

## The frequency of human herpes virus type 8 among blood donors and post-kidney transplant patients in two specialized centers in Khartoum

Shamsoun K. Kafi<sup>1\*</sup>, Reem T. Suliman<sup>2</sup>, Magdi Bayoumi<sup>2</sup>, Hassan A. Musa<sup>1</sup>

### Abstract:

**Background:** Human herpesvirus-8 (HHV-8) is an opportunistic virus proved to be the cause of AIDS associated Kaposi's sarcoma. Based on the emergence of HIV and its pandemic spread HHV-8 is expected to participate in increasing the risk of Kaposi's sarcoma in areas where the rate of infection is high. There is a great need to study the epidemiology of the virus.

**Objectives:** To find out the rate of infection with HHV-8 in Khartoum among blood donors and post-kidney transplant patients

**Methodology:** Venous blood samples were collected from 90 Subjects (60 blood donors from Elsalam Cardiac Centre (controls) and 30 kidney transplanted patients from IbnSina hospital). The blood specimens were tested for Human herpes virus anti- IgG using ELISA technique.

**Results:** The overall rate of infection with HHV-8 was found to be 2.2%. The highest rate of infection (20%) was in the age group 46 years and more. The seroprevalence of HHV-8 was found to be 6.7% in post-kidney transplant patients. None of the control group proved to be positive for HHV-8.

**Conclusion:** The rate of infection with HHV-8 was found to be relatively lower in the studied group.

**Key words:** Human herpes virus-8, Immuno-compromised patients.

**H**uman herpes virus type 8 is a herpes virus that belongs to the subfamily gamma herpesvirinae of the family herpesviridae. As early as 1994 scientists reported that, they had detected herpes like structure in Kaposi's sarcoma (KS) tumor by electron microscope<sup>1</sup>. They had then started searching the causative agent of Kaposi's sarcoma among more than 20 agents described as the possible causes; among these were cytomegalovirus and HIV<sup>1</sup>. Chang and Moore could successfully sequence the entire genome of a herpes virus which proved to be the causative agent of Kaposi's sarcoma<sup>2</sup>. That virus is known now as human herpesvirus type 8 in sub-Saharan Africa, intermediate levels of infection occur in Mediterranean countries and low levels of infection in most North European

and North American populations. The virus is opportunistic. Although it is wide spread it only causes tumor in immune-compromised patients as AIDS patients. With the global epidemic of HIV infection causing immunodeficiency; increasing incidence of Kaposi's sarcoma is anticipated. There is therefore a great need to study the epidemiology of the virus so that appropriate control measures will be taken for reducing the transmission of the virus and hence the incidence of Kaposi's sarcoma.

### Objective:

To find out the rate of infection with HHV-8, in Khartoum among blood donors, and post-kidney transplant patients.

### Materials and methods:

A total of 90 participants were recruited in this study. Sixty of them were blood donors in Elsalam Centre for Cardiac Surgery (healthy controls) and 30 post-kidney transplant patients attending IbnSina hospital for follow up (immune-compromised individuals). From each subject 3 mls of venous blood were

1. Dept of microbiology, The National Ribat University.

2. Department of microbiology, Faculty of medical laboratory science, University of Medical Sciences & technology.

\*correspondence: shamsounkafi@yahoo.co.uk

collected into sterile, clean and dry plain container. The collected blood specimens were allowed to clot. After clot retraction the sera were separated by centrifugation and stored in a deep freezer at  $-20^{\circ}\text{C}$  until tested. The stored sera were examined for human herpes virus type 8 IgG antibodies using ELISA technique. The test procedure involved three incubation steps:

The test sera, positive (in duplicate) and negative control sera were added into the corresponding wells of micro titer plate coated with optimal amount of solubilized HHV-8 whole virus extract. One well was left as blank. Specific HHV-8 antibodies present in the serum bound to the antigen-coated plates forming Ag/Ab complexes. After incubation the strips were washed to remove unbound samples components. Antihuman IgG conjugated to enzyme horseradish peroxidase (HRP) was added to the wells and during incubation bound to the Ag/Ab complexes already formed in the micro wells. After other incubation the micro wells were washed to remove unbound conjugate. HRP substrate tetramethylbenzidine (TMB) was added to the wells. During incubation, enzyme mediated cleavage of the substrate resulted in a color change. The reaction was then stopped using stop solution. The optical densities (OD) of specimens and control were read within 30 minutes using spectrophotometer (ELISA reader) at wavelength 450 nm. The test procedure was then validated according to the manufacturer instruction as follows:

OD of the blank  $\geq 0.100$ .

