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Research Article,

Seroprevalence of herpes simplex virus and toxoplasma gondii among schizophrenic patients attending psychiatric hospitals-Khartoum state.

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Abstract:

Background: Toxoplasma. Gondii can produce psychotic symptoms similar to those displayed by persons with schizophrenia· Some reports of acute toxoplasmosis in adults produce signs such as delusions and hallucinations also Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), also known as human herpes virus 1 and 2 (HHV-1 and HHV-2), are two members of the herpes virus family, Herpesviridae, that infect humans are ubiquitous and contagious.

Study objectives: The aim of the study to determine Sero- prevalence of Herpes Simplex Virus and Toxoplasma gondii among Schizophrenic patients attending psychiatric Hospital in Khartoum state.

Method:

A total of 140 samples from schizophrenic patients with different age groups. Personal and clinical data collected by questionnaire after verbal consent to co-patients, This study was evaluated a rapid sample (serum in plain container) to perform assay for detecting antibodies to Herpes simplex virus and Toxoplasma gondii by LAT {latex agglutination test} and {ELISA test}; dilutions of test sera were added to latex particles coated with Herpes simplex virus and Toxoplasm gondii antigen. 50 samples were positive for Herpes simplex virus ;these positive were tested by ELISA {Enzyme Linked Immunosorbent Assay}IgG; IgM and 70 samples positive for Toxoplasm gondii were also tested by ELISA IgG; IgM .

Results:

The results analyzed statistically and revealed that 50(35%) positive of Herpes simplex virus antibody by LAT ; of these 26 (52%) were positive for IgG and 3 (6%) were positive for IgM; and70(50%) positive 0f Toxoplasma gondii antibody by LAT were tested ; of these 30 (42%) were positive for IgG ,and 8 (11%) were positive for IgM, in serum from schizophrenic patient .The results of this study showed that the disease is most prevalent in the age group between (25 – 34) Years more than other groups. In the martial group the single were 80 samples of this (20(25%)samples showed positive results of HSV by LAT of these 8(40%) were positive for IgG; and 30(37%) were positive results for Toxoplasma gondii by LAT of these 6(20%) samples positive for IgG and 3(10%)were positive for IgM);for the married 60 samples(30(50%) were positive results showed of HSV by LAT;and of these 18(60%) were positive for IgG , 3(10%) were positive for IgM ; and 40(66%) were positive for Toxoplasma gondii by LAT of these 24(60%) were positive for IgG and 5(12%) sample positive IgM) ;the married more infected than sigle patients . 78 (55%)patients

have schizophrenia family history of this 20 (26%) were positive HIV LAT from this 2(10%)were positive IgG , 25(33%) were positive Toxoplasma gondii LAT from this 3(125) were positive IgG ; and 62(44%)samples were not schizophrenia family history and negative results .so patients with schizophrenia family history more infected than other .The patients infected by Toxoplasma gondii higher than those with Herpes simplex virus. The new finding of this study showed that infection with Toxoplasma gondii and herpes virus associated with some cases of schizophrenia.

Conclusions:

This study provides some prove that infection with Toxoplasma.gondii and Herpes virus may be associated with some cases of schizophrenia. Additional studies should focus on antibody to these agents in the sera of individuals with recent onset psychosis.

Introduction:

Schizophrenia is a mental disorder characterized by a breakdown of thought processes and by poor emotional responsiveness (Sternberg et al., 2006). It most commonly manifests itself as auditory hallucinations, paranoid or bizarre delusions, or disorganized speech and thinking, and it is accompanied by significant social or occupational dysfunction. The many possible combinations of symptoms have triggered debate about whether the diagnosis represents a single disorder or a number of discrete syndromes (Meltzer et al, 2004).

The term Schizophrenia means a "splitting of mental functions", because of the symptomatic presentation of the illness (Hutchinson and Haasen.2004).People with schizophrenia are likely to have additional (co morbid) conditions, including major depression and anxiety disorders; the lifetime occurrence of substance abuse is almost 50% (Beers and Mark. 2004). Social problems, such as long-term unemployment, poverty and homelessness, are common. A combination of genetic and environmental factors play a role in the development of schizophrenia. (Schiffer et al, 2014). People with a family history of schizophrenia who suffer a transient psychosis have a 20–40% chance of being diagnosed one year later (Meltzer et al., 2004).

