and gpUL4-gp48, they characterized by show genetic polymorphism, they recognized gC-I, gC-II, gC-III.

The gC-I is glycoprotein B is envelop components and it is important for replication essential member implicated in multiple levels of virus replication role in stick , entrance to cell, fusion, spread between cells, The ORF UL55 encoded glycoprotein B in early-late unplaced transcript and it is show locus genetic polymorphism, and there is four type of glycoprotein B recognize gB-1, gB-2, gB-3, gB-4, in addition other type as gB-5, gB-6, gB-7. The gB was determined in different form gBn and gBc this is prove that many number of non-prototypic strains occurs. The total number AA of sequence determines changeably, and it is are reported as 9.5%.

The gC-II is glycoprotein N and it is divided in to two other glycoprotein named gM and gN with highly immunogenic and major heparin binding, glycol protein N have ability to induce the neutralizing antibody or immune response and ordering first attachment to host cell also it is replicative viral sequence glycoprotein M performances as a chaperone for glycoprotein N giving out and it is result in glycosylated production with complexes developed of necessary protein.

gC-II, IgM component that play important role in replication of virus and most detected nucleotide changes of codons and role translational silent mutations, targeting of the host immune response, also maintain amino acid to variable in arrangement with convert glycosylated that locate in envelope structure essential of element of developed viral, and glycoprotein N also very important for viral replication in vitro and express late the body response cannot neutralize virus protein. gN polymorphism allow to identify type of gN-1, gN-2, gN-3, gN-4 of genotype, the total number of amino acids sequenced is 50%.

The gC-III is glycoprotein H it is very complex with gH (gpUL75), gL (gpUL115) and gO (gpUL74) they stimulate immune with free of virus neutralizing and have role for facilitates of CMV to host cell without sticking ability, in cell culture isolation gH play essential role for viral replication, but the expression of this glycoprotein are late in cell infection, there is two type of glycoprotein H genotypes identified glycoprotein H-1 & glycoprotein H-2.The gL component it is play role and it is necessary for transport glycoprotein H to the top of external surface tissue/or cell following invasive of intra-cellular spots next to virus assembly, the gL-1, gL-2, gL-3, gL-4 glycoprotein are main phylogenetic were recognized, O is the third component which play role in unnecessary duplication vitro of virus, following to create a small panel phenotype, virus-mediated cell fusion, there is four major component gO1, gO-2, gO-3 and gO-4 noted of genotypes. The complete picture of strain and location renowned antigenic epitopes for the gB, gH, gO, gM, gL and gN (Pignatelli, S., et al, 2004; Brañas, P., et al, 2015).

1.1.11. Viral Cell Tropism

The protein polymorphisms that interact with the viral replication stage or spreading between cells refereeing to invasion cells and tissue by the virus inside the host, the glycoprotein B, N or O are protein that involve and its lead to conjectures among imaginable association with both (genomic variants & tropism), there is same experiment working on different tissue and cells in order to mention genotype of the virus from patient who suffering CMV infection, and there was suggestion that deferent tropism associate with different genotype for example the most viability glycoprotein that connected with male seminal fluid samples are glycoprotein B-4 even more viable in semen than leukocytes (Pignatelli, S., et al, 2004).

1.1.12. Diagnosis

The diagnosis of CMV with development technology today give a chance to detect and search for active CMV virus nowadays by several ways such as nucleic acid detection, histopathology, isolation viral by culture, serological detection, also there is some methods detect CMV virus by quality and quantitate with several methods, some of methods are very important as rapid method which my helpful event for antivirals evaluation treatment in some specific cases such as various type of transplant recipients patients (De la Hoz, R., et al, 2002).

1.1.12.1. Histopathology

The CMV in side cells are characterize by intranuclear inclusions disease the virus appear like owl's eye shape during CMV infection, also there is inclusion in many different cell types and organs as esophageal, colon, lung, liver intracytoplasmic inclusions these are the most common biopsy section are valuable, false negatives are common turned to sampling mistake especially in lung biopsy however histopathologic

diagnosis is specific but it is very difficult to differentiate between non-viral inclusions and CMV viral inclusion (De la Hoz, R., et al, 2002).

1.1.12.2. Isolation

Isolation of viruses commonly is difficult and expensive but it is very specific method for culturing and it is gold standard can detect virus 100 percentage, the culture are usually done by taken infected sample fluid and cells, the culture of CMV viruses take around of 10 – 30 days because viruses are slow in growth (Ayensu, F., 2014).
Usually CMV infected tissue solation characterized by produces (CPE) which is cytopathogenic effect and make it easy stain with fluorescent antibody, the common samples or biopsy are respiratory swabs, tissues, body fluids, urine specimen especially in case of children, washes and blood, if we make comparison with polymerase chain reaction the culture have a lower sensitivity, the prolonged sample for inoculation due to false negative. There is a method which are more reliable and much more sensitive around 70 % - 100% when compare with viral culture the method termed as shell vial method which is rapid viral culture with the benefited of method is short period to get result from 7 to 14 days (De la Hoz, R., et al, 2002).

1.1.12.3. Serological Test

Serological method are depended on reactivation between the antigen and antibody it is useful for epidemiologic studies, the method characterized by incomplete diagnosis of CMV disease at seroconversion in primary infection, there is different method used in serological side such as TORCH, enzyme immunoassay, anti-complement immunofluorescence, fluorescent antibody, complement fixation. Most commonly serological test used for diagnosis primary infection IgM or secondary (reactive) IgG infection, but primary infection are difficult to diagnose by serologically (De la Hoz, R., et al, 2002), because detection CMV-IgM antibodies are not much sensitive in evaluating cases because it is occur in most primary infection and other

reinfection, latency reactive, except in some immunocompromised individuals, however the detection of IgG class of Ab of CMV give an idea to distinguish (Ayensu, F., 2014).

