Immunogentic Detection of Human Herpes Virus-6B, IL4and TNF-α in Male Patients Suffering from Infertility Disorders

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Abstract

The study aims to investigate HHV6-B, IL4 and TNF-α in infertility male patients. To achieve this goal, 200 samples (blood and semen to each patient) were collected from infertility center in ALSadr hospital and outside laboratory in Najaf Governorate (160 patients and 40 from normal person as control group). Patients were married and infertile. Samples were collected from patients during the period from 1 of December 2020 to 15 December 2021.

Polymerase chain reaction (PCR) technique was used to detect the occurrence of HHV6-B DNA among infertility male patients. Molecular assay results of this study revealed the presence of HHV6-B was 17 (10.63%) in infertility male patients. HHV6-B DNA not detected in seminal fluid of healthy persons.

The present study has detected that single nucleotide Polymorphisms (SNP) in IL-4 -590 C/T genotype frequency distribution in infertility male patients. The genotypes relative frequency in patients were as follow: CC (33.75%), CT (43.75%) and TT (22.5%) among them. C allele is higher frequency (65.32%) in patients than T allele (34.67%) in which there was no significant difference from statistical perspective (*P value* = 0.004) among infertility male patients and control group. The genotypes relative frequency in HHV-6B infections was: CC (29.41%), CT (41.17%) and TT (29.41%). There was no significant difference (*P value* = 0.849) among patients.

Also, in this study has detected that single nucleotide Polymorphisms (SNP) in TNF-α -308 G/A genotype frequency distribution in infertility male patients. The genotypes relative frequency in patients were as follow: GG (26.25%), GA (45.63%) and AA (28.13%) among them. G allele is higher frequency (56.2%) in patients than A allele (43.8%) in which there was no significant difference from statistical perspective (*P value* = 0.212) among infertility male patients and control group. The genotypes relative frequency in HHV-6B infections was: GG (23.53%), GA (52.94%) and AA (23.53%). There was no significant difference (*P value* = 0.9739) among patients.

Keywords: HHV6B, IL4, TNF-α, SNP and Infertility male patients.

1. Introduction

Infertility is defined as the failure to achieve conception while using full and regular sexual intercourse without the use of contraceptive measures for pregnancy over a period of one year or longer. Infertility is a prevalent disorder that affects around 15% of couples, according to estimates [1].

It is responsible for 40–50% of infertility in humans. Approximately 7% of all males are affected. Semen quality is utilized as a surrogate measure of male fecundity since male infertility is frequently caused by defects in the sperm [2].

Exanthema subitem is a frequent pediatric ailment caused by HHV-6B primary infection (also known as roseola infantum or sixth disease), It is handed down from generation to generation, and it is rare for adults to develop the disease because almost everyone has had it before kindergarten. An antibody is generated, which helps to prevent reinfection. In addition, HHV-6B reactivation is prevalent in transplant patients, and it can result in encephalopathy, bone marrow suppression, and pneumonitis, among other symptoms [3].

Cytokines have long been recognized as significant immune mediators, and they can play a role in a variety of activities in the male genital tract, including serving as immunomodulatory components inside the gonad [4].

Interleukin 4 (IL-4) is a cytokine that causes naïve helper T cells (Th0 cells) to differentiate into Th2 cells. When Th2 cells are activated by IL-4, they create more IL-4, creating a positive feedback loop. Mast cells, Th2 cells, eosinophils, and basophils generate IL-4, which is closely linked to and has activities comparable to interleukin 13 [5].

Blood samples by vein puncture were collected from infertility men which were included 4ml of blood put in EDTA tube When macrophages identify an infection, they produce tumor necrosis factor alpha (TNF-), a tiny protein employed by the immune system for cell communication. TNF is released to warn other immune system cells as part of an inflammatory response [6].

2. Method

200 samples (blood and semen to each patients)were collected from infertility center in ALSadr hospital and outside laboratory in Najaf Governorate (160 patients and 40 from normal person as control group) . Patients were married and infertile. Samples were collected from men infertility during the period from 1 of December 2020 to 15 December 2021.