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), also known as human herpes virus 1 and 2 (HHV-1 and HHV-2), are two members of the herpes virus family, Herpesviridae, that infect humans. Both HSV-1 (which produces most cold sores) and HSV-2 (which produces most genital herpes) are ubiquitous and contagious. They can be spread when an infected person is producing and shedding the virus. Symptoms of Herpes simplex virus infection include watery blisters in the skin or mucous membranes of the mouth, lips or genitals. Lesions heal with a scab characteristic of herpetic disease (Martin et al., 2009). Sometimes, the viruses cause very mild or atypical symptoms during outbreaks. However, as neurotropic and neuro invasive viruses, HSV-1 and 2 persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons. After the initial or primary infection, some infected people experience sporadic episodes of viral reactivation or outbreaks. In an outbreak, the virus in a nerve cell becomes active and is transported via the neuron's axon to the skin, where virus replication and shedding occur and cause new sores(Martin et al ., 2009).

Herpes simplex is divided into two types; HSV-1 causes primarily mouth, throat, face, eye, and central nervous system infections, whereas HSV-2 causes primarily conogenital infections. However, each may cause infections in all areas. (Schiffer et al (2014). In some psychiatric disorders environmental factors are involved in the pathophysiology of the diseases especially in schizophrenia. Research has focused on infectious agents as potential players in the etiologic pathway of chronic diseases, including psychiatric illnesses such as schizophrenia. Due to their potential neurotropism and latency, viral organisms in particular are considered possible agents in many chronic Central nervous system (CNS) disorders (Douglas et al., 2009) Epidemiological studies have revealed different microbial agents have been proposed as risk factors for schizophrenia, many recent studies have focused on members of the viral family of Herpesviridae, Borna virus, intracellular bacteria like Chlamydia as well as the protozoan organism Toxoplasma gondii (Roizman and Knipe .2001). Reasons for focusing on these agents include their ability to establish persistent infections within the central nervous system as well as the occurrence of neurological and psychiatric symptoms in individuals infected with these agents. Evidence for an infectious cause includes the 5-8% increased risk among those born in the winter-spring months, when infectious diseases are more prevalent and at times when other infections (measles, varicella, and poliomyelitis) show increased activity. Herpes simplex virus (HSV) has been implicated in schizophrenia as it has a tropism

for the nervous system and is capable of replication in the brain. Although post-mortem studies of brain tissue of schizophrenic patients have failed to detect the virus, these studies have been hampered by the unknown cellular localization of HSV genomes and by attempting to detect the virus years after the symptom onset analysis of stored blood samples showed an association between high levels of maternal antibody to HSV-2 and subsequent development of adult psychosis. Although laboratory-based research into Herpes family viruses as possible etiologic agents for schizophrenia goes back decades, ascertaining the nature of a possible etiologic association between infection and schizophrenia is highly challenging (Jones, 2003).T. gondii is an intracellular parasite in the phylum Apicomplexa. Its life cycle can be completed only in cats and other felids, which are the definitive hosts. However, Toxoplasma gondii also infects a wide variety of intermediate hosts, including humans. In many mammals, Toxoplasma gondii is known to be an important cause of abortions and stillbirths and to selectively infect muscle and brain tissue (Mitchell et al., 2003). A. variety of neurologic symptoms, including in coordination, tremors, head-shaking, and seizures, have been described in sheep, pigs, cattle, rabbits, and monkeys infected with Toxoplasam gondii (Chong et al ., 1989).

Humans may become infected by contact with cat feces or by eating undercooked meat. The importance of these modes of transmission may vary in different populations. Individual response to Toxoplasma infection is determined by immune status, timing of infection, and the genetic composition of the host and the organism (Mori et al., 1992).

Recent epidemiologic studies indicate that infectious agents may contribute to some cases of schizophrenia. In humans, acute infection with Toxoplasma gondii can produce psychotic symptoms similar to those displayed by persons with schizophrenia· Some cases of acute toxoplasmosis in adults are associated with psychiatric symptoms such as delusions and hallucinations (Shimojo et al., 2003).

Materials and methods:

Study design:

Prospective cross sectional study in which the disease had been occurred, and frequency had been determined.

Study area:

The study had been conducted in the psychiatric hospitals and center in Khartoum State.

Study period:

The study done during periods from March 2015 to September 2016.