1.1.12.4. Nucleic Acid Detection

Nucleic acid CMV viral detection are usually performed by polymerase chain reaction or real time polymeaser chain reaction, these method characterized by rapid and sensitive method and it is detect nuclear material in infected patient samples with CMV infection but it may cause the false positive in case of contamination, and there is different steps on different instruments for detection as DNA extraction, gel electrophoresis, primer, ..et, this method as previously mention is better than viral isolation also it can be used in case of controversial results, this method also expensive and take cost (Ayensu, F., 2014; De la Hoz, R., et al, 2002).

1.1.12.5. Antigen Detection

Van der Bij in 1988 for the first time develop CMV antigenemia to demonstrate viremia in transplant recipients, the test search in mixing WBCs of anticoagulated case fluid pertaining spinal /or patient blood to demonstrate CMV pp65 antigen, the counting and washing sample with duplicate onto a multiwells glass slide in order to recognize anti-pp65 after fixing following stain and then evaluating with monoclonal fluorescein, this method mention is better than isolation and more sensitive and it can detect the virus before start symptoms, for performing this test it is reading the result the technician must have more experience in order to manipulate and decide the final result within 8 to24 hours. There is also immunohistochemistry technique which is beneficial in open lung material in special in immunosuppressed patients (De la Hoz, R., et al, 2002).

1.1.13. Protection

There is always good person hayagen and prevention is better than treatment, but if you want to avoided to get CMV infection it is seemed almost impossible due to the global nature of the organism in worldwide, but there is some way to reduce CMV infection by controlling some sectors such as blood donation, close contact, surgical of organ transplantation and controlling pregnant women avoiding children to exposure and maintain CMV hyperimmune globulin to pregnant women and vaccines administered to female before pregnancy, following the experimental study on vaccine till under developing, Because the viral transmission by sexual contact also it should be preventing by use of condoms during sexual intercourse (Ayensu, F., 2014 ; Boeckh, M., & Geballe, A. P., 2011).

1.1.14. Treatment

In generally there is five of anti-viral drug available to treat CMV infection and mode of their action. First is Ganciclovir are deoxyguanosine analogue it was used first time in 1988 in order to treat CMV it was used for immune compromised patient at that time it was first drug choice, action of drug summarize in inhibition of viral DNA polymerase by competing with dGTP on binding enzymes spot and it is also stop DNA chine elongation (Gilbert, C., & Boivin, G., 2005).

The second anti-viral drug use for HCMV is valganciclovir.It is new generation upon formulation of GCV it is more stronger affective than GCV by ten times the way of taken drug is orally uses and it is also inhibited DNA viruses polymerase but it is mention that have toxicity profile. Therefore it is less using by physician, in case of useless of GCV on patient the valganciclovir may be useful (Gilbert, C., & Boivin, G., 2005).

The third anti-viral drug use for CMV is Cidofovir it is used for more recently against viral activity. It's also inhibited DNA chain polymerase. The fourth anti-viral drug use for CMV is Foscarnet. It's different in effected on virus the drug will stick and prevent the pyrophosphate binding site on the viral DNA polymerase and combination of deoxynucleoside triphosphates to DNA virus. The fifth anti-viral drug use for CMV is formivirsen, this drug will inter to viral replication in early stage of the virus and it is use to specific site especially in case of infection with HIV (Gilbert, C., & Boivin, G., 2005).

1.1.15. Vaccination

Nowadays sections of vaccine have been developing but until now there is no specific vaccine were developing to HCMV, but there is prophylactic vaccine for HCMV used and the action of vaccine on CMV still challengeable. The first vaccine development for HCMV founded by jennerian idea that associated with attenuated method of vaccine, there is many renewed co-development vaccine developed or under develop as a recombinant technology method, and we believe that the vaccine should in side of controlling disease of HCMV disease and stimulate strongly body immunity response such as innate immunity & adaptive immunity in an appropriate time (Crough, T., & Khanna, R. (2009).

Aim of the study was

i: Is the infection with CMV leads to changing seminal fluid parameters?

ii: Is CMV infection resulting to infertility? or it is related with the severity of the infection?

To examine the most recent popularity and hazard of different majority of CMV disease in men who, suffer from abnormal parameters in their semen just as the kinds of parental CMV contagion related with inborn CMV disease among their generations.

i: To detection of the effect of CMV infection on the seminal fluid parameters. .

ii: To find relationship between CMV infection and infertility.

CHAPTER 2

2. MATERIAL AND METHOD

2.1. Material

2.1.1. Devices and Tools

- Elisa Kit BioActiva diagnostica IgM = Germany
- Elisa Kit BioActiva diagnostica IgG = Germany
- Elisa Machine BioTek ELISA microplate reader = USA
- Elisa washer BioTek ELISA microplate washer = USA
- Piptte DRAGON microPieppet LAB = China
- Yellow Tip AL-Rawan = China
- Blue Tip AL-Rawan = China
- Epindrop Tube AL-Rawan Centerifuge tub = China
- Timer ISOLAB Laborgerate GmbH = Germany
- Incubator Galaxy CO_2 incubator = UK
- Microscope NOVEL = China
- Sterile Cup AL-Rawan sterile Cup = China
- PH meter paper CITOTEST Universal Paper test = Indonesia
- Slide AL-Rawan Microscopic Slide = China
- Cover Slip CITOGLAS Cover Glass = Malaysia
- OLYMPUS Stream 2.2 Efficient Microscopy imaging reader = France

2.1.2. Software Programs

In our study the Statistical Package Social Sciences (SPSS) application used in order to manipulate the each result parameters with other data. The SPSS is a widely used program for statistical analysis in social science. It is also used by many of other fields include health researchers, It can handling complex data manipulations and analyses them very easy and within minutes.

