Abstract: Infertility is a growing challenge worldwide that cause many affiliated socio-psychological problems in many cultures that carry a negative impact on many families. As a last resort, many physicians engage certain drugs to over stimulate the gonadotropins to hope for the production of healthy Sperms and Ovum to materialize the fertility. Also, Fertilization In Vitro is a growing solution for the rich but many patients are not able to afford. Over stimulation of the sperms or ovum does not constitute the chromosomal DNA/RNA to amicably contribute to the successful fertility. Normal fetal development, uneventful pregnancy and/ or normal delivery. Many countries have established the prominent link to the Cytomegaloviral and Herpes Simplex Virus to the infertility.

We studied the prevalence and the role of herpes simplex virus (HSV), cytomegalovirus (CMV) in the semen of infertile men. Data was collected from sixty nine infertile men. Serum Anti- HSV IgG & IgM, CMV IgG & IgM from the blood and the Real Time Polymerase chain reaction RT/ PCR test were performed on the semen to detect the presence of the HSV & CMV RNA’s in these infertile subjects. Anti- CMV IgG was found in 44 (62.9%), CMV IgM in 4 (5.9%), HSV IgG in 22 (31.4%), HSV IgM in (0%) and PCR of CMV in 26 (37.1%), HSV PCR in 13 (18.8%). Overall, results revealed that the RT/ PCR of CMV were relatively more prevalent in the semen of infertile men as compared to the HSV IgG & IgM, CMV IgG & CMV IgM in the blood of infertile men.

Key Words: Infertility, HSV, CMV, HSV & CMV PCR

I. Objective:

To test the hypothesis that Herpes Simplex & Cytomegalo Virus inflicted semen substantiates infertility among men.

II. Introduction

Infertility is considered to be a big human challenge worldwide. Couples in most societies and cultures deal with this challenge in many different ways. It has been observed clinically that among infertile couples the relationship tends to become strenuous leading to metabolic syndrome, hypertension, stress, depression, isolation anxiety, lower self esteem, psychological distress, as well as guilt feelings and suicidal ideation. Infertility becomes a stressful experience for both of the partners, effect relationships with each other and family members, occupational issues, helplessness and marital instability [1].

In many societies men’s infertility is just stereotypically associated with women problem. Females are stigmatized for this issue not only in Asian cultures but also western societies without knowing the core cause for infertility. Females are not only psychologically abused but also physically traumatized due to not be capable to produce baby [2].

Different researches have indentified the physiological causes of infertility, based on that different treatment strategies like In vitro fertilization (IVF) In vitro fertilization, Zygote intrafallopian transfer (ZIFT), Gamete intrafallopian transfer (GIFT) and many others been introduced but the actual cause behind the pathology is still unknown [3].

Therefore, the aim of our clinical study was to find the pathological link and possiblesolution to this growing challenge.

Great many scientists looked at this challenge in many different ways. Taiwanese, Malaysians and Greeks particularly looked at the involvement of the herpes family of viruses and hypothesized the phenomenon of its involvement in the infertility [4], [5], [6].

There are 8 herpes family of viruses recorded to have been involved in many prominent diseases, yet there are 48 viruses those are un categorized. These viruses have the ability to persist latently in the host cell and...
give rise to recurrent infections. The herpes viruses have been divided into three subfamilies (α, β, and γ) based on both virological and biological properties [7].

The most neuroinvasive viruses are Herpes Simplex Virus (HSV) and Cytomegalovirus (CMV). To see its prevalence, one has to look at the Chronicity by tracing the level of IgG and the acuteness or recurrence of the infection through IgM in the serum.

Human cytomegalovirus (CMV) belongs to the β family, has been known to contribute in the physical or mental retardation, hearing or vision impairment as well as neurologic impairments. When a person gets infected with this virus, it remains latent in individual’s body for life. Under specific conditions as viral infections become active, deprive the related functions of controlled area [8].

III. Materials & Methods:

Sixty nine (69) documented infertile men participated in this study. Participants were counseled with the scope of the study and the integrity of the overall project.

Measures:

Serum evaluation of Herpes simplex virus [HSV-IgG & IgM], Cytomegalovirus [CMV IgG & IgM] was made and HSV & CMV RT/PCR were measured in semen.

Procedure:

Prior to the laboratory tests, informed consent was taken from the participants. Subjects were assured to maintain the confidentiality, right to voluntarily participate and withdrawn was given to them. Participants were given full privacy to draw their sample in a container. All the measures were concluded one by one for the participants under the International standards and controlled conditions. At the end, all the participants were informed about the results.

All the samples were taken under the supervision of medical scientists, to ensure quality. World Health Organization (WHO) and Clinical Laboratory Standardization Institute (C.L.S.I) guidelines were followed. All the samples were sent to laboratory within 15 minutes of the ejaculation and the blood draw. All the semen samples were stored at minus 80°C before analysis. Enzyme linked immuno-sorbent assays (ELISA) were performed using commercially available kits, on blood samples for CMV IgM, CMV IgG, HSV IgG and HSV IgG. Real time PCR technique was used to detect nucleic acid of the target viruses using commercially available primers. Data was analyzed using SPSS 20.

