Serodiagnosis of Human Herpesvirus-8 among Iraqi Blood Donors

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ABSTRACT

Human herpesvirus type 8 (HHV-8) or known as Kaposi sarcoma-associated herpesvirus (KSHV) is the etiologic agent for all clinic-epidemiological forms of Kaposi's sarcoma (KS). Many studies have been documented that blood transfusion plays an important role in HHV-8 transmission. This study was prepared to determine the frequency of HHV-8 antibodies among blood donors. A cross-sectional study was conducted on ninety blood donors who attended the Iraqi National Centre for Blood Transfusion in February 2019 to detect HHV-8 IgG antibodies using an enzyme-linked immunoassay method. Anti-HHV8-IgG were detected in 78 out of 90 (86.6%) serum samples. A significant association has been found between anti-HHV-8 detection and associated risk factors in blood donors such as sexual relationships (legal and illegal), occupation, surgical and dental operations, blood transfusion, cupping, tattooing, smoking and numbers of blood donation. Our results confirmed that a high percentage of HHV8-IgG among blood donors may indicate an increased threat to HHV-8 infection via blood transfusion.

Keywords: HHV-8; KSHV; Blood donors; ELISA; blood transfusion.

INTRODUCTION

Human herpes virus 8 (HHV-8), or known as Kaposi's sarcoma associated herpesvirus (KSHV), is a member of herpesviridea, belong to gamma herpesvirus causes several neoplastic diseases and responsible of all clinic-epidemiological forms including Kaposi sarcoma, multicentric Castleman's disease and primary effusion lymphoma (Cesarman *et al.*, 1995) HHV-8 is clearly of crucial public health concern among immune-compromised individuals who are at higher risk of developing Kaposi sarcoma (Cattani *et al.*, 2001). However the existence of HHV-8 has been known for over a decade (Chang *et al.*, 1994), the detection of HHV-8 infection remains a challenge, when there are factors that make HHV-8 difficult to diagnose in peripheral blood among a large proportion of individuals who have been infected with HHV-8 but do not have detectable levels of the viral genome (Whitby *et al.*, 1995), for that the serological assays may remain the first line for HHV-8 detection, after subsequent development of antibody assays with reasonable sensitivity and specificity that paved the way for sero-epidemiological studies to indicate the spread of HHV-8 and the risk factors for HHV-8 infection. The geographical distribution of HHV-8 sero-positivity generally coequal with that of Kaposi Sarcoma (Pfeiffer *et al.*, 2010). The exact route of HHV-8 spread is still under discussion. Though, previous reports specify that HHV-8 can be transmitted via saliva, sexual and non-sexual routes, blood transfusion and during organ transplantation, HHV-8 can replicate during the acute state, enter latency, and then reactivate from this latent state (Toth *et al.,* 2010). Various studies provide strong evidence for HHV-8 transmission through blood transfusion, in addition to the evaluation of sero-prevalence and potential risk factors of HHV-8 infection in blood donors at different regions around the world (Wang *et al.*, 2010). In the past times it has been noticed that the spread of this virus has been recorded in most parts of the world and in neighbouring countries were provided information about HHV-8 detection in healthy individual and in blood donors, as in Saudi Arabia when the sero-prevalence was 1.7% in healthy individuals (Alzahrani *et al.*, 2005). A sero-prevalence of HHV8 had been reported at 2% among Iranian blood donors (Jalilvand *et al.*, 2011). Also seropositive blood donors of HHV-8 were reported at 29 (5.3%) among Turkeys (Altuğlu *et al.*, 2016).

MATERIALS & METHODS

Study group

Ninety blood samples were collected from seems to be healthy blood donors whom attended to Iraqi National Center for Blood Transfusion in Baghdad city. This study was conducted in the University of Baghdad/ College of medicine/ Department of Microbiology. The study extended from February 2019 to September.

Ethical consideration

The ethical approval of the study was obtained from the Ethical Committee in the Department of Microbiology and from the Council of Collage of Medicine/ University of Baghdad/ Iraq. All participants received a written and verbal data sheet explaining the purpose of the study. ritten consent signed by each individual participating in the study was obtained prior to a direct interview to record their information and history.