2.2. Sample Collection

This study was done in different hospital and private clinical in Erbil city-Iraq, Semen samples of 152 men (52 case control; 100 case infertility case) were collected in Erbil city- Iraq. For all participant's examination for seminal fluid analysis tests are performed such as sperm (color, liquefaction time, PH, volume, concentration, count, motility, morphology and cellular test, Agglutination). The case group were conditions are had history of 2 years infertilities, had no children and healthy partners with no identifiable cause of infertility (WHO source). The men who will control group conditions are had normal results of laboratory semen analysis according to the WHO standards. Also, serum samples will collect for the ELISA analysis. (Appendix 1)

2.3. Semen Analysis

Semen samples were collected from different patients by masturbation and collecting their semen into special sterile containers that use specially for semen sample collection, with avoiding different sexual activity approximately between 2 - 4 days. Samples were prepared for complete seminal analysis within 30 minutes to 1 hour after collection according to semen liquefaction. Seminal fluid examination (SFA) is divided into two part first macroscopic and second is microscopic examination.

2.3.1 Macroscopic Examination

According to world health organization (WHO) the macroscopically seminal analysis include (color, volume, liquefaction time, and PH). (1) Color: The first semen sample bring to lab then the doctor should foxing on the color and record it, there is different semen color according to the different situation. (2) Volume: Which is done event within cup because there is gradient marker in Milliliter on it or it is doing by syringe. (3) Liquefaction time: The semen should be handing in incubation normal semen should liquefy within 30 or 60 minutes, semen must be followed until it become liquid then test will perform. (4) PH: The pH were measured after liquefaction by gradient pH paper from 2 to14 pH indicator

2.3.2. Microscopic Examination

Microscopic examination of seminal fluid analysis usually done either by manual or spermatogram image reader that read all parameter automatically in our study both method were used. The microscopically seminal fluid analysis divided in to several parameter which include, (1) assessment of sperm motility (2) Calculation of sperm concentration and count (3) Valuation of sperm morphology (4) Assessment of cellular elements (5) calculate percentage of sperm cell clumping other than spermatozoa all these examination carefully calculated and arranged according to the World Health Origination (WHO) recommendations.

2.4. ELISA

The blood sample collected from all patient and indirect ELISA method used in evaluating a patient's serologic status to CMV-IgG and CMV-IgM. In this study, CMV ELISA kit are used to detect CMV infection in addition type of Ig, the kit also calculate the quantitative of CMV following both type of human antibody immunoglobulin M & G in vitro test.

With ELISA kit you can perform 96 test (plate contain 96 wells) for detection of Cytomegalovirus-specific antibodies, after procedure done the reaction between Ag and

Ab will take place and the ELISA reader automatically read the amount of these reaction, then evaluation the result and calculate it to get the final result in order detect positive samples and negative one.

BioActiva Diagnostica GmbH Human Anti-Cytomegalovirus IgM and IgG ELISA (enzyme-linked immunosorbent assay) Kit were used in order to diagnosis and detect type of immunoglobulin are prescience, CMV ELISA test system quantitative arrange and designed to detect IgM & IgG antibody to CMV in human serum, if test indicate positive that's mean Ab combine with monoclonal antibody and Enzymes which indicate CMV antigen forms complex to color change.

The anti-Cytomegalovirus (CMV) IgM & IgG Human *in vitro* ELISA kit are used for detection quantitative of IgM & IgG Ab. The human blood serum are used for evaluation, each well are contain amount of Cytomegalovirus Ag in order to bind with patient Ab to form color complex. The kit contain controls vial in order to arrange test for evaluation positive and negative result with positive vial and negative vial. Washer bottle use to wash sample in each step recommended. Labeling anti-Human immunoglobulin type M & G doing by (HRP) conjugate. HRP catalyzed by TMB the reaction between them result with producing color complex, stop solution eliminate reaction.

2.4.1. Sensitivity and specificity

Bioactica Diagnostica ELISA kit sensitivity measure by 86 samples, the diagnostic sensitivity and specificity was determined by a multicentric evaluation study of this test. Out of 86 Serum samples 63 CMV positive, 2 as brd line, the Bioactica Diagnostica has sensitivity 100% and specificity 100% when brd line sample are consider as positive, an analytic comparsion between two assay show $R^2 = 0.811$ which is acceptable consideration a serological assay.

2.4.2. Experimental calculated

The test must be read O.D and change intensity colorimetric at 450nm to quantitative rate with a microwell reader

The kit contain Cut-off O.D use foe calculation with assay result ＝Assay / Cut-off *2

Positive: Sample O.D must be equal/or more than result of Cut-off O.D

Negative: Sample O.D must be equal/or less than result of Cut-off O.D

In our experimental ratio for

Negative Control was = < 0.9

Positive control was = > 1.1

Cut-Off O.D = 0.75

Doubtful = if -/+ 10%of the Cut-Off

Not: The observable result suffer questionable you should repeat the procedure another time/or collect new sample repeat the test.

2.4.3. Positive and negative rates

According to kit Guideline the interpretation the result as following

Positive: Sample concentration > 10 U/mL1

Negative: Sample concentration ＜9 U/mL

2.4.4. Positive and Negative Control

According to our number of samples which is 152, the study analysis of samples performed in three steps, in first 50 sample analyzed in second 50, in final 52 sample were analyzed, because of separation work project in to three steps also the positive and negative control performed three time the O.D. was as following: (1) Positive control results (0.72, 0.75, 0.73), (2) Negative control was (0.20, 0.20, 0.22).

2.4.5. Experimental Procedure

1. Remove the kit components from storage and allow them to equilibrate to room temperature ~25C for more than 15 minutes before use.
2. Prepare a 1:101 dilution serum sample 10µL distribute in to 1 mL with distilled water.
3. Add Specimens 100µL into the ELISA wells accept the blank well, negative well and positive well.
4. Add 10µL specimen to the well.
5. Incubate samples at 37°C for 30 minutes.
6. Decant, Add Washer Buffer to each well. Repeat 4 times within 30 second.
7. Add 100µL of HRP Conjugate to each well, and mix well then Incubate at 37°C for 30 minutes.
8. Decant, Add Washer Buffer to each well. Repeat 4 times within 30 second.
9. Add 100µL of TMB, mix well, Incubate in room temperature for 15 minutes in dark place.
10. Add 100µL of Stop Solution.
11. Read the absorbance within 30 minutes, the result with a microwell reader at 450_{nm}.

