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# Diagnosis of human herpesvirus 6A association infertility by RT-qPCR and detection sanger sequencing

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***Abstract*---**Human samples of Human Herpesvirus 6A were collected during 13 October 2021 to 15 of December 2021. Human Herpesvirus 6A was including (15-49years). The real time polymerase chain reaction (RT-qPCR) method was detected of all samples, the results showed fifty five(55%) positive cases while (45%) negative cases. The Population groups studied samples subject groups were distribution into (4) groups including (15-25, 26-36 and 37-47 and 48-58) year, changed age too gender. The second groups (26-36 years) were high cases of human infected (43.6%) in compare of aged groups then [37-47 (32.7%); 15-25 (14.5%); 48-58 (9%) years]. The percentage of males(54.5%) is higher than females (45.5%) . the number of positive cases in infertility patients with 5months,1-5 years of infertility was(27)cases more than that in an infertility patients with 6-10years of infertility was(18)cases and at least in an infertility patients with 11-15 years of infertility was(10)cases. Regarding the sequences test, the results showed the percentage of similarity with the studied strains at a rate ranging between (96.63-93.64%) as shown in the table (2), two samples were selected based on the concentration of the virus, cycles, different age groups , gender in addition to the sample source areas at the time of collection.The samples were isolated from the hospital including (Al-Sadr/Infertility center).The first study in Iraq to diagnose of Human Herpes virus 6A with infertility.

***Keywords*---**human, herpesvirus 6, infertility, real time, qPCR.

**Introduction**

Human *Herpesvirus* 6 (HHV-6), which consists of two virus species (HHV-6A and HHV-6B), now belongs to the beta *Herpes virus* subfamily. Initially, HHV-6A and HHV-6B were classified as two variants based on their different genetic, antigenic, and growth characteristics (Ma *et al.*, 2020). Human *Herpesvirus*-6A and−6B (HHV-6A and HHV-6B) are linear, double-stranded DNA viruses and members of beta *Herpesvirus*, along with CMV and HHV-7. HHV-6A and HHV-6B were identified as two distinct *Herpes virus* as early as 1992 (1), and in 2014, they were formally classified as two separate species (Eliassen *et al.,* 2018). The microbial infections are major cause in abortion, of which viruses appear to be the most frequently involved pathogens. Human *Herpes virus* -6 (HHV-6) has been implicated in cases of poor pregnancy outcome (Dosh *et al.,* 2019). *Herpes virus* is large DNA viruses with a genome of 120–230 kbp in size. Their genome is enclosed by an icosahedral nucleocapsid, which is surrounded by the tegument and a lipid envelope containing viral glycoproteins. The linear double-stranded (ds) DNA encodes the so-called core genes that form seven gene clusters and are conserved among *Herpesvirus*. HHV-6A/B virions are approximately 200 nm in diameter and contain a dsDNA genome of about 160–162 kbp.

The genome contains a unique region (U) that is flanked by terminal direct repeats (DR) of 8–9 kb at each end. Both DNA strands contain coding sequences The capsid two fold symmetry has sixteen surfaces (icosadeltahedral)and its diameter from a hundred up to one hundred and twenty of partly depends on the thickness of the surfaces owns one hundred sixty-twocapsomeres (Denner *et al***.,** 2019; Fadyia, 2021). As with other *Herpesviruses*, HHV-6 persisted in the host in a latent form. Sera prevalence in general population exceeds 90%. Primary infection was acquired during the first two years of life; saliva being the most likely mode of transmission. Besides direct infection of the cells, HHV-6 particularly variant A was a powerful inducer of cytokines, e.g., tumor necrosis factor-alpha, interferon-gamma, and interleukin-1 beta It had proposed that the immunomodulatory and marrow suppressive effect of HHV-6 might partly be due to the production of these cytokines (Singh *et al.,* 2017). Human *Herpesvirus* 6 was confirmed by high viral DNA copy numbers in whole blood and somatic cells. The origin of integrated viral genome, paternal or maternal, was examined using the same method (Miura *et al.,* 2021). Infertility was defined as a couple's inability to conceive after a period of twelve months of regular unprotected intercourse. Infertility globally affected approximately 10-15% of couples. This study was carried out to find out the determinants of infertility among infertile couples (Tamrakar R and Bastakoti R, 2019).