Blood collection

Ten ml of fresh venous blood was aspirated from each participant; some sample was collected directly from blood

pouch by using 10 ml vacuum tube. The blood was put into a 5 ml of EDTA tube. The blood left to clot and the serum immediately separated after blood centrifuging, then frozen at -20 which stored for later serological detection.

Anti-HHV8-IgG detection

Elisa kit from MyBioSource (MBS2800428) was used for the qualitative detection of Human Herpes virus 8 IgG antibody (HHV8-IgG-Ab) concentration in serum. The procedure was adopted following instructions of the manufacturer. Frozen serum samples were dissolved in water bath at 37°C, Positive and negative control was added to the well of kit plate, Two µL of serum samples added with 200 µL of diluent to the wells then incubated in dark at 37°C after concealed with adhesive film for half hour, one hundred µL of horse radish peroxidase as conjugate was added then incubated for half hour, Automated washer was set up for 5 times washing with 250 µL of previously prepared washing buffer, The washing buffer was emptied into the sink and then confirmed that it was completely removed by inverting the plate on a clean paper towel, Each of 50 µL of substrate A and 50 µL substrate B was added to the well and incubated in dark for 10 minutes, To each well 50 µL of stop solution was added within 5 minutes, The optical density of each well was determined within 5 minutes by using microplate reader set to 450 following by calculation of results: After determining the optical density of each well by the reader, the result was printed on a sheet of paper, and then the samples were compared with the control:

1. For the negative control

Optical density of the negative Control must no more than 0.15. If the average (The sum of the OD value is divided by its number) value was less than or equal to 0.10 was calculated. If one of the negative OD values higher than 0.15, was discarded. If more than two negative OD values for the negative Control higher than 0.15, the test was repeated.

2. For the positive control

Optical density of the Positive Control must no less than 0.60. If one of the Positive Control OD values less than 0.60, was discarded. If the two Positive Control OD value less than 0.60, the test was repeated.

3. Calculation of Cut-off Value

Cut-off Value was calculated by the average of Negative Control OD values + 0.10:

1. While OD sample ≥ Cut-off Value: it mean Positive.
2. While OD sample ＜Cut-off Value: it mean Negative.

Statistical analysis

Statistical analysis tests were used to compare and explain the results of methodology applied and associated factors involved in this study, positive and negative results were recorded as percentages of total. Statistics for Windows software System used Statistical Package for Social Science (SPSS), Version 24.0 (IBM corporation, Armonk, NY,USA, 2016), program was used to detect the effect of difference factors in study parameters. The following tests were used in appropriate; One-way ANOVA analysis of variance was used for evaluation of differences between means of three groups. Chi-square test of independence with Fisher exact test was used to compare the significant between percentages. In this study each tests were two–sided significant; P value (0.05 and 0.01 probability).

RESULTS AND DISCUSSION

In the current study, enzyme immunoassay results were obtained for ninety serum samples that randomly taken from blood donors. 84 were males and 6 were females with a male to female ratio of 14:1 and Their ages ranged from 18-60 years with mean of (36.97 ± 10.186) years.

Serological detection of Anti-HHV8-IgG antibodies

Anti-HHV-8 IgG were detected in 78 out of 90 (86.6%), 73 (93.5%) were males and 5 (6.41%) were females. the highest rate of anti-HHV-8 IgG antibodies was detected in 31 (39.7%) of blood donors were among ages (31-43) years.

Risk factors associated with Anti-HHV8-IgG antibodies detection

In this study, serological results were significantly associated with many risk factors among blood donor's history the risk of HHV-8 infection increased significantly as the number of blood donations increased (O.R.= 1.355, CI 95%, P = 0.030) Table 1. The overall risk factors that significantly related to anti-HHV-8 sero-atus such as surgical and dental operations, blood transfusion, cupping, tattooing, and smoking that demonstrated in Table 2. Other crucial risk factor such as sexual relations, also was highly relevant to our results which increase proportionally with the number of sexual relationships as showed in Table 3, in both legal sexual relations (O.R.= 1.882, CI 95%, P = 0.002) and illegal sexual relations (O.R.= 1.761, CI 95%, P = 0.000) as demonstrated in Fig 1. Anti-HHV-8 IgG results were not significantly related to age, residence and blood groups of study group.

