Incidence of Cytomegalovirus and Epstein-Barr in IgG in Demyelinating Diseases

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***Abstract*—Of the demyelinating diseases, facial paralysis (FP) is the most frequent and benign. Multiple sclerosis (MS), Guillain-Barre (GB), transverse myelitis (TM) and amyotrophic lateral sclerosis (ALS) are neurodegenerative and disabling diseases whose origin is multifactorial. This study's objective was to determine the presence of cytomegalovirus (CMV) and Epstein-Barr (EB) viruses in IgG and IgM, in addition to the frequency of CD3, CD4, CD8 and CD19 lymphocytes in 447 patients with a diagnosis of demyelinating disease. In the results, CMV and EB were identified in IgG, in all patients. CMV-IgM was identified in ALS and EB-IgM in GB. Total lymphocytes increased in MS. B lymphocytes increased in MS, ALS and FP. CD3 lymphocytes showed no changes. CD4 lymphocytes increased in MS, ALS and decreased in GB, MT, and FP. CD8 lymphocytes were increased in all patients. CD4/CD8 ratio increased in MS and ALS and decreased in GB, TM, and FP. Conclusion: Results showed an alteration in the humoral and cellular immune system due to the elevation of the IgG and IgM and in turn the elevation of the CD4 + and CD8 + lymphocytes, which are possibly autoreactive clones, which could be the cause of the loss of myelin and/or damage to oligodendrocytes of the affected nerve.**

Keywords; Cytomegalovirus, Epstein-Barr, Facial paralysis, Multiple sclerosis, Amyotrophic lateral sclerosis

I. INTRODUCTION

Demyelinating diseases are characterized by the presence of myelin lesion in the course of its evolution; These lesions can occur in the central nervous system (CNS) or peripheral nervous system (PNS). The alteration can be primary, by a defect in the genetic codification of the enzymes of its formation, or secondary due to toxic effects, vascular diseases due to hypoxia or infectious and inflammatory processes. The location of demyelination determines the signs and symptoms of the disease [1,2].

Among the demyelinating diseases treated were mainly:

Multiple sclerosis.
Devic syndrome.
Marchiafava-Bignami disease

Pontine central myelinolysis
Acute disseminated encephalomyelitis.
Acute necrotizing hemorrhagic encephalomyelitis.
Facial paralysis.
Guillen-Barré.
Transverse myelitis.
Amyotrophic lateral sclerosis

The EM, GB, MT, ELA, and PF (and their relationship with the CMV and EB) are briefly described below, which are the subject of our study.

- **Multiple sclerosis:** Genetic susceptibility to environmental factors (infections by viruses and bacteria) determines that MS is expressed; this occurs through the genes of the molecules of the major histocompatibility complex class I (MHC-I), which consists of an alpha chain encoded by the genes in the human leukocyte antigen (HLA) system, this alpha chain is It binds to a beta-2 microglobulin chain, which consists of three polymorphic regions designated as 1,2 and 3, and its HLA-A3, the latter region is weakly associated with MS. The molecules of the major histocompatibility complex of class II (MHC class II) are composed of two encoded chains (alpha and beta) and three chains tightly linked to the locus on chromosome 6, marked as DR, DP, and DQ. The specific MHC-II alleles that are associated with the highest risk of developing MS are DRw15 and DQw6. This association between MHC genes and MS has not been established to date [3,4,5].
- **Guillain-Barré:** GB syndrome, also known as an ascending acute demyelinating polyneuropathy, is an autoimmune disorder, of which the cause is unknown. The syndrome can occur at any age, occurs in both sexes, with a higher prevalence between 30 and 50 years, although it can also occur in childhood. Since the elimination of poliomyelitis, GB

syndrome is the most frequent cause of acute dysreflexia paralysis. The world incidence is 1: 100,000 inhabitants/year [6,7,8]. Its main characteristic is the presence of multifocal lymphocytes in the peripheral nervous system associated with loss of myelin; axonal degeneration can occur as a secondary phenomenon where high serum levels of IL-6, IL-2, TNF-alpha have been found; after the antigen is recognized, the T lymphocytes are activated; which is mediated by chymosins, cell adhesion molecules and metalloproteinases, the inflammatory response ends with the production of IL10 and TGF beta [9,10].

