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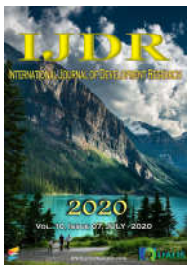

RESEARCH ARTICLE OPEN ACCESS

LOW OXIDATIVE DNA DAMAGE IN PATIENTS WITH TROPICAL SPASTIC PARAPARESIS BY HTLV-1

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ABSTRACT

Tropical spastic paraparesis is a myelopathy caused by HTLV -1 virus,whose pathogenesis is yet not clear, being the most likely hypothesis to be due TAX viral protein activity that increases the production of reactive oxygen species, resulting in transformation, cell immortalization, DNA damage and disease .The aim of this study was to evaluate oxidative DNA damage in patients with tropical spastic paraparesis by HTLV 1 treated at a referral service in Belém, Pará, Brazil. Methods: It was a retrospective study involving adults. The study participants were asymptomatic or with tropical spastic paraparesissimptoms, considering the following inclusion and exclusion criteria. The patients were studied according to the Castro-Costa classification (2006) of which eight were diagnosed,12 probable, 34 asymptomatic and 28 healthy. Results: the average age was 47,9 years, with 30.5% men and 69.5% women. The mean urinary 8 OHdG concentration in the defined patients was 2,5 ng / ml, probable 2,7 ng / ml, asymptomatic 2,6 ng / ml and healthy controls 2,5 ng / ml, showing no significant difference (p value = 0,989) between the groups. Conclusion: This study showed no difference between patients and healthy controls suggesting that oxidative DNA damage is not the most likely mechanism in the pathogenesis of tropical spastic paraparesis.

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INTRODUCTION

Human T Cell Leukemia Virus 1 (HTLV 1) is a retrovirus that has been implicated in the etiology of lymphoproliferative, inflammatory and degenerative Central Nervous System (CNS) disease and some immune disorders. It is estimated about 10 million infected people worldwide (Gessain, 2012). Infected individuals are asymptomatic in most cases, and the mechanism which the clinical manifestation of the disease occurs in not clear. HTLV1 is associated with tropical spastic paraparesis (TSP) in 1 to 5% of those infected, most commonly in women, with asymmetric progressive paraparesis with signs of pyramidal release, with greater sphincyer involvement (Gotuzzo, 2004). The diagnosis is classified as define, probable or possible according to clinical and laboratory characteristics (Castro-Costa, 2006). Tropical spastic paraparesis is a disease of the immune mediated nervous system whose precise mechanism of its development remains unknown (Castro-Costa, 2006). The most widely accepted hypothesis would be to the activity of the Tax viral protein that increases the production of reactive oxygen species through the activation of nuclear factor kB (NK-Kb) resulting in cell transformation, immortalization, DNA damage and disease (Kinjo, 2010). The 8 hydroxydeoxyguanosine (8OHdG) has been extensively used as a biomarker of oxidative stress, DNA damage, carcinogenesis and degenerative diseases according to the literature (Kasai, 1997; Rahimpour, 2018 and Valavanidis, 2009). Due to the difficult

on knowledge of tropical spastic paraparesis pathogeneses and some evidence of oxidative DNA damage by HTLV1, the present study investigated the association between the 8 OHdG biomarker with clinical forms of this disease.

METHODS

We did a retrospective cross-sectional study, through data analysis of medical records of patients treated from March 2017 to March 2018 at the HTLV outpatient clinic of the Tropical Medicine Center of the Federal University of Pará in Belém, Pará, Brazil. The study participants were asymptomatic or with tropical spastic paraparesissimptoms, considering the following inclusion criteria: 1- have positive serology for HTLV1 by PCR; 2- clinical diagnosis of probable and definitive tropical spastic paraparesis according to the criteria of Castro – Costa, (2006), according to neurological evaluations contained in the patient's medical records; 3- asymptomatic carriers with neurological evaluation; patients treated during the research period who were negative serology for HTLV1 and who underwent clinical, neurological and laboratory tests. Patients with other concomitant diabetic, neurological diseases, rheumatological diseases, uncontrolled hypertension, previous history of cancer, nephropathy and liver diseases were excluded. Socio-demographic and clinical-epidemiological data were obtained and recorded in Biostat Program database, 2007 (Ayres, 2003).

et al(1992). Biochemical including fasting glucose, creatinine, TGO and TGP were performed to rule out other diseases that have known oxidative DNA damage. The 8 OHdG analyses were performed at Evandro Chagas Institute (IEC) Toxicology Laboratory by high performance chromatography (HPLC) by electrochemical method (Mei Su-Rong, 2003 and Sabatini, 2005). Data were presented using analytical –descriptive statistics, using mean and standart deviation. Significant differences were considered when p value< 0.05. Statistical analyzes were performed using Biostat 5.0 software [9]. We used the G test for categorical variables and the Anova test and Kruskal-Wallis test for quantitative variables and Spearman Correlation. The protocolof this study followed the norms established by Resolution 466/2012 of the National Health Council of Brazil and was approved by the Research Ethics Committee of the Tropical Medicine Center and all participants signed a statement of informed consent.

RESULTS

A total of 82 patients were analyzed : 20 symptomatic (8 classified as defined and 12 probable), 34 asymptomatic carriers and 28 healthy seronegative personas. There was no statistical difference in age and sex between the groups, predominantly in women, and biochemical data were normal (Table 1). The clinical neurological profile were show in table 2. Values are n (%) or mean ± SD (standard deviation).