As far as we can tell, there is no study or statistics conducted in Iraq to test blood donors or investigate the risk factors for HHV-8 transmission. To date, HHV-8 sero-detection is routinely recommended only in an endemic area as many facts that refer to the possibility of HHV-8 role in the pathologic process of many diseases. The decision to test blood donors came from the ascending spread of HHV-8 in the Arab world and specifically in the surrounding countries. Serological assays that determine the existence of antibodies against HHV-8 is the main method to investigate the prevalence of HHV-8 in a population for large epidemiological studies (Minhas & Wood, 2014). The present study adopted ELISA technique to detect the HHV-8 IgG antibody in the serum samples of blood donors as they are more sensitive, easier and faster. It necessary to detect the HHV-8 in the blood not only to find the HHV-8 antibodies but also to relate the transmission of infection to recipients via blood transfusion. The high rate of serological results was also reported in two studies done in Peru with 56.25% and in Tanzania with 56.9% among blood donors (Mohanna *et al.*, 2007; Lidenge *et al.*, 2020). In addition to our findings, we are in line with the increasing incidence of

Kaposi's sarcoma among different populations at risk in all surrounding countries (Alzahrani *et al.*, 2005; Jalilvand *et al.*, 2011; Hussein *et al.*, 2012; Altuğlu *et al.*, 2016; Shokri *et al.*, 2020). Meanwhile, different studies have been conducted using various tests to measure HHV-8 existence in different populations in order to identify the HHV-8 risk factors and to investigate possible routes of transmission. It's well documented that the prevalence of HHV-8 were recorded at high rate in men than women, which coincided with our research and many other findings are in line with studies conducted to inspect the role of gender in HHV-8 infection in prevalent and in non-prevalent regions and among healthy and diseased patients (Wang *et al.*, 2010; Jalilvand *et al.*, 2011; Shokri *et al.*, 2020). Once more, male predominance can be explained to reflect the socio-behavioural state of the studied group, high risk exposure to HHV-8 infection, Moreover, as they are healthy and asymptomatic, they acts as a carrier for transmitting the virus to others. In this study the highest frequency of positive results were observed in age group of (31-43) years. That is in agreement with a study done among blood donors in Brazil, HHV-8 sero-prevalence was 31.3% in those above 35 years of age (Nascimento *et al.*, 2009). Similarly, a study in Peru reported nearly parallel results as the highest sero-positivity of (68.75%) was recorded in age group of (30–34) years among blood donors and plateaued among those above 35 years old with range from (50%–64.7%) (Mohanna *et al.*, 2007). HHV-8 serostatus appeared in the very active age group who are sexually active and are exposed to blood-borne infection by newly introduced practices of tattooing, cupping, and possibly due to needle sharing. Non-sexual route can also be the cause; exposure to infected saliva through specific acts by this age group through the sharing of food dishes, glass water, smoking pipes or cigarettes as recorded in Egypt (Mbulaiteye *et al.*, 2008). Of the non-HIV-infected group that was included in this study, HHV8 is likely to have constant and multi-transmission methods, as in endemic pathogens, and is not limited to subjects at risk for the development of Kaposi's sarcoma. Our results indicated an important statistical association of seropositive blood donors with occupation but not with residence, a recent study pointed out to a close association between serological detection of HHV-8 with patient's occupation (Lee *et al.*, 2018). Occupation of the free workers group was the most associated group in this study, and may highlight the socioeconomic status of this group when there's a lack of awareness and knowledge about viral diseases which increases the potential risk of HHV-8 transmission, this is inconsistent with the research done in north-western China (Wang *et al.*, 2010), another study reported highly significant association between occupation and HHV-8 seropositive males in Egypt (Mbulaiteye *et al.*, 2008). This study analyzed multivariate risk factors between the history of blood donors with history of any surgical operations including dental surgery or previous blood transfusions, which showed threatening signs for HHV-8 infection. As for their history in terms of smoking, cupping, tattoo and drug use; they were also directly related to HHV-8 detection. Eventually, these risk factors may responsible for the transmission of many pathogens and each donor should be questioned about before donating blood, as it was previously recorded that blood transfusion was the cause in two HHV-8 seropositive children under five years old who had received blood (Kamiyama *et al.*, 2004), as well as Ugandan research established the seroconverting of seronegative children after receiving blood (Hladik *et al.*, 2006). Chinese study registered a significant increase HHV-8 sero-prevalence among blood donors in Xinjiang, which relates to the high prevalence of Kaposi sarcoma in the region, indicating the possibility of HHV-8 blood-borne transmission route (Wang *et al.*, 2010). From another cohort study critically reported that the risk of death related to the receiving short-stored HHV-8 seropositive blood (Hladik *et al.*, 2012). Conspicuously, a recent Iraqi study recorded fourteen women with breast cancer were HHV-8 IgG positive with previous history of blood transfusion (Shakir 2018), these evidence has raised the concern that HHV-8 might be transmitted by blood. In term of smoking (cigarettes and pipes); our results were consistent with the study (Cesarman *et al.*, 2019). These findings suggest salivary HHV-8 transmission in our study group and a potential biologic explanation for geographical variation of HHV-8 sero-positivity and Kaposi's Sarcoma. Also cupping (known as Hijama in the Arab world) and tattooing were associated significantly with anti-HHV-8 IgG detection, consistent with earlier reports in Egypt concluding that sero-positivity of HHV-8 was associated with tattoos (Mbulaiteye *et al.*, 2008). In the present study all diabetic blood donors have been infected with HHV-8, this was in agreement with a study that analyzed viral infections in diabetics including HHV-8 (Shakir, 2018) A case–control study was performed in China among type two Diabetes mellitus patients and pointed out the risk of HHV-8 infection increased with the concentration of glucose and HHV-8 antigens was more likely to express in diabetic patients (Cui *et al.*, 2019). Patients who have diabetic at higher risk of tumours that associated with HHV-8 infection, as it was approved that the HHV-8 life cycle had been modulated by high glucose levels in different types of cells (Chang *et al.*, 2017). These results led us to issue whether diabetes is a disease that prefers HHV8 infection either by reduction of immune system efficiency, or, conversely, alike HHV-8 infection produce conditions that make metabolic modifications headway to diabetes mellitus (Angius *et al.*, 2020) Our findings indicated that the state of blood donor donation, usually analyzed through various researches, showed the link between HHV-8 detection and the number of donations recognized as an important risk factor for HHV-8 transmission, and most blood donors who donated up to ten times and more over their lifetime, all of which were heavily related to the HHV-8 detection. The increasing frequency of blood donation, raise the possibility of increasing the infection as mentioned in the study (Wang *et al.*, 2010). Furthermore, our study pointed out that the legal and illegal sexual relationships of Iraqi blood donors suggest a direct risk factor of HHV-8 infection, as some participants who have had sexual relations with more than a dozen partners reach the maximum positive measures of the cut-off value among qualitative immunological detection