- **Transverse myelitis:** The cause of transverse myelitis (TM) is not known, but most research supports that it is due to an autoimmune process; It frequently develops in an environment of bacterial and/or viral infections, especially those associated with rashes such as measles, chicken pox, smallpox, rubella. Approximately one-third of patients with TM report fever near the onset of neurological symptoms. In some cases, there is evidence of direct invasion and damage to the marrow by the infectious agent (virus). However, in many cases the infection causes an alteration in the immune system, producing autoimmune injury of the spinal cord. This abnormal activation of the immune system against human tissue is called molecular mimicry; this hypothesis postulates that an infectious agent can synthesize a molecule (in the antigen presenting cells) which mimics another homologous molecule or target cell (spinal cord), causing inflammation and damage [11].

- **Amyotrophic Lateral Sclerosis:** Amyotrophic lateral sclerosis (ALS), also known as Lou-Gehrig, is a disease where nerve cells (neurons) die or have lesions in the myelin of the central nervous system or the spinal cord that control the movements of voluntary muscles, causing muscle weakness, fasciculations and inability to move the arms, legs, and body. The condition slowly gets worse and when the muscles in the chest area stop working, it becomes difficult or impossible to breathe on its own, causing death [12].

Causes, incidence and risk factors: 10% of patients with ALS cause it because of a genetic defect, while in the rest of the cases, the cause is unknown. ELA affects approximately 5 out of every 100,000 people around the world. There are no known risk factors, except for the fact of having a family member who has a hereditary form of the disease [13]

- **Facial Paralysis:** The etiology of facial paralysis (PF) is still unknown, however, infectious, ischemic, hypertension, diabetes, immunological and genetic processes play an important role in its pathogenesis. The investigations carried out during the last 20 years have shown and concluded that the cause may be of viral origin. Herpes virus type I, such as varicella zoster virus (VZV), herpes simplex virus (HSV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are among the most studied causative viruses; as well as the rubella virus and recently the human immunodeficiency virus (HIV). Herpes viruses have double-stranded DNA whose function is restricted to the establishment of a latent infection in their hosts and cause disease by reactivation. HSV-1 and 2 and VZV are neurotrophic, that is, they are established in the sensory ganglia of the peripheral nervous system for life in the host. Although experimental evidence has shown that HSV-1 infections in IPP have increased in recent years, the pathophysiological mechanism of infection has not yet been clarified, however, two mechanisms of damage have been postulated by reactivation of the virus: 1) by the stimulus through the skin (ultraviolet light) and 2) by systemic stimuli (fever, stress, menstruation, among others [14,15,16].

1.1. Pathophysiology of demyelinating diseases

As previously mentioned, the initial phenomenon of myelin loss in the central and peripheral nervous system is the presence of acute inflammation, which consists of perivascular and parenchymal infiltration of B lymphocytes, T lymphocytes, macrophages and destruction of myelin. In the central and peripheral nervous system, axonal cylinders in acute lesions are conserved in 90% of cases; the remaining 10% in acute injuries and those chronic injuries are accompanied by axonal degeneration [17,18,19].

2. JUSTIFICATION

The demyelinating diseases have in common the presence of the virus and/or the immunological alteration, which could be the cause of the direct and indirect lesion of the myelin or death of the oligodendrocytes. For this reason, it is necessary to determine the etiology of demyelinating diseases to reduce their incidence, administer timely drugs that modulate the immune response or antiviral drugs, start their rehabilitation to prevent disability and be more independent in their daily lives.

3. OBJETIVES

• Determine the presence of CMV and EB viruses by serological levels of IgG, IgM.

• To assess the frequency of leukocytes, subpopulations of B lymphocytes (CD19), CD3 + T cells, CD4 +, CD8 + lymphocytes and their CD4 + / CD8 + ratio.

4. MATERIAL AND METHODS

4.1. Study design

An observational, cross-sectional and descriptive study was carried out.

447 patients with definite demyelinating disease from the National Rehabilitation Institute were invited to participate in the study, who were informed that the investigation did not present any risk to their health and undersigned consent; They were classified as follows: 200 patients with Multiple Sclerosis, 7 patients of Guillain-Barré, 8 patients of Transverse Myelitis, 20 patients of Amyotrophic Lateral Sclerosis and 212 patients of Facial Paralysis, agreed to donate a blood sample to perform the following studies:

A) Determination of the frequency of leukocytes and lymphocytes: A 447 patients were taken 1 ml of heparinized peripheral blood to realize differential leukocyte count through the manual blood technique stained by Wright dye, for a differential count of leukocytes and total lymphocytes / 100 cells, in an Olympus light microscope, 100X objective.