1. Collection of blood sample

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and stored at -20°C for human DNA extraction to study gene polymorphism of IL4(-589C/T) and TNF-α(−308 G/A)

2. Collection of seminal fluid sample

on the size and density of the source material.

Conventional PCR used to detect HHV6-B and the Single Specific Primer-Polymerase Chain Reaction (SSP-PCR), which can detect a known SNP in IL4 and TNF-α consist of two complementary reactions: one containing a primer specific for the normal DNA sequence and cannot ampli fy mutant DNA at a given locus and the other one containing a mutant-specific primer and does not amplify normal DNA.

The Primers which used in this study illustrate in table (1). Seminal fluid samples collect from male patients in tubes at room temperature. First, for microscope examination of semen is done by using 100X magnification. And second, centrifuged the remaining of seminal fluid samples at 3500 r.p.m for 5 minutes to obtain pellet and add phosphate buffer's to pellet and make centrifuge at 1500 r.p.m for 20 minutes and take pellet to storage in deep freeze until uses to diagnosis HHV-6 infections by viral DNA exraction.

3. Detection by Molecular detections by DNA extractions and Polymerease chain reaction (PCR)

DNA extraction from semen for viruses and from blood for Human has been done using a modified protocol which is compatible with Blood genomic DNA extraction kit (Solarbio Cat No.: D1800). The protocol can be used for fresh or frozen semen samples with equal efficiency. Frozen samples must be thawed thoroughly before use. Please note that lysis time will vary depending

Table (1): Primers.

Gene	Primer	PCR product length
IL4 -590 C/T	F: AGG CTG AAA GGG GGA AAG C R1: CTG TTC ACC TCA ACT GCT CC R2: CTG TTC ACC TCA ACT GCT CT	253bp
TNF-α -308G/A	F: AGG CAA TAG GTT TTG AGG GCC AT R1: TCC TCC CTG CTC CGA TTC CG R2: ATA GGT TTT GAG GGG CAT CA	107bp
HHV6-B	F: GAC AAT CAC ATG CCT GGA TAA TG R: TGG TAA TGG ACT AAG TGT GCG TTA TTT TC	134bp

3. Ethical Committee

The Faculty of Sciences, University of Kufa, provided supervision and suggestions for this work. All samples included in this study were acquired according to the research methods for each kind, with no extra materials or alteration, approved by the Iraqi Ministry of Health's Medical Ethics Committee.

4. Statistical Analysis

The information was entered into a digital database. The statistical studies were carried out with the help of Microsoft Excel 2010 and the Graph Pad Prism (version 6) computer software. The distribution of infertility in patients with. Directed counts were used to estimate allele and genotype frequencies of the IL2 and IL10 genes, which were expressed as a percentage. The Chi-square test was also used to compress the data. The test was conducted using a significance level of 0.05.

5. Result and Discussion

The detection of HHV-6 B infections in samples has occurred by using sensitive molecular techniques which include conventional PCR . To our knowledge, there is many studies have been published on HHV-6 using conventional PCR.

The HHV-6B DNA has been detected in (17 out of 160) or (10.63%) in infertility male patients as positive infections with virus, and (143 out of 160) or (89.38%) as negative infections to virus while in control group the number is (0 out of 40). There is significant difference (Chi-square = 4.645; P value = 0.0311) among study groups (infertility male patients and control group) according to HHV-6B detection in Figure (1)and distribution as displayed in Figure (2).