Infertility was a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse (World Health Organization,2018 World Health Organization., 2021). Sanger sequencing was a targeted sequencing technique that used oligonucleotide primers to seek out specific DNA regions. Sanger sequencing begins with denaturation of the double-stranded DNA. The single-stranded DNA was then annealed to oligonucleotide primers and elongated used a mixture of deoxy nucleotide triphosphates (dNTPs). As the dNTPs and ddNTPs had an equal chance of attaching to the sequence, each sequence would terminated at varying

lengths (Gomes A and Korf B, 2018). Sanger sequencing used the SBS approach in which a DNA polymerase generated DNA reads from a template that was the DNA molecule to be analyzed. The nature of the nucleotide at a given position was determined using specific dyes (Vernet G, 2017).

## Material and Methods

### Collect affected specimens of Human *Herpes virus 6A*

Samples were collected of *Human Herpesvirus 6A* through a start interval 13 October 2021 up to 15 December 2021. Fifty five positive cases including (30 (54.5%) males and 25 (45.5%) females) with infected human patients of age ranged fifteen up to fifty nine years of specimens.

### Real Time -qPCR Technique

This method was used to diagnose Human *Herpes virus 6A*, via (this primer was designed based on the NCBI which is gene U95, GoTaq®qPCRMaster mix kit (Cat. Number: 023484574400, abm, canada).Viral DNA was extracted by using Viral Nucleic Acid Extraction Kit (gSYNC TM DNA extraction kit)(Geneaid, Lot No.FA30411-GS,USA ). This technique was performed in the postgraduate laboratory of the Department of Life Sciences at the faculty of Education for Girls and in faculty of Veterinary by using (Analytik Jena/Qtower3G) advice**.**

Table 1
Primers of human *herpesvirus* 6A depended to NCBI

| Primer\Name | Sequence | Bases | PCR product size |
|---|---|---|---|
| F | 5-CTGCAAAGTGGTACGCTCAA-3 | 20 | 181 Pb |
| R | 5-GCATACGTGCACCAATCATC -3 | 20 | |

## Results

### Genetic analytic method for diagnosis of *Human Herpes virus 6A* through RT- qPCR

Fifty-five cases appear positive from 100 samples of collected serum and semen of different areas were diagnosis by real time- q PCR, while 45 cases were negative as show in figure (1). Thirty cases of males infected consider the highest of twenty four females as in figure (2). The age group (15-49 years) compared to other totals in terms of age in fig. (3)