B) Determination of the concentrations of the B lymphocytes, CD3, CD4, CD8 and CD8 lymphocytes and their CD4/CD8 ratio: The subpopulations of lymphocytes were determined by means of flow cytometry, for which the following antibodies conjugated with allophycocyanin were used, chlorophyllin peridinin protein, phycoerythrin or fluorescein, using a flow cytometer with anti-CD3 (clone SK7), anti-CD4 (clone SK3) antibodies. Anti-CD8 (clone SK1). For the analysis of the surface molecules, 10,000 mononuclear cells containing the antibodies were read, washed, fixed with 1% paraformaldehido. The process of the samples was carried out in a FacsCalibur flow clitometer equipped with CellQuest 3.1, software (Becton-Dickinson).

C) Determination of the presence of antiviral antibodies of the Cytomegalovirus and Epstein-Barr virus: The determination of antiviral antibodies of CMV class IgG, IgM was carried out through the ELISA technique. The analysis of these data was carried out in a statistical program of Instat-3 with Kruskal-Wallis and ANOVA, a nonparametric test for non-paired samples.

To select the possible candidates for the study, the following criteria were taken into account.

- Inclusion criteria: Patients with a defined diagnosis of MS, GB, MT, ALS, PF of the National Institute of Rehabilitation who come to the Neurological Rehabilitation service, who give their signed consent to donate 5 ml of peripheral blood.
- Exclusion criteria: Patients of the Neurological Rehabilitation service, whose diagnosis of demyelinating disease is not defined and they do not accept to participate.

5. RESULTS

Figure1 shows the distribution by disease of the 447 who participated in the study. The distribution of gender vs disease is shown in Figure 2.

