Presence of Herpes Simplex Hominis type I virus in the regions of interest (ROIs) of the brain of schizophrenic patients.

In agreement with previous reports the advances obtained in understanding central nervous system (CNS) viral infections make viruses attractive etiological candidates for schizophrenia. Among these candidates is Herpes Simplex Hominis type I Virus (HSV-1) due its capacity to remain latent with periodic reactivation, its affinity for the limbic system, the part of the brain involved in schizophrenia, its reactivation by endocrine changes, stress and immune alterations and for its relation to genetic predisposition that makes some individuals more susceptible to chronic viral infections for HSV-1. Presumptive evidence for viral etiology requires the demonstration of a virus, antigen or viral antibody. Neuropathological examination from post-mortem studies of the brain has provided definite diagnosis in many slow viral infections of the Central Nervous System and the technology used at cellular level appears to be an adequate tool for future research in schizophrenia. Electron microscopic techniques due to their higher resolution power are among those techniques that might be used in future research workup since the macroscopic examination of the brain through the current imaginologic techniques as positron emission tomography and magnetic resonance imaging are inappropriate for study at cellular and molecular level the structures that have been implicated in the physiopathology of schizophrenia: amygdala, hippocampus and auditory cortex. An electron microscopic analysis of these structures (the unique in the medical literature) was done in a post-mortem study of 16 schizophrenic patients and 10 controls. The findings of intranuclear inclusion bodies were suggestive of viral etiology in our first observations together with the presence of viral like particles which reacted in a positive form with anti-herpes viral antibody. The presence of a virus does not always mean that it is etiologically related to the disease under study specially related to HSV-1 as many normal persons carry this virus in their brain, but the observation of similar neuropathological findings in the brain of fetuses from schizophrenic mothers and in experimental animals inoculated with cerebrospinal fluid (CSF) from schizophrenic mothers made us to consider this virus as an etiological agent since Koch postulates had been partially fulfilled.

Keywords: schizophrenia; post-mortem studies; electron microscopy; herpes virus; limbic system; brain
INTRODUCTION.

BACKGROUND. Karl Menninger wrote in 1922: “I am persuaded that dementia praecox (schizophrenia) is at least in most instances a somatopsychosis; the psychic manifestations of an encephalitis. The acuteness or chronicity, the benign or malignant nature of the encephalitis perhaps determines the degree of reversibility of the schizophrenia” (1). By 1930 the cerebrospinal fluid and nasopharyngeal washings from schizophrenic patients were being injected into rabbit brains in attempts to produce sepsis in the brain (2).

DIRECT EVIDENCE OF VIRAL INFECTION. Direct testing of a viral hypothesis of serious mental illnesses began in the 1950s. Utilizing the technology that was then available, Morozov (3) and his colleagues in the Soviet Union claimed to have microscopically seen “virus-like corpuscles” in the CSF and nasal secretions of many patients with schizophrenia. In Italy, Mastrogiovanni and Scarlato (4) inoculated CSF from patients with schizophrenia into chicken embryos and also claimed to have microscopically visualized “virus-like particles”. Since that time, the only researches who have claimed to have found virus particles in patients with serious mental illnesses have been Castillo and his colleagues in Havana, Cuba. They have described intracytoplasmic encapsulated structures similar to herpesviruses in freshly obtained postmortem brain tissue from patients with schizophrenia (5) and also in brain tissue from aborted fetuses from mothers with schizophrenia (6). Cited by Fuller Torrey (7).

VIRAL GENOME. Studies of viral genome in postmortem brain tissue from individuals with schizophrenia started in 1979. Sequiera et al. found HSV-1 positive genome by solution and in situ hybridization in a patient with schizophrenia (8). Taylor et al. in 1985 obtained negative results to HSV-1 studying the temporal lobe by dot blot hybridization (9). Carter et al in 1987 also obtained negative results studying samples from caudate, putamen, hippocampus, temporal cortex and frontal cortex by dot blot hybridization to HSV-1 (10). Hayward et al. also reported negative results to HSV-1 in temporal cortex by Southern blot hybridization. Cited by Torrey (11). Rajcani et al. (12) found positive genome results for HSV-1 by blot hybridization studying amygdala samples from schizophrenic patients in 1991. Alexander et al. found negative results in temporal cortex by PCR in 1992 (13). Taller et al. studying temporal cortex samples from 63 schizophrenics and 7 controls by PCR found negative results except 1 HSV-1. It is interesting to highlight the positive results in samples obtained from the amygdala in the study done by Rajcani in 3 of 18 patients (17%) and in 4 of 26 controls (15%) which relates the amygdala to herpes simplex hominis type I virus. The negative results may indicate that HSV-1 is not present in other brain regions apart of the ROIs obtained by imagenology.

MATERIAL AND METHOD

The method of patient selection, diagnosis and procedure have been previously described (14).

RESULTS

The particles observed in the ultrastructural study of the samples obtained from the central nervous system (in the three experiences already mentioned) had a virus morphology, with a central core and several envelopes. The diameter of the particles were from 90 to 120 nanometers (nm). The results obtained are in congruence between them and with those reported in the literature for virus of this size. The technique used, allowed us, to put in evidence those structures of the nervous system that relate to the particles. In this sense we have confirmed the relation of the particles with membranes structures, either in the nuclei or in the cytoplasm of neurons and endotelial cells. The spherical particles within the nucleus of neurons and axons were identified by immuno-electron microscopic techniques as herpes simplex virus hominis type 1.