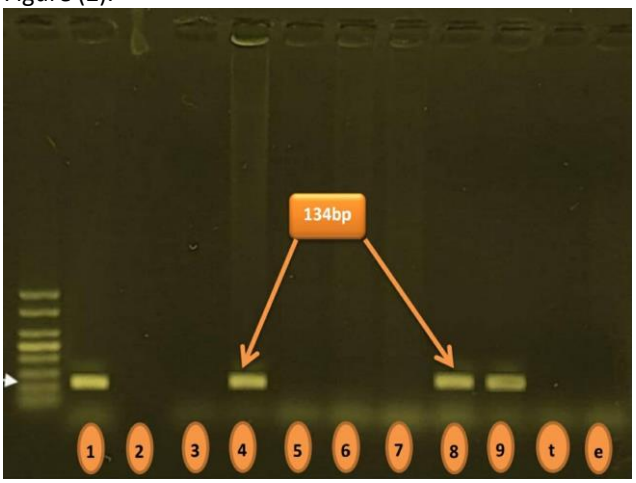

Figure (1): Agarose gel electrophoresis image that show the PCR product analysis of HHV6-B gene in infertility seminal fluid samples. PCR product was analysis by 1% agarose gel . Where M: marker (50bp – 1000bp), lane (1,4,8,9) showed positive bands to HHV6-B (134 bp) in samples.

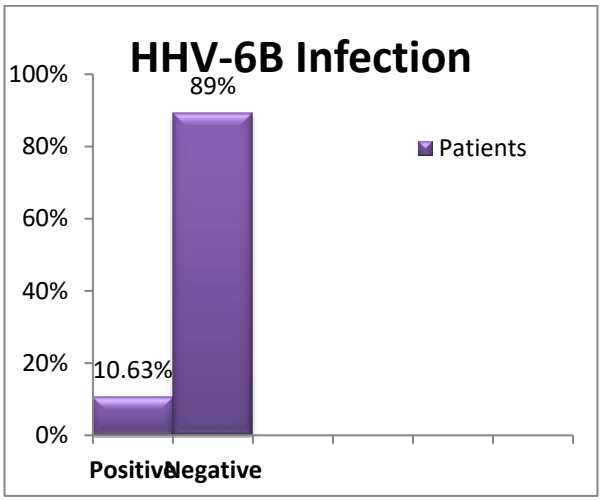

Figure (2): Distribution of infertility male patients and control according to according to HHV6-A, P<0.05.

The current study shows that HHV-6B can be transmitted to the uterus by sperm, but this may argue against infection of the oocyte during normal fertilization because the acrosome is dissolved before the sperm enters the egg. However, HHV-6B integrates chromosomally at a frequency of 0.8 percent, and it is unknown how this integration occurs. As a result of HHV-6B chromosomal integration, it is estimated that PCR-based detection will detect about 1% of semen samples [7].

The poor detection of HHV6-B in seminal fluid samples from infertile male patients groups might be linked to seasonal distribution and environmental factors that influence HHV6-A transmission.

Cytokine Polymorphism Analysis of IL-4

The genetic polymorphism of the IL-4 gene has been determined at one position, the IL-4 -590 C/T which is present with three genotypes (CC , CT and TT) in patients and control. SSP-PCR method has been used to genotyping of IL-4 -590 C/T in infertility male patients and control. The PCR products have been well resolved and sized by agarose gel electrophoresis , allowing easy identification of different genotyping. Heterozygotes and homozygotes have been unambiguously assigned from the gel profilewas suitable for the separation on 1 % agarose gel. Which shown in Figure (3). male patients with (65.32%) than T allele (34.67%). While T allele is also higher frequency in control with (63.33%)than C allele (36.66%)There was a no significant differences (Chi-square =8.247 ; *P value* = 0.004) in the IL-4 allele frequency distribution between control and patients groups, as shown in Figure (5).