Figure 1. Disease distribution

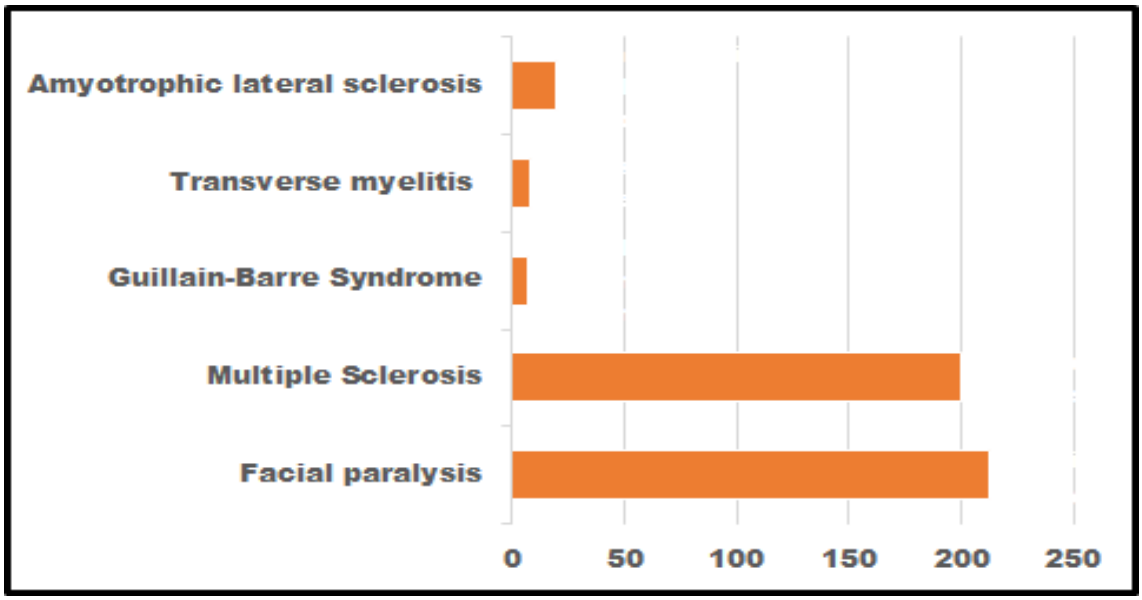
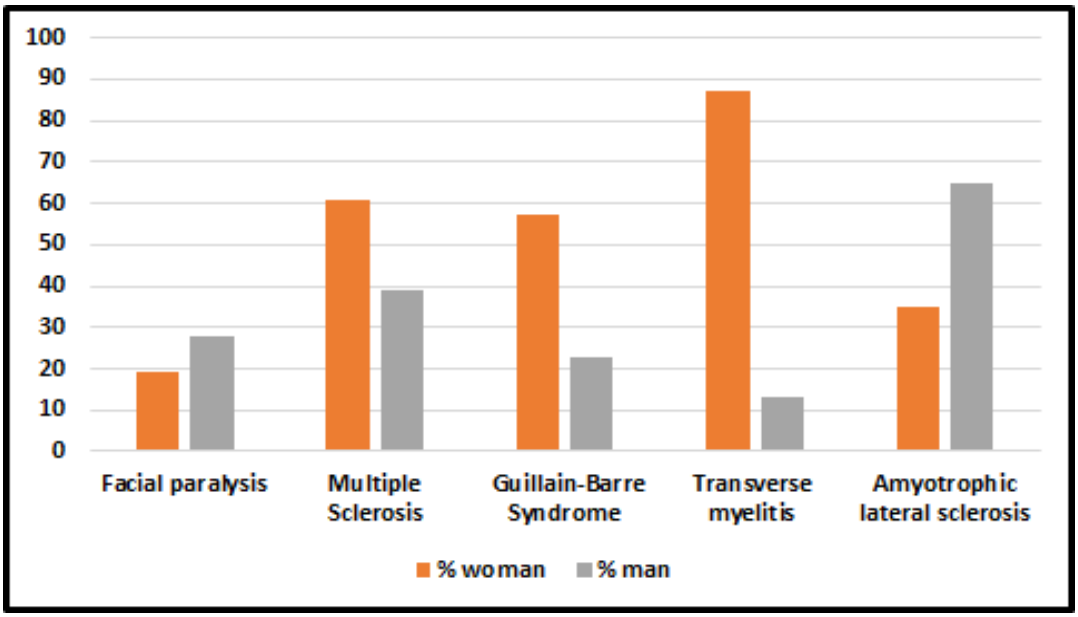


Figure 2. Incidence of the disease by gender



In all patients diagnosed with the demyelinating disease, the results showed that the CMV and EB antiviral antibodies were identified in the IgG, which increased their values significantly (p <0.05), compared with the reference levels.

The values of CMV antiviral antibodies for IgM showed a significant increase (p <0: 05), in patients diagnosed with amyotrophic lateral sclerosis, and EB antiviral antibodies for IgM, significantly increased in patients with Guillen-Barre, compared with reference values (Table 1).

TABLE 1.

The reference values were: Negative when the reading at 450 nm is > 0.9 optical density (OD) and positive when the reading at 450 nm is < 0.9 OD

Disease	Average Age	CMV IgG	CMV IgM	EB IgG	EB IgM
Multiple Sclerosis	31±11.5	*2.9± 0.12	0.7± 0.06	0.6 ± 0.09	0.4± 0.02
Guillain-Barré	40±13.0	*3.6± 0.19	0.3± 0.04	*4.9± 0.58	* 2.4± 0.47
Transverse Myelitis	29± 10.3	*5.6± 0.28	0.5± 0.08	*2.9± 0.43	0.47± 0.07
Amiotrophic Lateral Sclerosis	46±12.3	*4.8 ± 0.76	*1.8 ± 0.06	*2.8 ± 0.26	0.7± 0.21
Facial Paralysis	38± 13.4	*2.4 ± 0.81	0.4 ± 0.02	*2.3 ± 0.05	0.4 ± 0.02
Reference values		< 0.9 OD	< 0.9 OD	< 0.9 OD	< 0.9 OD

****Student's «T» versus control. *Statistically significant difference with respect to the reference values, ANOVA and Student's t-test (P=0.05)**

The frequency of leukocytes in all patients with a diagnosis of the demyelinating disease was normal, compared to the reference values (Tab.2).

The results of the frequency of the total lymphocytes had a significant increase (p <0: 05) in the patients with MS, compared with the reference values see (Tab.2).