2.4.6. Elisa Kits Firm Name

Name of company = Bioactiva Diagnostica LISA Kit
Kit code = CMVM02 for IgM
Kit code = CMVG01 for IgG
Lot = S-020
Boiactiva Diagnostica GmbH: louisenstrasse 137
61348 BadHomburg: Germany
WWW.bioactiva.de

2.5. Statistical Data Analysis

Because of the data pertaining non-parametric and there is some parameter relate to quality (category) data the Chi-square test were used to solve the relation variable on semen parameter to CMV. But other parameter contain quantitative (continues) data therefor Mann-Whitney test was the best option for comparison case group with control group and evaluate CMV variations of positive and negative to the semen parameter in order demonstrate the relationship/or affectivity of CMV to semen. And the color parameter with time liquefies were categorizing data, for analysis them we use chi-square test to get signification between two groups choosing Fisher exact test, for other Mann-Whiney test are use. SPSS program are used in order to manipulate the significant rate by evaluation each of (P value, mean, standard deviation).

2.6. Ethical Acceptance

The proposal form project under the title INVESTIGATION CYTOMEGALOVIRUS AMONG MALE INFERTILITY DISORDER have been reviewed by regulator of Erbil Polytechnic University / Erbil Technical Health College (Scientific Committee) and Patients right consent and Confidential is respected according to Helsinki guidelines. (Appendix 2)

CHAPTER 3

3. RESULTS

3.1. Study Population

In this study was 152 patient were participate as a population from different clinical and different hospitals, the experiment divided in to two groups one are relate to control group and another one are case (Patients) group, All participants in this study they have history with infertility period 2 years, the patient Age parameter was divided in to three category, Age below 18 years was 7 patients (7%) for patients group and for control group was 2 person (3.8%), Age between 18 to 50 for patient group was 90 patient (90%) for control group age was 50 person (96.2%) as a control group, Age above 50 years for patient group was 3 patient (3%) and for control group was zero (0%). And there is some feature recorded that may have role in sub-infertile such as (Alcoholism 12%, Smoker 45%, varicosele 21%, radiation 0%, surgical of teste 8%) for patient group, and control group was zero (0%) for each of Alcoholism , Smoker , varicosele , radiation , surgical of teste), the (Table 1) summary percentage of study population.

Table 1: The percentage of questioners paper for patient and control group.

	Age			Alcoholism		Smoking		Varicocele		Radiation		Testicular Surgery	
	<18	**18-50**	**>50**	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Control	**3.8**	**96.2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Patient	**7**	**90**	**3**	**12**	**88**	**45**	**55**	**21**	**79**	**0**	**100**	**8**	**92**

*All numbers resemble as a percentages

For choosing participant as a control group we eliminate those who answer YES to questioners form statement such as (Alcoholism, Smoking, Varicocele, Radiation, Testicular Surgery) because this statement consider as a sub-infertility, so we planning to remove these sub-infertility in our study especially in case of age because age over 50 normally in semen appear RBCs in samples and it is not suitable choosing it as a control group.

3.2. Control Group

The 52/152 are selected as a control group that's mean they have Negative result in CMV examination in both type IgM and IgG in addition to normal result of all semen parameters and they used as a control group in the experiment, the control semen analysis contain 13 parameter (Color, Liquefy time, Volume, PH, Concentration, Count, Motility, Morphology, RBCs, WBCs, Epithelial cell, Germ cell, Presence of agglutination), and all 52 controls parameter found in normal form (100/100% have normal quality of seminal fluid analysis parameter), and there is no abnormal semen sample used in this study (0/100% have abnormal semen quality of seminal fluid analysis), the (Table 2) show of normal and abnormal rate of SFA parameters control group.

Table 2. The normal and abnormal rate of SFA parameters of the control group

Seminal Fluid Analysis	Normal Semen	Ab-Normal Semen
Color	**100%**	**0%**
Liquefaction Time	**100%**	**0%**
Volume	**100%**	**0%**
PH	**100%**	**0%**
Concentration	**100%**	**0%**
Count	**100%**	**0%**
Activity	**100%**	**0%**
Morphology	**100%**	**0%**
Red Blood Cell	**100%**	**0%**
White Blood Cell	**100%**	**0%**
Epithelial Cell	**100%**	**0%**
Germ Cell (immature cell)	**100%**	**0%**
Agglutination	**100%**	**0%**

3.3. Patients Group

3.3.1. Semen Analysis Results

The patient group is 100/152, examination test for seminal fluid analysis perform according to world health organization (WHO) semen standard, in addition of semen analysis result was found all 100 patient have semen abnormality at least 5 parameter out of 13 parameter, (normal semen analysis require normality in all parameter as a normal fertile semen quality), the most abnormal semen parameter are Leukocytospermiya (77%), Sperm Activity (69%), Semen concentration (65%), Sperm cell morphology (64%), the (Table 3) summary all normal and abnormality of semen parameter.

Table 3. The Normal and Abnormal rate of SFA parameters of the Patient group

Seminal Fluid Analysis	Normal Semen	Ab-Normal Semen
Color	**92%**	**8%**
Liquefaction Time	**93%**	**7%**
Volume	**95%**	**5%**
PH	**97%**	**3%**
Concentration	**35%**	**65%**
Count	**81%**	**19%**
Activity	**31%**	**69%**
Morphology	**36%**	**64%**
Red Blood Cell	**79%**	**21%**
White Blood Cell	**23%**	**77%**
Epithelial Cell	**78%**	**22%**
Germ Cell (immature cell)	**73%**	**27%**
Agglutination	**85%**	**15%**

If we make summary to (Table 3) it show that all macroscopically examination of semen analysis parameter (color, liquefy time, volume, PH), the percentage number of normal form around (94.2%), and abnormal form will be (5.8%), revers to microscopically seminal fluid analysis section will be around (57.8%), and abnormal percentage will be around (42.2%).