this may propose the pivotal evidence of HHV-8 transmission during sexual route, as supported by (Zakari *et al.*, 2012). Since travel activity has increased in recent years that coincide with the increase in illegal non-conservative relationships and homosexual behaviours that directly affect KSHV infection according to other study (Kumar and Nautsch, 2016). In addition, another study mentioned the possibility of the sexual transmission of HHV-8 among Iranian population (Kakavand-Ghalehnoei *et al.*, 2016), also an increase HHV-8 detection in semen of Iranian healthy people was reported by (Rakhshan *et al.*, 2019). Altogether, results of the studies have made a tantalizing image of the source of transmission and without obvious recommendations on the incipient prevention of HHV-8 infections.

In conclusions, The present study provides further evidence that blood transfusion carries a potential risk for HHV-8 transmission, since that the percent of serological detection were high among Iraqi blood donors, many risk factors play an important role in the HHV-8 transmission. Further studies are needed to explain plausible existence of HHV-8 infection among Iraqis, which helps to explain many of unclear aspects of HHV-8 epidemiology, viral transmission factors and the related diseases.

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Preparing an original draft of writing: Mohammed ZB and Abdullah SF; writing-review: Mohammed ZB; editing: Abdullah SF. All authors read and approved the pre-submitted version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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Table 1: Donation status in relation to anti-HHV8 IgG results.

Donation status	Anti-HHV-8 IgG results		Total NO
	Positive NO (%)	Negative NO (%)	
First time	17 (73.9)	6 (26.1)	23
(2-10) times	47 (90.4)	5 (9.6)	52
More than 10 times	17 (68.0)	8 (32.0)	25
ANOVA (F = 3.638, df = 2, P = 0.030)			

Table 2: Risk factors associated with Anti-HHV8 IgG results of Iraqi blood donors.