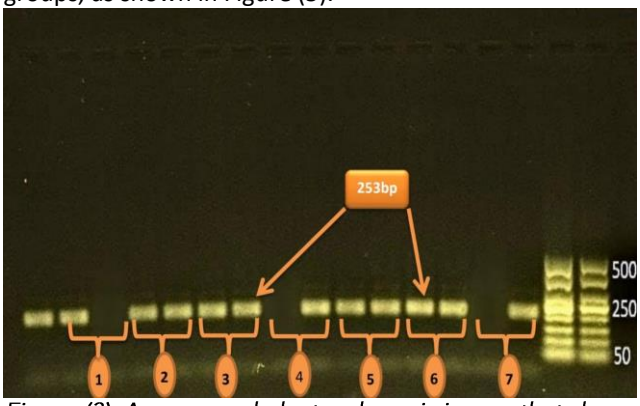

Figure (3): Agarose gel electrophoresis image that show the SSP-PCR product analysis of IL-4 gene (-590 C/T) in infertlity male samples. SSP-PCR product was analysis by 1% agarose gel . Where M: marker (50bp – 1000bp), lane CC wild type homozygote was shown at the bands sample (1), lane CT mutant type heterozygote was shown at the following bands samples (2,3,5 and 6) and lane TT homozygote was shown in bands samples (4 and 7).

The IL-4 genotype frequency distribution in infertility male patients and control groups. The genotypes relative frequency in infertility male patients were as follow: CC (33.75%), CT (43.75%) and TT (22.5%).While in control subjects was: CC (27.5%), CT (37.5%) and TT (35%). There was no significant difference (Chi-square = 2.679 ; *P value* = 0.262) among patients and control group according to IL-4 genotype detection and distribution as shown in Figure (4).

C allele is in higher frequency in infertility

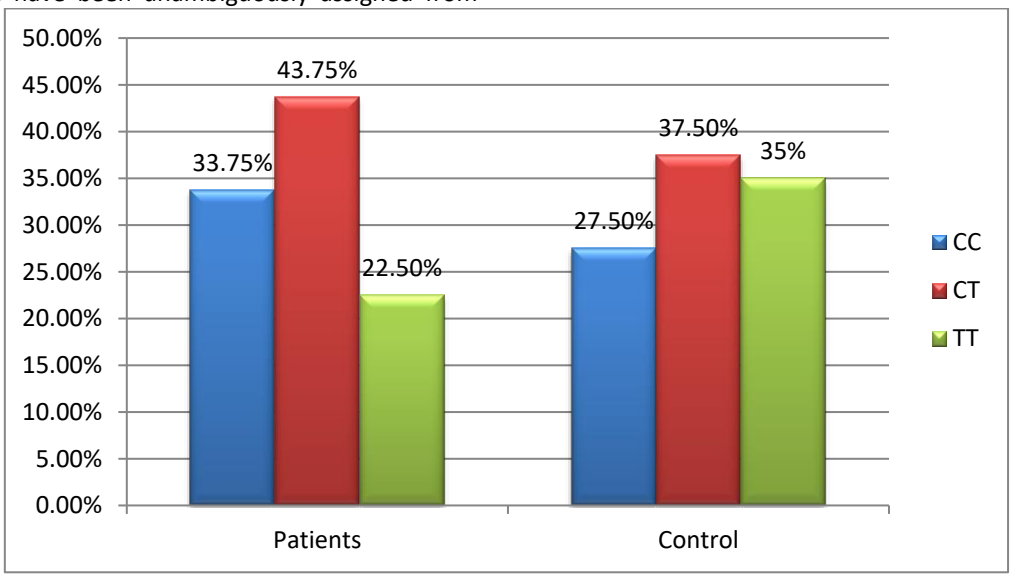

Figure (4): Detection of IL-4 genotype among infertility male patients and control group.