DISCUSSION

The viral hypothesis of schizophrenia has been considered by several elements: the seasonality of birth with a modest excess - related to population control -about 8% of spring and winter births among individuals who become schizophrenics (15-17). This seasonality relation suggests an association with the well known increased occurrence of viral epidemics in spring and winter and therefore of viral replication. Increased of viral antibodies particularly of the herpes class, have been elevated in schizophrenic patients (18-20). Viral isolation and Transmission of illness (21-26). Immunological measures. Initial evidence from several sources indicates abnormal functioning
of the immune system (27-33). Brain pathology related to the imaging observations. A significant proportion of schizophrenic patients have evidence of structural brain abnormalities (34-39) that may be consistent with a viral etiology. Gene and virus. A viral hypothesis can coexist with the clear evidence for genetic factors in schizophrenia since viruses can integrate into the genome and be transmitted to offspring as genetic material or could interact with cells in events occurring prenatally, perinatally or later during puberty due to hormone endocrine changes at this stage of life that facilitates virus replication. Viruses may remain latent and transmitted from generation to generation in a pattern that would make it appear to be a genetic disease. The concept of latent viral infections with periodic reactivation is now well established. Among the viruses that have this property are many of the herpes family. The presence of virus like particle in the studied fetuses from schizophrenic mothers favors the possibility of virus insult to the brain at crucial times in the growth and differentiation of areas of limbic regions: amygdala e hippocampus. It is interesting the observation that the changes observed in the amygdala-hippocampal volume start with the psychotic symptomatology. Medial temporal structural changes are not seen until after the onset of a psychotic illness (40). Nevertheless other authors have observed these changes even in the prodromic stage, observing that some of the grey-matter abnormalities associated with psychotic disorders predate the onset of frank symptoms, whereas others appear in association with their first expression (41,42). This aspect could be related to the neurobiology of slow virus infections affecting the ROIs after a period of latency starting their replication under stress u hormonal changes at puberty. A delay between transmission in the perinatal period and symptom onset in late adolescence is compatible with chronic, latent, slow, or persistent viral infection and with the neurodevelopment hypothesis of schizophrenia with involvement of the regions of the limbic system mainly in the left temporal lobe. These areas that at present have been called regions of interest (ROIs) in macroscopic examination of the brain were stalking since the use of the imaging techniques that started to develop in the 70s and 80s. As one observed said: “such approaches give us a broad sense of how the brain looks anatomically and how it functions physiologically. To understand how genes affect the brain in a way that puts it at risk, we need to examine in a much more finely detailed way the actual cells of the brain. We can do this with living brain tissue to some degree, and we can do this with postmortem brain tissue to a much greater degree” cited by Weinberger DR (43). Viruses may selectively attack specific cells types or locations in the CNS and alter their chemical functions. It is reasonable to postulate specific viruses that may selectively alter only one enzyme system of one cell type or one area of the brain. It has been shown that HSV infection alters the dopamine system in animal models. Several lines of evidence have been cited suggesting that schizophrenia might begin as an in utero infection. In spite of that commented it should be kept in mind that a virus presence in any illness can be the result of the succession of biological events that happen for that illness and not to be related specifically with the etiology of the same one. Nevertheless the fact that the present investigation is the only one carried out in the calls regions of interest in the brain in schizophrenia (ROIs) using a higher resolution power technique and of being the reported findings to be able to be related with the viral etiology, the necessity it is imposed of replying these studies in other populations with different ethnical, cultural and socio economic conditions in order to discarding other coincident factors that could alter the obtained results. Then, the presence of a virus does not always mean that it is etiologically related to the disease under study specially related to HSV-1 as many normal persons carry this virus in their brain, but the observation of similar neuropathological findings in the brain of fetuses from schizophrenic mothers and in experimental animals inoculated with cerebrospinal fluid (CSF) from schizophrenic mothers made us to consider this virus as an etiological agent since Koch postulates had been partially fulfilled.

**REFERENCIAS**

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NEUROPATHOLOGICAL RESEARCH IN SCHIZOPHRENIA

ELECTRON MICROSCOPY

Brains from dead patients
Aborted fetuses
Experimental animals
Controls

Left temporal lobe
Compatible alterations with the viral hypothesis: particles with herpes simplex hominis type I viral antigen, intranuclear bodies, membrane alterations.

Figura 1.

Main findings:
• Particles with viral morphology which react to peroxidase and colloidal gold conjugated with herpes hominis type I antibodies
• Nuclear bodies
• Membrane proliferation

Figura 2.
Figura 3.- Viral particles in the CNS of the main region of interest (ROIs) of schizophrenic patients.

Figura 4.- Viral particles in the temporal lobe of aborted fetuses, chicken embryos and rats.
Figura 5.-

Inmunoelectron microscopy. Particle (vertical arrow) related to membrane structure (oblique arrow). Digital analysis—right superior inset—shows the morphology of a virus in the antigen-antibody reaction (yellow and green).