3.3.2. CMV IgM and CMV IgG Results

The patient group is 100/152, and examination test for CMV show that 25 % of them have CMV IgM positive and other 75 % were CMV-IgM Negative, and all 100 patient have CMV IgG positive. According to our experiment statistical analysis the results between CMV and seminal parameter in macroscopically section were showing as following:

Table 4: The prevalence and P value CMV-IgM infection on semen color and liquefy time of patient group.

		CMV-IgM (n=25)		
Semen Parameter		**Positive**	**Negative**	**P value**
Color	**Milky**	**22%**	**123%**	**0.088**
	Other	**3%**	**4%**	
Liquefy-Time	**30 min**	**25%**	**121%**	**0.590**
	60 min	**0%**	**6%**	

1: Color milky is resemble as normal semen color.

2: Other color meaning patients that have Ab-normal semen color.

3: The normal semen liquefy time should be within 30 minutes

4: Within 60 min is acceptable

The statistical analysis result according to (Table 4) between CMV on semen color show that there is no significant, and there is no relation between color and CMV-IgM infection. Test analysis among Liquefy data show that there is no statistical signification of CMV-IgM to liquefaction time

Table 5: The analysis between CMV-IgG among semen Color and Liquefaction time of patient group.

		CMV-IgG (n=100)		
Semen Parameter		**Positive**	**Negative**	**P value**
Color	**Milky**	**93%**	**52%**	**0.096**
	Other	**7%**	**0%**	
Liquefy-Time	**30 min**	**94%**	**52%**	**0.950**
	60 min	**6%**	**0%**	

1: Color milky is resemble as normal semen color.

2: Other color meaning patients that have Ab-normal semen color.

3: The normal semen liquefy time should be within 30 minutes

4: Within 60 min is acceptable

The statistical analysis result of affecting viruses CMV-IgG on semen color according to P value show there is no relation between color and CMV-IgG infection. Test analysis among the data show also there is no statistical signification of CMV-IgG to liquefaction time

Table 6: The mean, Std and P.value of SFA parameter among CMV-IgM of patiemt group.

	CMV-IgM (n=25)		
	Positive	Negative	
Semen Parameter	**Mean ± Std**	**Mean ± Std**	**P value**
Volume	**2.728 ± 1.1667**	**2.203 ± 1.0333**	**0.988**
PH	**8.016 ± 0.2882**	**8.013 ± 0.1797**	**0.510**
Concentration	**19.28 ± 17.468**	**27.78 ± 16.120**	**0.001***
Count	**66.60 ± 59.999**	**111.61 ± 80.179**	**0.003***
Motility	**26.56 ± 21.280**	**33.06 ± 17.327**	**0.110**
Morphology	**46.64 ± 17.380**	**53.83 ± 20.003**	**0.020***
Hematospermiya	**2.48 ± 2.275**	**2.07 ± 1.831**	**0.308**
Leukocytospermia	**7.76 ± 5.840**	**6.36 ± 8.409**	**0.005***
Epithelial Cell	**2.52 ± 1.711**	**2.36 ± 3.436**	**0.003***
Germ Cell	**2.88 ± 3.321**	**2.20 ± 2.075**	**0.59**
Agglutination	**12.88 ± 15.667**	**21.75 ± 17.868**	**0.001***

*resemble as a signification foundation

According to the (Table 6), the statistical observation between all semen parameter among CMV-IgM, and the statistical analysis demonstrate there is no relation between CMV-IgM on semen volume. Whatever there is also no signification found between CMV-IgM to semen PH.

The microscopical analysis of semen section versus CMV-IgM tests, we found that there is a signification demonstration relate to semen fluid concentration to the CMV-IgM. Also, the statistical analysis proved there is significance between Virus CMV-IgM and seminal fluid Count and morphology. But there was no significant fount in motility section according to demonstration of p value (<0.05). In addition to presence RBCs (hematospermia) in semen sample relate to CMV-IgM also there is no significances to Germcell (Immature cell). But there were found that there is statistical signification between CMV-IgM to the leukoctospermia (WBCs), Epithelial cell and Agglutination when comparing to control groups mean and Std.

Table 7. The mean Std and P.value of SFA parameter among CMV-IgG of patient group.

	CMV-IgG (n=100)		
	Positive	**Negative**	
Semen Parameter	**Mean ± Std**	**Mean ± Std**	**P value**
Volume	**2.590 ± 1.0879**	**2.933 ± 0.9499**	**0.039***
PH	**8.021 ± 0.2471**	**8.000 ± 0.000**	**0.822**
Concentration	**23.04 ± 18.838**	**32.81 ± 7.894**	**0.000***
Count	**83.90 ± 77.268**	**143.27 ± 66.730**	**0.000***
Motility	**25.90 ± 18.898**	**43.69 ± 8.154**	**0.000***
Morphology	**45.77 ± 19.695**	**65.87 ± 11.315**	**0.000***
Hematospermiya	**2.71 ± 2.114**	**1.04 ± 0.484**	**0.000***
Leukocytospermia	**9.30 ± 8.778**	**1.42 ± 0.572**	**0.000***
Epithelial Cell	**3.19 ± 3.703**	**0.85 ± 0.50**	**0.000***
Germ Cell	**3.07 ± 2.520**	**0.87 ± 0.658**	**0.000***
Agglutination	**22.09 ± 21.057**	**16.83 ± 7.558**	**0.599**

*resemble as a signification foundation

According to the (Table7) expression the illustration of significant study of statistical analysis observation between all semen parameter among CMV-IgG, in this experiment study we noted according P.valus that there is no signification between the viral CMV-IgG type to PH and Agglutination of seminal fluid analysis. Following to other semen parameters relationships to CMV-IgG virus, our statistical analysis show us that the all P. values are less than (P 0.05), moreover, there is signification between semen (Volume, Concentration, Counts, Motility, Morphology, Hematospermia, Leukocytospermia, Epithelial cell, Germ cell), when comparing to control groups mean and Std.