The frequency of B lymphocytes in patients with multiple sclerosis, amyotrophic lateral sclerosis, facial paralysis values increased significantly (p <0: 05) compared to the reference values (Tab.2).

TABLE 2.

The frequency of B lymphocytes in patients

	Multiple Sclerosis	Guillian Barre	Transverse Myelitis,	Amniotrophic Lateral Sclerosis	Facial Paralysis
Leukocytes *5-10000	7019±140	7300±283	8287±407	65875±382	69635±145
Total Lymphocytes *25 - 30%	**32.1± 0.83	25.3± 0.60	32.6±2.4	31.9± 2.2	28.7±0.37
Lymphocytes B CD19: *5 - 15%	** 27.4±1.69	14.8±1.18	13.1±2.27	**19.7±1.7	**17.2±0.25
Lymphocytes T CD3: *70-90%	**50 ±0.62	71.3±1.09	78.5±2.29	81.8±11.2	71.3±0.8
Lymphocytes T CD4: *33-58 %	***30 ±35%	40.0±1.33	***25.8±0.98	*** 22.1±3.6	45.9±4.5
Lymphocytes T CD8: *13-35%	20± 25%	38.6±1.19	**51.7±0.03	** 61.62±4.2	**43.8±3.9
CD4 / CD8 ratio *2.0	1.7±134	***0.09±0.50	***0.03±0.36	*** 0.09±1.52	***0.41±0.89

***Reference values. **Student's «T» versus control. *Statistically significant difference with respect to the reference values, ANOVA and Student's t-test (P=0.05)**

The values of CD3 lymphocytes in all patients remained within normal limits (Tab.2).

The frequency of CD4 lymphocytes decreased significantly (p <0.05) in patients diagnosed with MS, ALS. However, patients with GB, MT, and PF decreased their frequency significantly (p <0.05), compared with the reference values (Tab.2).

In all patients, the values of CD8 lymphocytes increased significantly (p <0.05), with respect to reference levels, see (Tab.2). The relationship of the CD4 / CD8 frequency was significantly reduced (p <0: 05) in the patients with GB and MT diagnosis, see (Tab.2).

6. DISCUSSION OF RESULTS

6.1. Multiple sclerosis

Of the 447 patients who participated in the study, 45% were diagnosed with multiple sclerosis, of which 39% were men and 61% were women, as well as many autoimmune diseases, Comston et al. Mention that the disease is more common in women and the trend could be increasing [10, 27]. The minimum age for the presentation of multiple sclerosis in our patients was 10 years and the maximum age was 42 years; in previous research conducted by Alonso et al. showed that multiple sclerosis occurs between 20 and 40 years [20].

6.2. Guillain Barre

Of the 447 patients with a diagnosis of the demyelinating disease who gave their consent, there were 7 male patients, with a minimum age of 27 years and a maximum age of 53 years. The literature reports that GB occurs in both sexes between the ages of 30 to 50 years. Although in our study only presented in male and a child of 4 years [21.22].

6.3. Transverse myelitis

Of the 447 patients who participated, 8 patients were diagnosed as transverse myelitis, of which 1 man and 7 women of the average age of 29 years, however, in the literature acute transverse myelitis has an incidence of 1 to 4 new cases. 100000 inhabitants per year and affects individuals of all ages with bimodal apices between the ages of 10 and 19 years and 30 and 39 years. There is no familial or sexual predisposition to transverse myelitis [23].

6.4. Amyotrophic Lateral Sclerosis

Of the 447 patients in our population, 20 patients with a diagnosis of amyotrophic lateral sclerosis participated, of which 7 men and 13 women with a

minimum age of 24 years and a maximum of 59 years. It has been reported that the incidence is higher in men than in women between 40 to 70 years of age and each year there are 5.0 cases in 100 000 inhabitants [24, 25].