OD of positive control at least 1.5 times of the cutoff serum.

The ratio between OD of negative control and cutoff serum must be  $\geq 0.6$ .

The absorbance of the cutoff must be  $< 0.2$ .

#### Calculation of the cutoff value:

The cutoff value was calculated according to the formula: Cutoff value =  $1,227 \times 0.10$  (= 0.123) Where 1,227 was the mean of the OD of the two positive control sera.

#### Interpretation of the results:

Specimens with OD less than the cutoff value were considered to be negative. Those with

cutoff values greater or equal to the cutoff were considered positive.

#### Results:

A total of 2 (2.2%) were found to be positive for HHV-8 IgG antibodies. The two positive cases were both post-kidney transplant patients giving seroprevalence of 6.7% in this group. None of the control group was found to be positive. The positive cases constituted 20 % of the age group 46 years and more (table 1), they were both kidney transplanted 7 -12 months before testing (table 2).

Table 1: Distribution of HHV anti IgG according to age

| Age group (in years) | Total number tested | Number positive | Percent age positive |
|----------------------|---------------------|-----------------|----------------------|
| 15 – 25              | 21                  | 0               | 0                    |
| 25 – 35              | 40                  | 0               | 0                    |
| 36 – 45              | 19                  | 0               | 0                    |
| 46 ≤                 | 10                  | 2               | 20                   |
| Total                | 90                  | 2               | 2.2                  |

Table 2: Distribution of HHV-8 IgG among post-kidney transplant patients according to the duration of transplant

| Duration of transplant (months) | Number tested | Number positive | Percent positive |
|---------------------------------|---------------|-----------------|------------------|
| 1 – 6                           | 21            | 0               | 0                |
| 7 – 12                          | 7             | 2               | 28.5             |
| 13 – 19                         | 0             | 0               | 0                |
| 20 – 24                         | 2             | 0               | 0                |
| Total                           | 30            | 2               | 6.7              |

#### Discussion:

The overall seroprevalence of HHV-8 IgG antibodies among the studied group of blood donors and post-kidney transplant patients was found to be 2.2%. This figure is comparable with previous studies done in USA and Northern Europe (5-10% & 5% respectively)<sup>3,4</sup>.

None of the blood donors was found to be

positive for HHV-8 which is in disagreement with previous reported figures from Belgium and China (3.3% and 20.4%, respectively)<sup>5, 6</sup>. This variation in the rate of infection with HHV-8 can be attributed to the different socioeconomic conditions and the small sample size in the current study.

In Greece, Azavitsanou and his colleagues reported seroprevalence of HHV-8 of 7.2% among patients on haemodialysis<sup>7</sup>. He also reported that patients younger than 50 years had an increased probability for HHV-8 infection.

### Conclusion:

The rate of infection with Human herpesvirus type 8 is 6.7% among the studied group of post-kidney transplant patients and zero% among the control group of blood donors. The rate of infection was found to be higher (20%) after the age of 46 years. Further in depth studies including different populations and larger sample size are needed to validate these results.

### Acknowledgement:

Sincere gratitude to staff of Ultra lab (in Khartoum and Cairo) who facilitated the practical work. Thanks extend to the nursing staff of IbnSina and Salam Cardiac Centres for their great help in specimens collection.

### References

1. Chang Y, Cesarman E, Pessin M S. Identification of Herpes-like DNA sequences in AIDS – associated Kaposi's sarcoma. *Science* 1994; 266: 1865 -9.
2. Moore PS, Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. *The new England Journal of Medicine*. 1995; 332(18):1181 – 5.
3. Massambu C, Pyakurel P, Kaaya E et al. (2003). Serum HHV-8 DNA and Tat antibodies in Kaposi's sarcoma patients with and without HIV-1 infection. *Anticancer Res*. 2003; 23(3B):2389 - 95.
4. Simpson GR, Schulz TF, Whitby D. Prevalence of Kaposi's sarcoma associated herpes virus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. *Lancet*; 1996; 348: 1133 – 1138.
5. Schulz TF. Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8). *J Gen Virol*. 1998; 79: 1573 – 91.
6. Fu B, Sun F, Li B. Seroprevalence of Kaposi's sarcoma-associated Herpesvirus and risk factors in Xinjiang, China. *J Med Virol*. 2009; 81: 1422 - 1431.
7. Azavitsanou V, Sypsa M, Petrodaskalaki M et al. Human herpesvirus-8 infection in hemodialysis patients. *Am J kidney Dis*. 2006; 47: 1667 – 70.