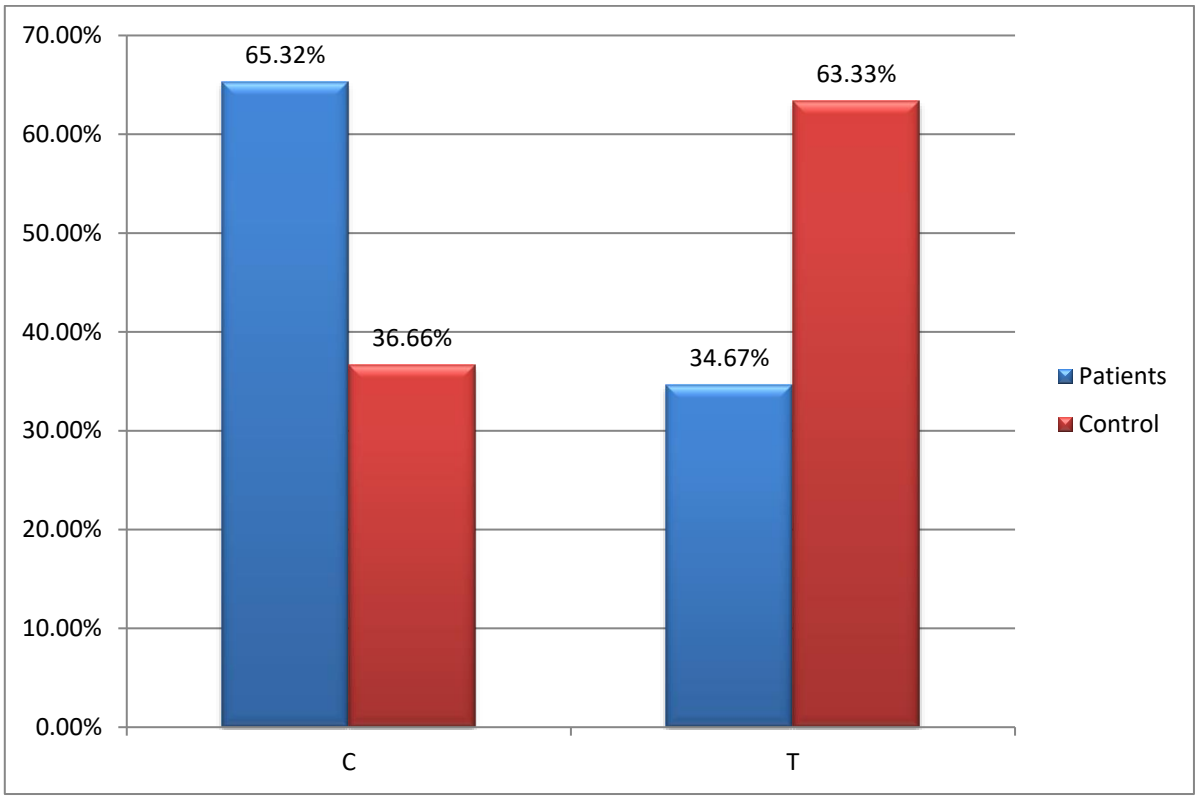

Figure (5): Detection of IL-4Allele frequency among infertility male patients and control group.

The IL-4 level in seminal plasma is directly associated to male reproductive capacity, according to Li et al. The rise or decrease in these cytokines reflects the condition of reproductive system immunity and/or infection, and influences sperm function [8].

According to the findings of the current study, the distribution of genotypes and allele frequencies in the endometriosis group did not differ statistically from the control group (27.5 percent C/C, 55.0 percent C/T, and 17.5 percent T/T vs 40.0, 45.0, and 15.0, respectively). The chance of detecting no significant variations in genotype distribution was 0.496, while the likelihood of detecting the presence or absence of the mutant allele was 0.928[9].

In another study, allele T and genotype TT of the IL-4 rs2243250 single nucleotide polymorphism were linked to allergic rhinitis susceptibility [10].

Polymorphism Analysis of TNF-α

The genetic polymorphism of the TNF-α gene has been determined at one position, the TNF-α -308 G/A which is present with three genotypes (GG , GA and AA) in patients and control. SSP-PCR method has been used to genotyping of TNF-α -308 G/A in infertility male patients and control. The PCR products have been well resolved and sized by agarose gel electrophoresis , allowing easy identification of different genotyping. Heterozygotes and homozygotes have been unambiguously assigned from the gel profilewas suitable for the separation on 1 % agarose gel. Which shown in Figure (6).