CHAPTER 4

4.1 DISCUSSION

The infection with CMV may cause several desiase complication to human body disorder therefor CMV one that consider as an important microorganism which more interesting in to scientist/or doctors in field of medical, clinicians and experimental study, especially in field of human male sub-fertility or fertility, also CMV one of those infection agent that the treatment are very hard and it is more dangerous with immune dysfunction, also CMV may associate mortality with other disease condition, in previous study mention CMV has been transmitted through human insemination procedures (Wortley, P. M., et al, 1998). Furthermore, CMV lead to a life-threatening risk to human life. In many study mention around 99% of population suffering from CMV the main cause turned to vertical transmission during pregnancy thought placenta and it is cause of congenital disease in Childs and their fetus (Jean Beltran, P. M., & Cristea, I. M., 2014), this vertical transmission lead to cause wide range of human suffering CMV especially in men may lead to defect in male genital tract and it is reproductive system therefore CMV genetic material has been found in male seminal fluid in many previous experiment without expression any symptom to the patient (Dejucq, N., & Jégou, B., 2001; Hamdad-Daoudi, F., et al, 2004; Mohseni, M., et al, 2018).

The field of male infertility which relate to CMV and it is effect of semen parameter until nowadays are contentious issue and it is still debatable, the main drive in our design of study was evaluation of the pathogenicity of CMV in an infertility cases in order to evaluation it is influence with seminal fluid parameters quality. In previous study, the prevalence of CMV and its association with male infertility was examined, infertility in men is commonly without specific identify reason. There is also some other experimental (Mohseni, M.) conduct to investigate the CMV in fertile and infertile men's semen examined in the finally the relationship between viral presence and semen

parameters was investigated and the results of this study showed a high prevalence of CMV DNA in semen samples in total men observed that most of them were infertile men and little of them are fertile men (Mohseni, M., et al, 2018). In our study we fox on men how have historical with infertile. Followed, examination prevalence of CMV in both type imunoglobin (IgM and IgG) and it is related to Seminal fluid analysis parameters.

In this study the statistical analysis divided in to 2 part as a (macroscopically and microscopically), and examination among CMV-IgM and IgG infection. The macroscopic parameter are include the semen color, in one of study that made in Turkey mention that herpesviruses have also been isolated from subfertility male with semen abnormalities consisting of a discoloration (Benfield, D. A., & Adldinger, H. K., 1984), in addition of few section of study mention many factors that effect on semen color such as hematospermia, prostate and vesicular cancer, leukocytospermia,…ect. (Mulhall, J. P., et al, 2014), So the CMV also cause these diseases and it mean may cause discoloration, in some other books mention one of the discoloration due to inflammation (Jeyendran, R. S., 2000), and according to CMV inflammation (CMV produce inflammation) in our study we foxing more in CMV inflammation and it is affecting on color, so we screen semen color in our study in order to demonstrate affectivity of CMV to semen color appearance, but we found CMV IgM and IgG didn't have any relation to color change of semen (Non-signification found).

In some article mention that presence of CMV positive reduce testicular volume. The one of outcome that affect standard semen analysis is CMV when penetrate testicular barrier and rich to seminiferous then influence with male seminal fluid, whatever in some study pointed there is no effectively/or relation between CMV to the semen ejaculate volume was found (Eggert-Kruse, W., et al, 2009). But in other as (McGowan, M. P., et al, 1983; Benfield, D. A., & Adldinger, H. K., 1984) mention CMV effect on semen volume and it cause reduction of amounts of spermatozoa. Whatever in our study demonstrate there is significances CMV-IgG type to volume revers CMV-IgM there was no any significant. Also there was no signification were

found in some previous experiment, which is agree with our study idea on the relation between CMV-IgM and IgG infection and semen PH (there are no relation was found in our study) (Eggert-Kruse, W., et al, 2009).

CMV can be found in male seminal fluid and it is affecting semen quality parameters, in many of published paper mention CMV causing no changing in increase or decrease of liquefactions time (Viscosity) (Kapranos, N., et al, 2003; Neofytou, E., et al, 2009; Eggert-Kruse, W., et al, 2009), the statistical analysis result according to our P value that obtained between CMV and liquefaction time show that there is no significant was found and there is no relation between effecting CMV-IgM and CMV-IgG on liquefaction time of semen.

The microscopical data analysis depending on P value demonstration in our experiment and it is discover that CMV can effect on semen concentration, also there is some paper shown the infection of CMV leads to the reducing of seminal fluid concentration (Oligospermia abnormality) (Chen, M., et al, 2013; Benfield, D. A., & Adldinger, H. K., 1984). In other experiment also show the reducing of sperm cell concentration in infected samples with CMV compared with controls group it was provides and an indication the capability of CMV to change sperm value (Wu, K. H., et al, 2007; Naumenko, V., et al, 2014). Whatever in our study the P value obtain in semen concentration parameter to CMV-IgM and IgG was lower than (0.05), that's mean CMV correlate with semen concentration parameter quality.

There is an experimental perform previously in duke medical center Durham, N.C,27710, US and it is mention patient how demonstrate CMV in their semen which is term as (viruseminia) was relate to abnormality in semen analysis especially the spermatozoa count (Lang, D. J., et al, 1974). In our study according to the data analysis of the results which is based on statistical analysis of CMV among to studied groups parameters in semen count it was determined that there was signification obtained according to P value less than (0.05), so there are relationship between effecting CMV prevalence on sperm count in both type Ig, also there are many of other experimental is compatible with our result as they demonstrate that yes there are relation between

semen count and CMV (Bezold, G., et al, 2007; Mohseni, M., et al, 2018; McGowan, M. P., et al, 1983).