Study population:

All schizophrenic patients attending psychiatric hospitals in Khartoum State during conduction of the study.

Sampling technique:

Non Probability sampling had been selected randomly to meet the objective of the study.

Sample size:

The below equation had been used for calculating sample size:

N=PQ (Z) 2/E

Where:

N=Sample size

Z=Level of confidence interval at 95%, so Z=1.96

P=estimated percent of population [1]

E=acceptaple sample error expressed as apercent [5%]

Q=1-P Then;

N=10*.90*(1.96)2 / (.05)2 =139.29

So the minimum number of schizophrenic Samples under detection had been 140.

Tools of data collection:

Primary data were collected from the respondents in the form of close questionnaire.

Samples collection:

The Blood samples from each participant had been taken intravenously then separated by centrifugation at the collection site. The separated sera were split into aliquots, for stored at −20 °C for further use in plain container

Laboratory work:

1preparation of samples:

Blood samples were allowed to clot and then centrifuge at 3000rpm for 5-10minetes to obtain sera .Then the obtained sera were preserved at -20°C until serological analysis.

Lab Investigation of Herpes Simplex Virus and Toxoplasma gondii:

The samples were analyzed by for the presence of Herpes Simplex Virus and Toxoplasma gondii Immunoglobulin (IgM &IgG) antibody by commercially available ELISA kit

Principle:

The HSV 1or2 ELISA test kits is solid phase enzyme immunoassay based on indirect principle for the qualitative detection of IgG or IgM antibody to HSV in human serum. The mice well plate is coated with HSV antigens. During testingthe specimens are added to the antigen coated micro well plate and then incubated .If the sample contain antibodies to HSV it will bind to the antigen coated on the micro well plate. After incubation, the micro well plate was washed to remove unbound materials. The enzyme conjugated anti human has been binded to immobilized antigen HSV antibody complexes present .After incubation, the micro well plate is washed to remove un bound materials .Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of HSV IgG or IgM antibody present in the sample .Sulfuric acid solution is added to the micro well plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of HSV antibodies present in the sample,is measured with a microplate reader at 450/630-700nm or 450nm.

Procedure:

Prepare working wash buffer by diluting the concentrated wash buffer 1:25.puor the contents of the bottle containing the concentrated wash buffer graduated cylinder and fill it with freshly distilled water to 1250ml for 96 wells/plate testing .If crystals arepresent in the concentrated wash buffer ,warm it up at 37 until all crystals dissolve.

Leave A1 as blank well.

Add 100 micro liter of negative control in wells B1 and C1.

In D1 and E1 Add 100 micro liter Cut-Off calibrator

F1 and G1: Add 100 micro liter positive control.

Add 5 micro liter sample to H1.

Mix gently.

Cover the micro wells plate with the plate sealer and incubate at 37 for 30 minute.

Wash each well 5 times with Working Wash Buffer,.and turn the micro well plate upside down on absorbent tissue.

Add 100 micro liter of conjugate to each well except for the Blank well, and cover the micro well plate with the plate sealer and incubate at 37 for 30 minute.

Wash each well 5 times with Working Wash Buffer,.and turn the micro well plate upside down on absorbent tissue.

Add 50 micro liter of substrate A to each well, and add 50 micro liter of substrate B to each well, blue color had been developed in wells contain positive specimen.

Mix then Cover the micro wells plate with the plate sealer and incubate at 37 for 10minte. Remove the plate sealer and add 50 micro liter of stop solution to each well, yellow color had been developed in wells contain positive specimen**.** Read at450/630-700nm within 30min.

Interpretation of results:

Result: compeer the specimen with cut of value.

Cut of value = Mean absorbance of cut of calibrator –Blank absorbance.

Blank absorbance =Well A1.

Mean=cut of calibrator: Well D1+ cut of calibrator: Well E1.

Latex Agglutinatio Test slid:

In this test, soluble antigen is coated on latex particles, and agglutination is observed when the positive serum is added. LAT is rapid and easy to perform to detect anti-Toxoplasam -gondii IgG or IgM antibodies.(Oncel T, et al.2005).

Ethical consideration:

Verbal consent was taken from patients with schizophrania **.**Ethical clearance obtained from Omdurman Islamic University College of postgraduate studies and the agreement was taken from any co-patient before sample and data collection.

Data analysis

Data were analyzed by used Statistical Package for Social Science (SPSS).