There was a no significant differences (Chi-square =1.557 ; *P value* = 0.212) in the TNF-α allele frequency distribution between control and patients groups, as shown in Figure (8).

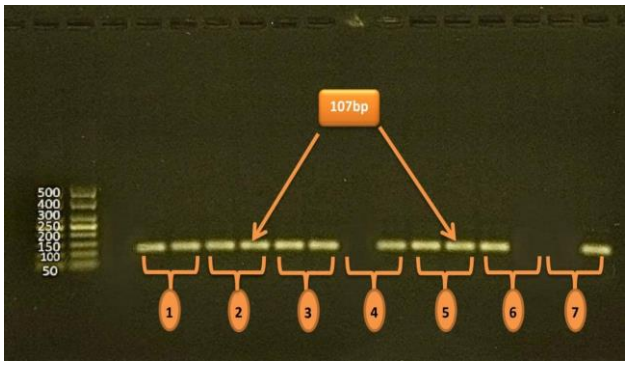

Figure (6): Agarose gel electrophoresis image that show the SSP-PCR product analysis of TNF-α gene (-308 G/A) in infertlity male samples. SSP-PCR product was analysis by 1% agarose gel . Where M: marker (50bp – 1000bp), lane GG wild type homozygote was shown at the bands sample (6), lane GA mutant type heterozygote was shown at the following bands samples (1,2,3and 5) and lane AA homozygote was shown in bands samples (4 and 7).

The TNF-α genotype frequency distribution in infertility male patients and control groups. The genotypes relative frequency in infertility male patients were as follow: GG (26.25%), GA (45.63%) and AA (28.13%).While in control subjects was: GG (27.5%), GA (42.5%) and AA (30%). There was no significant difference (Chi-square = 0.128 ; *P value* = 0.938) among patients and control group according to TNF-α genotype detection and distribution as shown in Figure (7).

G allele is in higher frequency in infertility male patients with (56.2%)than A allele (43.8%). While A allele is also higher frequency in control with (55.88%)than G allele (44.11%)

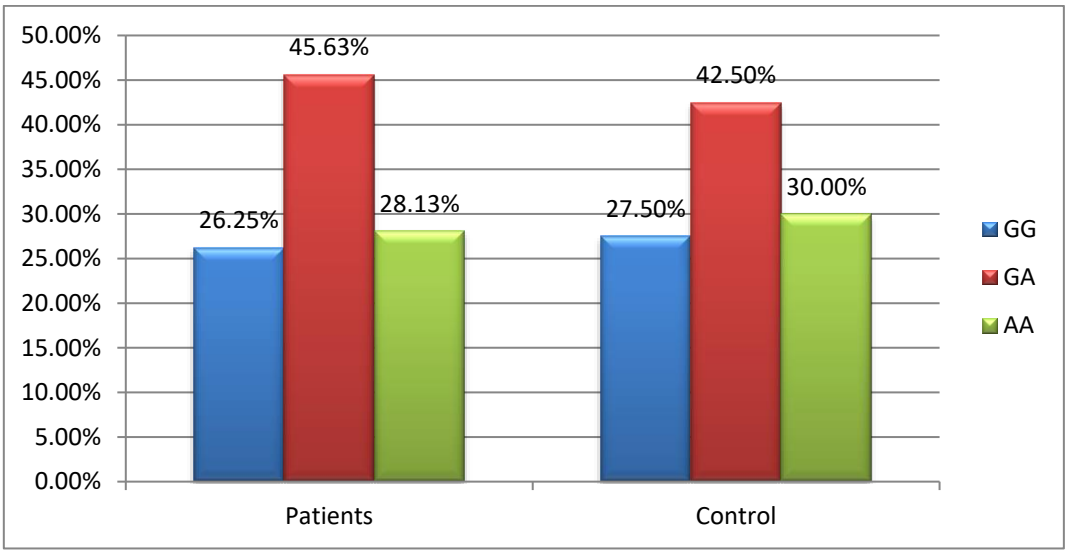

Figure (7): Detection of TNF-α genotype among infertility male patients and control group.