In experiment study demonstrated that there is no change in activity of sperm cell motility as a result of sperm which incubation with CMV for a period (Pallier, C., et al, 2002; Baghdadi, K., et al, 2016). But some studies mentioned that CMV also leads to lowing sperm activity (Lai, Y. M., et al, 1997; Mohseni, M., et al, 2018; Mansat, A., et al, 1997), so in our study the results are different between type of CMV Ig to sperm cell activity, first the correlation between CMV-IgM among to spermatozoa motility and it is according to statistical analysis there was no significant have been established, second is relation between CMV-IgG and it is affecting on sperm cell motility the statistical analysis P value was less than (0.05) and it is clarify that there is significant of CMV-IgG to sperm activity was found.

Various studies mention and conducted that CMV infection and its effect on the functional parameters morphology and the relationship between infection of Herpes virus family and sperm morphology especially CMV has been described, such as presence of teratospermia phenomenon (Abnormal sperm morphology) and its relationship with sperm cell structure of contents (Neofytou, E., et al, 2009; Mansat, A., et al, 1997), the our study results of statistical analysis among to the CMV in two type of IgM and IgG correspond by studied groups sperm cells morphology was significant the P value was less than (0.05).

It mention that the age of male who aggrieved with hematospermia are divide between 30 to 40 years and men above 40 years have been detected with express the symptoms, and it has been clarify that the presence of CMV in male semen may leading to appearance of RBCs in semen sample which know as (hematospermia) (Suh, Y., et al, 2017). Also In some other study mention that presence or RBCs also relate to vitality of orchitis especially cytomegaloviruse (Elem, B., & Patil, P. S., 1987; Fuse, H., et al, 2011), Therefor, hematospermia was association whit CMV action, as a result there was liveliness of CMV to presence RBCs in sperm samples (signification P value <0.05) As an investigational result to CMV-IgG. But there was no significant relation

was found between Hematospermia and CMV-IgM, the reason may due to IgM is new infection still no strong as possible to invade testicular barrier or patient may still immunocomptent and CMV not active more than patient immune system because in some article they mention hematospermia relate to chronic CMV infection (Koment, R. W., & Poor, P. M., 1983).

Leukocytospermia abbreviate as (LCS), but it is also termed as leukospermia, pyospermia or pyosemia to prescience of extraordinary numbers of leukocytes in human seminal fluid which (WHO), and the high prevalence of leukocytospermia concentrations presence in male seminal fluid may influence with male fertility because WBC in semen impact with semen parameter in male seminal fluid also vitality of leukospermia in infertility patients differs from 2% to 40% according to published reports (McGowan, M. P., et al, 1983), and some study suggests that CMV is associated with chronic urogenital tract inflammation which study has been identified CMV viruses detection by a PCR assay and the result that obtained was significantly associated with increase leukocyte count in seminal fluid samples (Naumenko, V., et al, 2014; Xiao, J., et al, 2013). There was cross idea also in our study toward the CMV infection causing change in semen function quality, the signification was found in our study and P value observation between CMV-IgM and CMV-IgG among leuocytospermia was (<0.05).

To support our study in epithelial parameter signification there is also some articles mainly focused on CMV infection, also they mention there is some viruses attack cells/tissue and penetrate it result with destroy and change structures of genital territory with resulting of releasing germinal epithelial cell (Eggert-Kruse, W., et al, 2009), in other paper mention that LCS was strongly related to raised immune particle as (PMN-elastase & IL-1β), and when epithelial cell stimulate result with manufactured proinflammatory cytokine (Bezold, G., et al, 2007; Keck, C., et al, 1998). In a research experiment mention the effect of CMV on spermatogenesis nearly after 14d may result with variations of sperm germ cell moreover damage tests manner and slackening epithelium (Naumenko, V. A., et al, 2011). As comparing to our result there was also

signification found concerning CMV in both type of immunoglobulin (IgM and IgG), the statically analysis was (P value < 0.05).

The CMV have ability to affect immature germ cell at different stages of maturation, the spermatocytes and spermatides are the most sensitive to cytomegalovirus and decrease in the immature germ cell (IGC) semen population is a possible factor for fertility disorders (Wu, K. H., et al, 2007; Pallier, C., et al, 2002; Naumenko, V., et al, 2010). CMV affecting both localization developing sperm cell (mature germ cell) and un-developed sperm cell which is known us (immature germ cell). There was compatibility in our study to other study in affectivity of CMV to reduce germ cell to be mature, there is some paper mention data obtained assume that CMV encouragement immature germ cells (Naumenko, V. A., et al, 2011). In our study and it is according to statistical P value (< 0.05) we found that CMV-IgG impair with developing sperm germ cells, in addition no signification found for CMV-IgM among decrease of immature germ cell.

It has shown that sperm antibody in semen may affect sperm function. Also In some research it has been showed some viruses may influence with blood testes barrier which lead to stimulate anti-sperm antibody, one of these viruses is CMV (Keck, C., et al, 1998), and ASA finally may result in agglutination of sperm cells together, in our study CMV-IgM give signification to agglutination (AGG), but in other experimental study mention the presence of cytomegalovirus in human seminal fluid was not significant associated with semen antisperm antibody (Eggert-Kruse, W., et al, 2009), as same as our result because the result obtain according to statistical analysis among CMV-IgG to antisperm antibody the P value was found more than 0.05).

CHAPTER 5

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

According to statistical analysis most of seminal parameter the significant correlation was obtained between the cytomegalovirus infection and male infertility. In addition a few parameter indicate non-significant, although the results achieved may vary with changes in population size. The prevalence of this CMV was reported in reducing semen function and relationship was found between reduced quality of sperm parameters and infertility. Our study showed that prevalence of CMV infection was higher in infertile men compared to control group men and CMV infection can be considered as an important part of male infertility. So antiviral treatment of positive cases can be effective in improving sperm quality and help to reduce affectivity of virus in fertility. The relationship between CMV infection in semen and infertility was obtained in previous studies and was confirmed by our study.