Results:

Out of the 140 blood samples examined (26 for IgG &3 for IgM) were found positive to HSV and (30 samples for IgG&8 samples for IgM) were found

positive to Toxoplasm gondii in schizophrenic patients.

Distribution of schizophrenia in the patients according to age groups:

In the different age groups, the frequency was 23 % in the age group 15-24 years. In the age group 25-34 years, the frequency was 34% while in the age group 35-44 years the frequency was 24%. While more than 45 years old were 17 %.(Table 1) (figure 3).

Table 1: Distribution of schizophrenia in the patients according to age groups.

Age groups	Frequency	Percent
15-24	33	23.6
25-34	48	34.3
35-44	34	24.3
more than 45	25	17.9
Total	140	100%

As shown in figure 4 and Table 2 the highest infection rate was reported among males (71.4%), and the lowest infection rate (28.6 %) was reported among females.

Table 2: Over all percenteg of schizophrenia in the patients according to gender

Test	Frequency	Percent
Male	100	71.4
Female	40	28.6
Total	140	%100

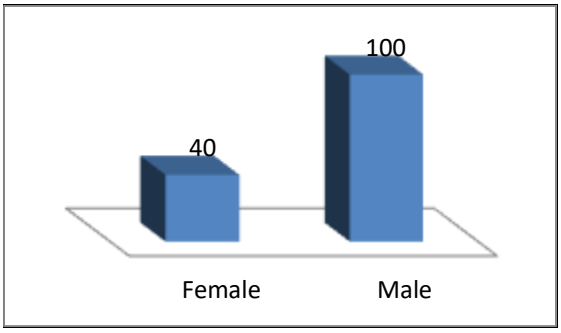

Figure 4: Over all percentage of schizophrenia in the patients according to gender.

Detection of schizophrenia in the patients according to marital status:

Out of the 140 individuals examined, 60 were married and 80 were single . The prevalence of schizophrenia infection among the single (57.1 %) exceeded the prevalence among the married (42.9 %).(figure5) (Table 3).

Table 3: Detection of schizophrenia in the patients according to marital status.

Marital status	Frequency	Percent
Single	80	57.1
Married	60	42.9
Total	140	100%

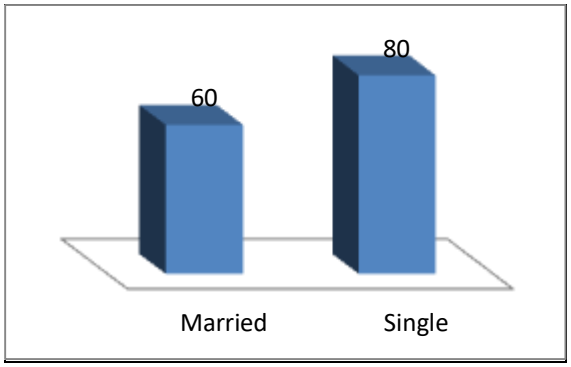

Figure 5: Detection of schizophrenia in the patients according to marital status

Detection of schizophrenia in the patients according to the family history :

Out of the 140 individuals examined, 78 (55.7.6%) were have schizophrenia family history and 62 (44%) were not . (figure 6) (Table 4).

Table 4:Detection of schizophrenia in the patients according to the family history.

Result	Frequency	Percent
Yes	78	55.7
No	62	44.3
Total	140	100%

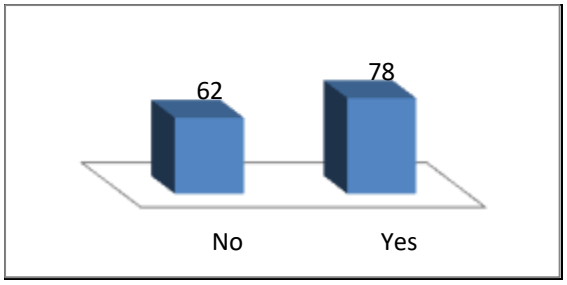

Figure 6: Detection of schizophrenia in the patients according to the family history.

Detection of Herpes *simplex virus* in the patients samples by LAT techniques:

As shown in Table 5, out of 140 patients, 50 patients (35.7%) were found to be positive by LAT technique while 90 (64.3%) were found to be negative.