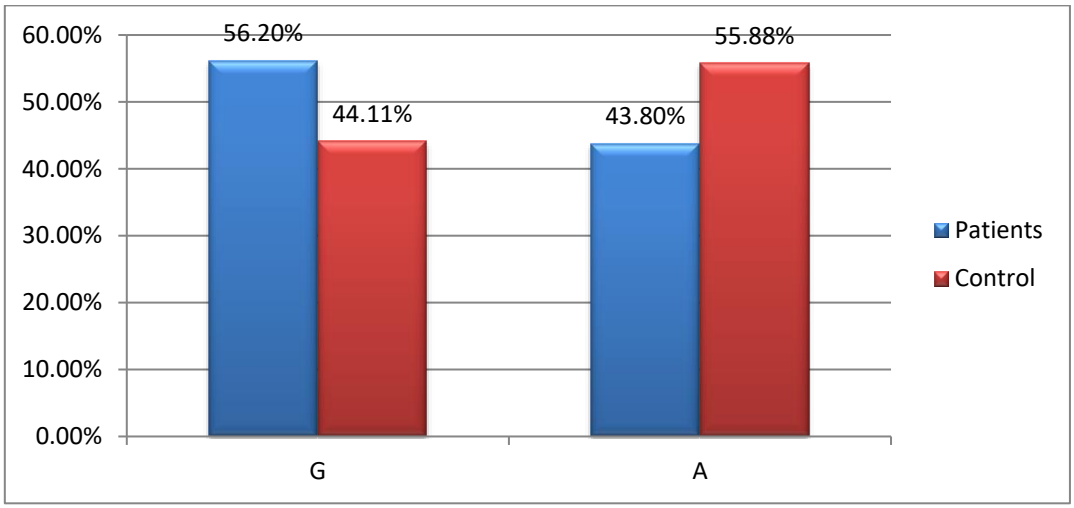

Figure (8): Detection of TNF-α Allele frequency among infertility male patients and control group.

The current study was the first to show a connection between four polymorphisms in the promoter region of the TNF- gene and endometriosis in women of Iranian descent. The A allele may play a role in the occurrence of primery infertility in both women and men, according to new study[11] .

The current investigation in an Indian community found that infertile people had much greater substitution levels from G to A in the TNF- gene than healthy fertile controls. Infertile oligozoospermic and asthenozoospermic patients had greater levels of apoptosis and necrosis, which were linked to higher amounts of reactive oxygen species [12].

In the current investigation in Asians, Li et al. found that infertile men with normal sperms had significantly lower frequency of GA/AA at position 308 in the promoter region of the TNF- gene than infertile men with asthenozoospermic or oligoasthenozoospermic sperms. They discovered a link between the GA + AA allele of the TNF-308 gene and sperm motility progression. They found that asthenozoospermic and oligoasthenozoospermic men had much greater seminal plasma TNF- levels than infertile men, with normal sperm levels being significantly higher in the GA + AA type of the TNF-308 allele than in the GG type [13].

In another investigation in the Egyptian population, the TNF-GG genotype was shown to be more common in fertile males than asthenozoospermic, asthenoteratozoospermic, or oligoasthenoteratozoospermic men. Infertile patients had a considerably higher prevalence of the A allele than fertile controls. When compared to men with the TNF-GG genotype, men with the TNF-AA genotype showed a substantial drop in sperm concentration, motility, normal sperm morphology, acrosin activity, and seminal-glucosidase, as well as a significant rise in seminal plasma caspase-9 apoptotic factor [14].

6. Conclusions

Based on this study ,the following conclusions could be made this research describes the prevalence of HHV6-B in infertility male patients.The current study revealed that HHV6-B is more abundant among patients with infertility disorders. Genetic study of IL-4 -590 C/T and TNF-α −308 G/A alleles do not consider a risk factor for effection in infertility male patients with and without HHV6-B infections.

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