6.5. Facial paralysis

Of the 447 patients in our study population, 47% had a diagnosis of facial paralysis, of which 19% men and 28% women, with a minimum age of 25 years and a maximum age of 51 years; with respect to other studies, they indicate that the incidence is 11-40 cases per 100,000 people per year and men are equally affected like women with a peak incidence between 4 and 70 years [26, 27]

6.6. Determination of cytomegalovirus antiviral antibodies in immunoglobulin G

The presence of cytomegalovirus antiviral antibodies in immunoglobulin G was detected in 100% of the patients with diagnoses of demyelinating disease. It has been studied that immunoglobulin G is a surface protein expressed by B lymphocytes, and they are increased in the presence of antigens such as CMV and EB viruses, as shown in [28]. Monteyne and Johnson showed that acute CMV infection in adults is a rare event but when it occurs with active liver disease of the type of infectious mononucleosis, patients have fever, lymphadenopathy and hepatitis or encephalitis, and have been linked to other degenerative diseases such as chronic fatigue syndrome, Guillain Barré, lymphoproliferative syndrome, Sjogren's syndrome, sarcoidosis, systemic lupus erythematosus and recently with multiple sclerosis, as was detected in our patients with degenerative diseases [29]

6.7. Determination of cytomegalovirus and Epstein-Barr antiviral antibodies in immunoglobulin M

The antiviral antibodies of CMV and EB in the IgM were present in the patients with ALS and GB diagnosis, the CMV seropositivity results have been described by other investigators in 67% of the patients with ALS diagnosis, this diagnosis was made by capture ELISA techniques [30, 31]

6.8. Determination of Epstein-Bar antiviral antibodies in immunoglobulin G

Regarding the detection of the antiviral antibody of Epstein-Barr in IgG, we observed that in 100% of the patients it is present in immunoglobulin G. However, the virus was detected in IgM in the patients diagnosed with Guillen- Barre. Previous studies have reported that it is a widely distributed virus, it is estimated that about 90% of adults have been infected. Previous studies have shown that this virus is the main cause of infectious mononucleosis (MI), a disease of adolescence and childhood. It can also cause certain forms of cancer, such as Undifferentiated Nasopharyngeal Carcinoma (CNI), Burkitt's Endemic Lymphoma (LBE), or B-cell lymphomas in patients with acquired or congenital immunodeficiencies and there is great controversy about the role of this virus as a cause of chronic disease, especially in regard to Chronic Fatigue Syndrome, multiple sclerosis, amyotrophic lateral sclerosis [32,33,34].

6.9. Determination of the frequencies of leukocytes, b lymphocytes, t lymphocytes, cd3 lymphocytes, cd4, cd8 and the cd4 / cd8 ratio

The results of the leukocyte frequency were kept within normal limits. However, total lymphocytes increased in patients with multiple sclerosis, the rest of the patients remained within normal limits. Of the B lymphocytes (CD19) in patients diagnosed with multiple sclerosis, amyotrophic lateral sclerosis, and facial paralysis, they increased their concentration. The CD3 lymphocytes in 100% of the patients remained at normal values. CD4 + lymphocytes in patients diagnosed with multiple sclerosis and amyotrophic lateral sclerosis increased their frequency, however, in patients with Guillain-Barre diagnosis, transverse myelitis and facial paralysis decreased their values. In contrast, CD8 + lymphocytes in 100% of patients increased their values. The CD4 + / CD8 + ratio in patients diagnosed with Guillen-Barre, transverse myelitis and facial paralysis decreased their concentrations. Previous studies mention that possibly the CD4 + and CD8 + lymphocytes are activated in the cervical ganglia of cerebrospinal fluid drainage, and once activated they cross the blood-brain barrier through different epithelial cells that constitutively express molecules such as selectins and adhesion molecules, are those that allow the passage of activated T cells (CD4 +) to the central nervous system such as multiple sclerosis, transverse myelitis, and also those that [35,36,37,38].

7. CONCLUSIONS

The alterations in the frequency of the B lymphocytes, the increase in antiviral G antibodies of CMV and EB, and the presence of the CMV antiviral antibodies M, indicating that the CMV and EB viruses activated the humoral immune system. Also, the cellular immune system was activated since the CD4 + 7 and CD8 + subtypes showed alterations in all the patients, which indicates that these lymphocytes possibly behaved as autoreactive clones; and together the activated humoral and cellular system are responsible for myelin destruction and/or death of oligodendrocytes, resulting in demyelinating diseases, as shown in Fig. 1, 2 and 3

CONFLICT OF INTEREST

The authors guarantee responsibility for everything published in this manuscript, as well as the absence of a conflict of interest and the absence of their financial interest in performing this research and writing this manuscript. This manuscript was written from an original research work and has never been published, neither is it under consideration for publication elsewhere.

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