Table 5: Detection of Herpes *simplex virus* in patients samples by LAT:

Result	Frequency	Percent
Positive	50	35.7
Negative	90	64.3
Total	140	%100

Detection of Herpes simplex virus (IgM) in the patient's samples:

As shown in Table 6, out of 50 patients, 3 (06%) Patients were found positive. While 47 (94%) were found to be negative.

Table 6: Detection of Herpes simplex virus (IgM) in patient's samples:

Result	Frequency	Percent
Positive	3	6
Negative	47	94
Total	50	%100

Detection of Herpes *simplex virus* (IgG) in the patient's samples:

As shown in Table 7, out of 50 patients, 26 (52%) Patients were found to have positive, while 24 (48%) were found to be negative.

Table 7: Detection of Herpes simplex virus (IgG) in patient's samples:

Result	Frequency	Percent
Positive	26	52.0
Negative	24	48.0
Total	50	%100

The percentg of Herpes simplex virus (IgG) and IgM in the patients samples:

As shown in Table 8, out of 50 patients ,26 Patients were found to have positive IgG , while 3 were found to be positive IgM.

Table 8: Detection of Herpes simplex virus (IgG) and IgM in patients samples:

Result	IgG	IgM
Positive	26	3
Negative	24	47
Total	50	50

Detection of Herpes *simplex virus* by LAT and ELISA in the patient's samples:

As shown in Table 9, out of 140 patients, 50 Patients were found to have positive LAT, of these 3 were found to be positive IgM and 26 positive IgG.

Table 9: Detection of Herpes *simplex virus* by LAT and ELISA in patient's samples:

Test	Frequency
Positive(LATEXE)	50
Positive (ELISA)	26

Detection of Toxoplasma gondii by LAT in the patient's samples:

As shown in Table 10, out of 140 patients, 70 (50%) Patients were found to positive. While 70 (50%) were found to be negative by ELISA technique.

Table 10: Detection of Toxoplasma gondii by LAT in the patient's samples:

Test	Frequency	Percent
Positive	70	50
Negative	70	50
Total	140	%100

Detection of Toxoplasma *gondii* (IgM) in the patient's samples:

As shown in Table 11, out of 70 patients, 8 (11%) Patients were found to positive, while 62 (88%) were found to be negative.

Table 11: Detection of Toxoplasma *gondii* (IgM) in the patient's samples:

Test	Frequency	Percent
Positive	8	11.4
Negative	62	88.6
Total	70	100%

Detection of Toxoplasma *gondii* (IgG) in the patient's samples:

As shown in Table 12, out of 70 patients, 30 (42%) Patients were found to positive. While 40 (57%) were found to be negative.

Table 12: Detection of Toxoplasma *gondii* (IgG) in the patient's samples:

Result	Frequency	Percent
Positive	30	42.8
Negative	40	57
Total	70	%100

Detection of Toxoplasma gondii (IgG) and IgM in the patient's samples:

As shown in Table 13, out of 70 patients, 30 Patients were found to have positive IgG, while 8 were found to be positive IgM.

Table 13: Detection of Toxoplasma gondii (IgG) and IgM in patient's samples:

Result	IgG	IgM
Positive	30	8
Negative	40	62
Total	70	70

The percent of Toxoplasma gondii by LAT and ELISA in the patient's samples:

As shown in Table 14, out of 140 patients, 70 Patients were found to have positive LAT, of these 8 were found to be positive IgM and 30 positive IgG.

Table 14: The percentg of Toxoplasma gondii by LAT and ELISA in patient's samples:

Test	Frequency
Positive(LATEXE)	70
Positive (ELISA)	30

Discussion:

From the results I has been suggested that different microbial agents have been proposed as risk factors for schizophrenia, many recent studies have focused on Toxoplasma gondii. Reasons for focusing on these agents include their ability to establish persistent infections within the central nervous system as well as the occurrence of neurological and psychiatric symptoms in individuals infected with these agents. (Coulson JM .2005). Herpes simplex virus (HSV) has been implicated in schizophrenia as it has a tropism for the nervous system and is capable of replication in the brain, acute infection with T. gondii can produce psychotic symptoms similar to schizophrenia such as delusions and hallucinations.(Coulson JM .2005).This study has been assessed the Seroprevalence of Herpes Simplex Virus and Toxoplasma gondii among Schizophrenic patients attending Psychiatric Hospitals in Khartoum State, and it is association with Schizophrenic.In our study Herpes simplex virus (IgM) was detected in 6% of schizophrenic patients and herpes simplex virus IgG was detected in 52% of schizophrenic patients , and Toxoplasma gondii (IgM) was detected in 11% of schizophrenic patients, while Toxoplasma gondii (IgG) was detected in 42% of cases .Similar to study carried out by Juanah in Hospital Kajang. Which found the anti-T. Gondii IgG antibodies 51.0% in patients with schizophrenia (Juanah, et al 2013). Also the results was disagree to study carried out by Rania in Sudan in which HSV1/2 IgG antibodies were detected 95% in patients with schizophrenia. (Rania et al., 2010) Another study also nearly similar to my study in which the seroprelevence rate for anti-Toxoplasma IgG antibodies among schizophrenia patients (66%) (Zafer et al., 2007).

Reference:

1-Zafer Cetinkaya2, Suleyman Yazar1, Omer Gecici4 and Mustafa N .2007. Namli5. Schizophr: Bull. 33 (3) April 2.

2- Douglas J and Berman S (2009). Screening for HSV-2 infection in STD clinics and beyond: a few answers but more questions. Sex Transm Dis: 36:729—31

3-Nawaz Khan, A. 2015. Imaging in CNS Toxoplasmosis. Presented at the 103 annual meeting of society of experimental results Omdurman, Tejany Elmahe hospital.

4-Dubey JP, Hodgin EC and Hamir AN (2006). Acute fatal toxoplasmosis in squirrels (Sciurus carolensis) with bradyzoites in visceral tissues. Parasitology 92 (3): 658–9.

5-Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T and McAuley JB. 2003. Toxoplasma gondii infection in the United States: sero- prevalence and risk factors. Epidemiology: 154(4) : 357–65.

6-Montoya, J. G.and Liesenfeld, O. 2004. Toxoplasmosis. Lancet 363.

7-.Martin ET, Krantz E and Gottlieb SL. 2009. A pooled analysis of the effect of condoms in preventing HSV-2. Acquisition; 169:1233--40.

8-Schiffer JT, Mayer BT, Fong Y, Swan DA and Wald A 2014.Herpes simplex virus-2 transmission probability estimates based on quantity of viral shedding.J R Soc Interface 11 (95): 0160.

9-Beers, Mark H., MD, and Robert Berkow, MD.2004. Schizophrenia and Related Disorders." Section 15, Chapter 193 .

10-Hutchinson, G., and C. Haasen.2004. Migration and Schizophrenia: The Challenges for European Psychiatry and Implications for the Future. Social Psychiatry and Psychiatric Epidemiology 39: 350-357.

11-Meltzer, H. Y., L and Arvanitis, D. 2004.Placebo-Controlled Evaluation of Four Novel Compounds for the Treatment of Schizophrenia and Schizoaffective Disorder. J. Psychiatry 161: 975-984.

12-Mori N, Schoenherr C, Vandenbergh DJ, Anderson DJ 1992. A common silencer element in the SCG10 and type II Na+ channel genes binds a factor present in no neuronal cells but not in neuronal cells. Neuron; 9:45–54.

13-Mitchell BM, Bloom DC, Cohrs RJ, Gilden DH, Kennedy PG 2003. Herpes simplex virus-1 and varicella-zoster virus latency in ganglia. Virology 9:194–04.

14-Jones C. 2003. Herpes simplex virus type 1 and bovine herpes virus 1 latency. Microbiol; 16:79–95.

15-Roizman B, Knipe DM. Howley PM, Knipe DM 2001. Herpes Simplex Viruses and their replication, pp Microbiol. P2399–2460.

16-Juanah, Lovetta Yatta; Jalaludin, Juliana; Osman, Malina; Osman, Zubaidah Jamil .2013. J of Infectious Diseases; 2013, Vol. 9.

17-Rania M. Abdelgfour, Mustafa E. M. Yassin, Nazar A. Wadidy and Nafi, M.2010 . J Of Current Research..

18 Sternberg MR, Kottiri BJ . 2006. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. Microbiol : 296:964--73.

19- Shimojo M and, Hersh LB .2003 . Regulation of the cholinergic gene locus by the repressor element-1 silencing transcription factor/neuron restrictive silencer factor (REST/NRSF) Life Sci. 74:2213–2225.