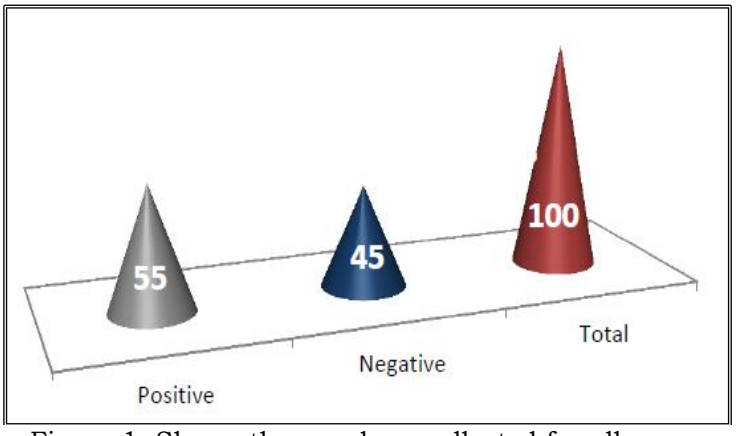


Figure 1. Shows the numbers collected for all cases

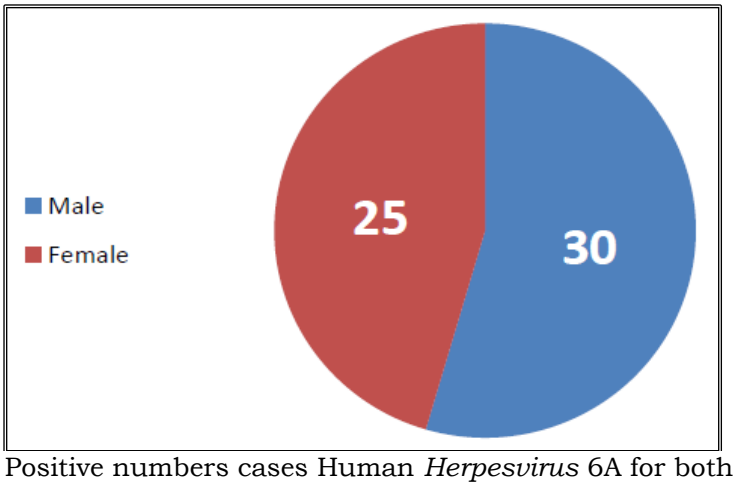


Figure 2. Positive numbers cases Human *Herpesvirus* 6A for both genders

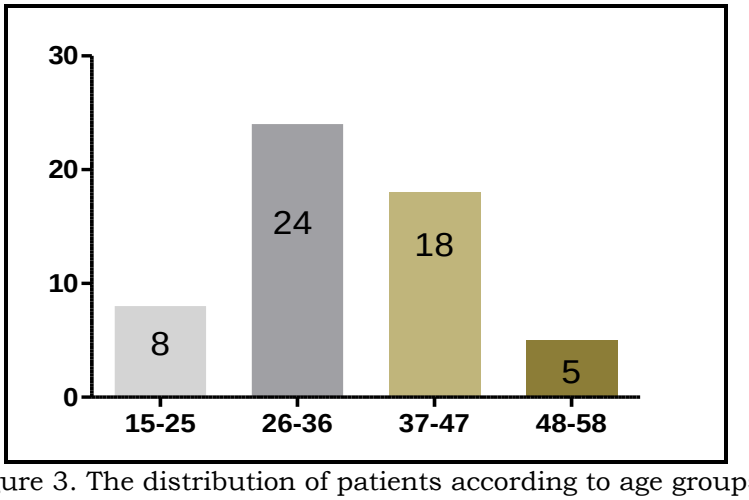


Figure 3. The distribution of patients according to age groups.

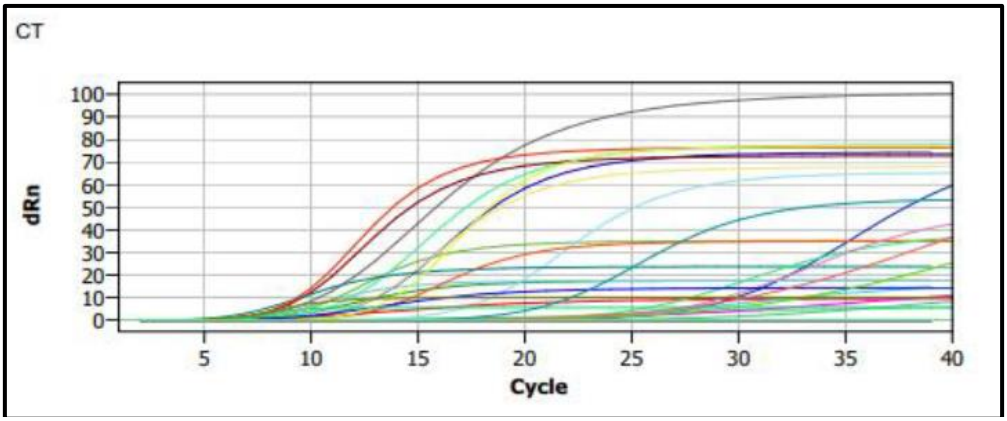


Figure 4. The figure shows the method for diagnosing of human *Herpesvirus 6A* through real time –qPCR.

Regarding the sequences test, the results showed the percentage of similarity with the studied strains at a rate ranging between (96.63-93.64%) as shown in the table (2) two samples were selected based on the concentration of the virus, cycles, different age groups, gender in addition to the sample source areas at the time of collection.

Table 2
The NCBI-BLAST Homology Sequence identity (96.63-93.64%) between local human *Herpesvirus 6A* isolate and NCBI-BLAST submitted human *Herpesvirus 6A* isolate

| Local isolate No. | NCBI-BLAST Homology Sequence identity (%) | | |
|---|---|---|---|
| | NCBI-BLAST identical Genotypes | Genbank Accession number | Identity (%) |
| Human *Herpesvirus*6A in Infertility isolate No.1 | Human betaherpesvirus 6A strain HHV6A_303035. | MW049315.1 | 96.63% |
| Human *Herpesvirus*6A in Infertility isolate No.2 | Human betaherpesvirus 6 strain HP73C5. | KY290183.2 | 93.64% |

**Discussion**

Diagnosis of Human *Herpes virus 6A* with infertility the study is considered in Najaf Governorate and at the level of Iraq as well by designing primers depending on the Location "NCBI" by RT-qPCR technicality which resembled with karmoff *et al.* (2020) which show that males are more susceptible to injury compared to females, and also agreement with study Alazzam *et al.* (2022) .In our study

examine the similarity and variance between strains by observing the genetic deficiency with Sanger Sequencing technology was amplified from the serum and semen samples and results of the examination displayed a percentage going between (96.63-93.64%)according to National Center for Biotechnology Information (NCBI) is agreement with study (Lino *et al.,* 2022).

**Conclusion**

This is the first study in Iraq to diagnose of Human *Herpes virus 6A* with infertility.

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