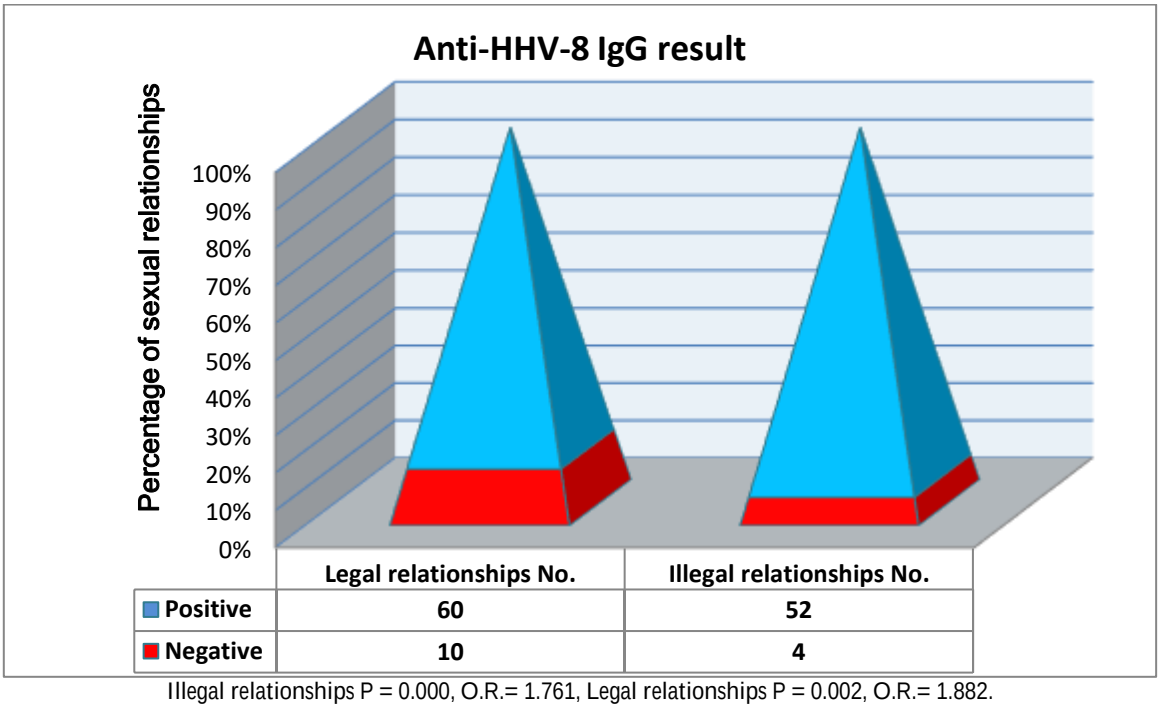
Risk factors	Anti-HHV-8 IgG results Positive NO (%)	Negative NO (%)	Total NO	P value by chi-square
dental operation	48 (53.3)	29 (32.2)	77	
surgical operation	24 (26.6)	4 (4.4)	28	P = 0.033
blood transfusion	14 (82.4)	3 (3.3)	17	
Smoking	48 (53.3)	6 (6.6)	54	
Cupping	36 (40)	11 (12.2)	47	
Tattoo	10 (11.1)	6 (6.6)	16	P = 0.011
Drug used	3 (3.3)	2 (2.2)	5	

From total 90 blood donors, each donor may have more than one risk factors.

Table 3: Number of Sexual relations in association with Anti-HHV 8 IgG result among Iraqi blood donors

Sexual relations	HHV8 PCR results Positive NO (%)	Negative NO (%)	Total NO
No relation	10 (11.1)	5 (5.6)	15 (16.7)
1-9 times	49 (54.4)	4 (4.4)	53 (58.9)
More than 10 times	19 (21.1)	3 (3.3)	22 (24.4)
Total NO.	78	12	90

ANOVA (F = 6.742, df = 2, P = 0.002)



Illegal relationships P = 0.000, O.R.= 1.761, Legal relationships P = 0.002, O.R.= 1.882.

Figure 1: Association of Legal and illegal sexual relations with anti-HHV-8 IgG results of Iraqi blood donors.

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