The findings of this study indicate that infection with CMV can effect on the sum of essential and important parameter in seminal analysis that may cause possibility infertility in the infected men the one of the important parameter that CMV-IgM type was affected on the sperm (concentration, count, morphology, leucocytospermia, epithelial cell and agglutination). But, there was most strongly CMV-IgG correlate with semen parameter that's mean more affecting male infertility because our result show that CMV-IgG more significant on semen parameter as (Volume, concentration, count, motility, morphology, haematospermia, leukocytospermia, epithelial cell and immature germ cell).

There was few parameter didn’t show any signification in both type of Ig such as semen color and liquefaction time. In final conclusion we should mention that late CMV infection was more signification than recent infection.

5.2. RECOMMENDATION

According to the results of the current study, we recommended the following:

1. All adult individual should have screen CMV test with seminal fluid analysis in order avoiding any effective of virus.
2. All new couple should have screening for both IgM and IgG of CMV.
3. The study recommended to perform further study by recent method as PCR and compare between two methods are very necessary.
4. Further studies should be intended on different types of viruses specially those have significant with male infertility.

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APPENDIX

Appendix A

King Laboratory

For Clinical Analysis

KING Laboratory

For Medical Analysis

تاقیگەی کینگ

بۆ شیکردنەوەی
نەخۆشیەکان

31.Aug.2019

To whom it is concern of the Near East University.

I would like to inform you the project under the title (Investigation of Cytomegalovirus Among Male Infertility Disorders), where accepted. which be conducted by (Aras Othman Rasool), in King laboratory for medical analysis.

With respect.....

Dr.Rabar M.Abdulrahman
PhD.Bioengineering

Bahaadin A. Sabir
PhD. Student of Mol. Biology

Dr.Jutyar Sh.Ali
MB.CHB

Appendix B

Ministry of Higher Education and Scientific Research
Erbil Polytechnic University
Hawler Health Technical College
Scientific Affairs Department

زانکۆی پۆلیتەکنیکی هەولێر
ERBIL POLYTECHNIC UNIVERSITY

Erbil Health Technical College
(Dean Office)
No.
Date

No. : 923
Date: 29.9.2019

To/ **Near East University, Medical Faculty**

Greeting,

We have reviewed the proposal of the project (Aras Rasul Othman) that supervises by one of our academic staff (Assistant Prof. Dr. Najat Jabbar Ahmed) as a co-supervisor with (Assoc. Prof. Dr. Meryem Güvenir) from Near East University. We found that the process of the project above going with ethically sound according to our regulation in Erbil Technical Health College (Scientific committee) and Patients right consent and .confidential is respected according to Helsinki guidelines

Scientific committee of Erbil Technical Health College/Kurdistan Iraq.

Assistant. Prof.: Aumed Arshad Hawezy
The dean

Assistant. Prof Dr. Najat Jabbar Ahmed
Head of MLT Dep.

Assistant. Prof. Dr. Twana Ahmed
Head of Scientific affairs

Dr. Mahdi Khaled
Head of Physiotherapy Dep.

CURRICULUM VITAE

Personal Information

Full name	**Aras Othman Rasool**
Surname	**Rasool**
Gender	**Male**
Marital State	**Single**
Date of birth	**20/7/1990**
Place of birth	**Erbil-Iraq**
Nationality	**Kurdish**
Phone number	**+964 750 7559349**
Email	**osman.aras.osman@gmail.com**

Education

University \ College	Department	Degree	Year
Erbil Polytechnic University / Erbil Technical Health College	Medical laboratory Technology (MLT)	Diploma	2012
Knowledge University / College of Science	Pathological Analysis	Bachelor	2018

Previous Experience

Three Years Working In Blood Bank Laboratory / Al-Rahma Hospital
Four Years Skill In Private Clinical Lab. / Family Safe Clinic
Two Years Employed In Medical Lab. Diagnosis / Selar Laboratory

Knowledge languages

Language	Speaking	Writing	Reading
Arabic	Good	Good	Good
English	Good	Good	Good

Courses and certification

	Place	Year
Certification Of (Business Development Service)	FCYI-IOM Organization	2012
Certification Of (Infection Control &West Management)	Ministry Of Healthy / Erbil-Iraq	2013
Certification Of English Language Courses (Beginners, Elementary, Intermediate Level)	AMIDEAST &Macos Organazation	2016. 2017, 2018
Certification Of (Youth For Peace)	T.O.S.D & F.E.S Organization	2012
Certification Of Aggression Replacement Training (Listining, Starting Convasation, Asking Question, Saing Thank You, Apologizing, Anger Management And Problem Solveing)	QANDIL Swidish Humanitarian Aid & GIZ Gmbh Organization	2018
Certification Of (The Road To NOBEL) Symposium	Near East University / Cyprus	2019
Microbiology Laboratory Practical Training	Near East University / Cyprus	2019

Computer Knowledge

Microsoft office word	**Good**
Microsoft office power point	**Good**
Adobe Photoshope	**Intermediate**
Internet using	**Good**
SPSS software statistical program	**Good**

Hobbies

Medical laboratory, Reading, Teaching in university, Environment and Earth, Travel, Hand work, Football, Sewing.

Future Dream

Medical laboratory professional

QUESTİONARY PAPER

Name:

Age:

Married ☐ **Single** ☐

City:

Occupation:

Alcoholism:

Smoking:

Medication:

Last sexual intercourse: **day**

Varicocele:

Radiation:

Surgery of tests:

CMV: IgM

IgG

Color:	**Milky**
Liquefaction time:	**60 min**
Volume:	**1-5 ml**
Concentration	**$39x10^6$ /ml**
Count	**$15 x10^6$ /ml**
PH:	**> 7.1**
Motility (Active Motile):	**32%**
Sluggish (Intermediate):	
Non motile (Dead):	
Normal sperm cell (Vitality):	**58%**
Abnormal sperm cell:	
Red blood cell:	**1-2 /HPF**
Pus cell (WBC):	**< $1x10^6$/ml**
Epithelial cell:	**1-2 /HPF**
Immature cell:	**1-2 /HPF**
Agglutination:	**50%**