IV. Results:

Descriptive statistical analyses were carried out including frequencies, means, and standard deviations. A total sample of 69 candidates were selected, CMV IgG antibodies were found positive in 44 males 63.8%, on the other hand, CMV IgM antibodies were found positive only in 4 infertile males were positive that is 5.8% candidates were positive for CMV IgM. For HSV IgG antibodies, 22 males were found positive and none of the candidate was found positive for HSV IgM antibodies. It precipitated 39.9% and zero percent respectively. Total sero positivity for CMV, HSV was 69.6% and 39.9% respectively.

PCR showed 56.5% positivity in total for HSV and CMV, out of which 18.8 % male were positive for HSV nucleic acid compared with 37.7% for the CMV nucleic acid in the semen samples. Comparing CMV PCR results with HSV showed that CMV positivity was 50% greater in the semen samples. Independent 2 tail T-Test showed a highly significant value, $p^{***} < 0.0001$

Graph. 1> Frequencies of the HSV, CMV antibodies in blood.
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Graph. 2 Frequencies of the HSV, CMV PCR in semen samples.

V. Conclusion:

Significant presence of the antibodies against any virus is the direct reflection of the war against that viral nucleic acid at the least. Nucleic acid of the herpes family of viruses that continue to proliferate is the origin of leading challenges related to infertility. This most important reason behind the infertility has been left out of focus. This study is a paradigm shift in understanding of the etiology and viral pathophysiology of infertility.

We have found the greater influence of the cytomegalovirus (CMV) in defiance of the sperm’s capability of fertilization. Therefore, the progenesis of healthy sperms is defied by the chronic infection instigated by the CMV.

We believe that CMV infection must be eradicated to overcome the continuous infection in the body. This may help the production of the healthy sperms to enhance the chances of fertility.

VI. Discussion:

As the viral nucleic acid proliferation invades the controlling neurons that are responsible of secreting the neurosecretory enzymes to stimulate the pituitary gland, the molecular integrity of these enzymes becomes malified with the viral nucleic acid influence. Therefore, now the stimulating hormones secreted by the pituitary gland i.e. FSH, LH, also carry a grave influence of these viruses. Now, the Testosterone carrying the proliferating viral DNA influence of the HSV and/ or CMV begins to stimulate the production of the sperms from sertoli cells to undergo through further differentiation and maturation to become a viable sperm. Therefore, the presence of the antibodies of the HSV & CMV in the serum has been found due to the antibodies war against the viral genome proliferating in the seminal fluid. This substantiates the very potential of the infection process taking place within the neospermogenesis, leaving a variety of conditions of the spermatozoa.

According to Late Dr. Edward Wagner of University of California at Irvine in 2004, Viral RNA proliferation takes place continuously in Central nervous system under three major conditions (high heat, internal injury and downtrend of immunity) [9].

Because, the heat plays a significant role in the viral genome proliferation, therefore, the atmospheric and environmental heat may play a significant role in the enhancement of this Pathophysiology. Heat factor may acts as a complimentary effect in view of the local atmospheric conditions and its influence on spermogenesis and viral genome proliferation in the testes of male.

A Study carried out on 250 infertile men in Taiwan concluded that Anti- CMV IgG was present in 249 (99.6%) of the 250 male serum samples and in 247 (98.9%) of the 250 female serum samples. Viral shedding was detectable in 83 (33.5%) of 248 semen samples and 83 (33.7%) of 246 cervical mucus samples. It is concluded that the seroprevalence and genital tract viral shedding were relatively high in infertile couples in Taiwan [4]. This study is very close to our study in respect of viral shedding among the infertile population.

A descriptive clinical study to investigate the prevalence of herpes simplex virus cytomegalovirus and Epstein-Barr virus in the semen of men with fertility problems was reported. This study concluded that Herpes simplex virus plays a significant role in male Infertility [5]. Our present study do compliments the mentioned study in terms that HSV plays a role in the infertility. At the same time we concluded that HSV is of lesser prevalence compared with the CMV.
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Another study reported in literature reveal that, if CMV does not contribute to infertility then in future it gives rise to other obstetric complications. Study result of the serum samples from 125 apparently healthy pregnant women proved that Cytomegalovirus plays significant role in later challenges in fertile women. CMV IgG was found in 84% of the cases and CMV IgM in 7.2%. Both CMV IgM and IgG were also found in another 37 women with various obstetric complications that included 17 cases of spontaneous abortions, 15 cases of fetal anomalies, 1 case of incomplete abortion, 3 cases with premature delivery of infant with congenital anomalies and 1 case of infertility [6]. This study signifies total CMV seropositivity 91.2% for the infertility.

Whereas we have concluded it is 68.8% infertility due to CMV total seropositivity.

Many nations reported a variety of seropositivity of CMV & HSV in relation to Infertility. From the overall literature review, it can be concluded that Cytomegalovirus (CMV) in relation to infertility has rarely been reported in the literature. Not even a single study was reported from Pakistani context. So, further studies in that dimension are required, for not only enlightenment of knowledge but also to make the therapeutic services more effective and beneficial for the human beings.

VII. Future Guidelines

Further studies are required in order to find out the role of viral pathogenesis in infertility. Role of antiviral therapy shall be studied among infertile patients.

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References