Table 1 . Demographic and laboratory characteristics of the patient sample (total = 82 patients)

Variable	*A* (n=8)	*B* (n=12)	*C* (n=34)	*D* (n=28)	*p*value
Age (years. mean ± SD)	53.9 ± 9.4	47.3 ± 14.8	49.7 ± 13.0	44.3 ± 16.0	NS
Sex (%)					NS
Male	4 (50.0)	2 (16.7)	8 (23.5)	11 (39.3)	
Female	4 (50.0)	10 (83.3)	26 (76.5)	17 (60.7)	
Blood glucose (md/dl)	90.6 ± 8.3	107.7 ± 37.4	96.5 ± 16.2	92.5 ± 11.1	NS
TGO (U/L)	19.0 ± 2.7	24.0 ± 7.1	23.4 ± 6.8	25.5 ± 11.1	NS
TGP (U/L)	18.0 ± 4.9	26.2 ± 14.5	24.5 ± 11.8	24.7 ± 10.2	NS
Creatinine (mg/dl)	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	0.8 ± 0.2	*B* x *D*/*C* x*D* (p<0.05)

Values are n (%) or mean ± SD (standard deviation). The ANOVA test was used to compare mean ages. The G test was used for sex. The Kruskal-Wallis was used for blood glucose, TGO, TGP and creatinine concentration. *Group A* Defined HAM/TSP, *Group B* Probable/oligosymptomatic, *Group C* Positive carrier, *Group D* Negative, *NS* not significant.

Table 2. Clinical characteristics of the HTLV positive sample (total = 54 patients)

Variable	*A* (n=8)	*B* (n=12)	*C* (n=34)	*p*value
Diagnosis time (yearsago)	9.5 ± 5.4	5.5 ± 3.8	3.8 ± 3.4	*A* x *C* (<0.05)
Muscleweakness				
LL	8 (100.0)	2 (16.7)	0 (0.0)	*A* x *B*/*A* x *C* (p<0.001)
UL	1 (12.5)	1 (8.3)	0 (0.0)	NS
Spaticity				
LL	8 (100.0)	3 (25.0)	0 (0.0)	*A* x *B*/*A* x *C*/*B* x *C* (p<0.05)
UL	0 (0.0)	0 (0.0)	0 (0.0)	NS
Babinski signal	8 (100.0)	4 (33.3)	0 (0.0)	*A* x *B*/*A* x *C*/*B* x *C* (p<0.05)
Clonus	8 (100.0)	2 (16.7)	0 (0.0)	*A* x *B*/*A* x *C* (p<0.001)
Hyperreflexia				
LL	8 (100.0)	12 (100.0)	0 (0.0)	*A* x *C*/*B* x *C* (p<0.001)
UL	0 (0.0)	2 (16.7)	0 (0.0)	0.041
Uninary disturbance	7 (87.5)	4 (33.3)	10 (29.4)	*A* x *B*/*A* x *C* (p<0.05)

The variables considered included: age, gender, previous infectious and neoplastic diseases clinical history, time since diagnosis and neurological examination. The HTLV-1 infection was confirmed in the Celular and Molecular Biology Laboratory of the Tropical Medicine Nucleous of the Federal University of Pará by Polymerase chain reaction (PCR), according to the methodology of Saiki et al (1988)[10] and Tuke

The *p* value column shows the adjusted *p* value for multiple comparisons if the respective variable was significantly associated with the outcome variable. The Kruskal-Wallis test was used to compare the infection time between groups. The G test was used in all other cases (both for general association

Table 3. 8 OHdG levels of the patient sample (total = 82 patients)

Variável 8 OHdG (ng/ml)	*A* (n=8)	*B* (n=12)	*C* (n=34)	*D* (n=28)	p-valor
					NS
Minimum	0.0	0.0	0.0	0.0	
Maximum	6.1	5.3	6.3	5.3	
Median	2.1	3.0	2.4	2.6	
Mean ± SD	2.5 ± 1.9	2.7 ± 1.8	2.6 ± 1.7	2.5 ± 1.6	

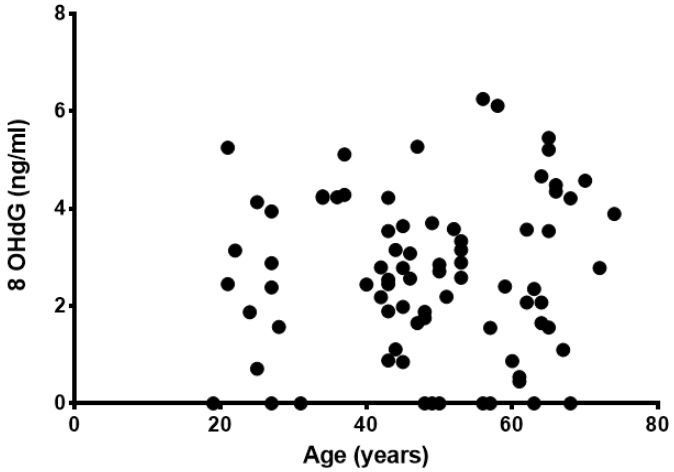

Figure 1. Dispersion diagram between 8 OHdG levels and age in all groups taken together

and multiple comparisons). In the case of Hyperreflexia UL, none of the multiple comparisons were significant, after *p* value adjustment. *Group A* Defined HAM/TSP, *Group B* Probable/oligosymptomatic, *Group C* Positive carrier, *NS* not significant, *LL* lower limbs, *UL* upper limbs. The results of 8 OHdG were show in table 3. No statistical differences were found between the sick group and the healthy controls, as well as no difference between the symptomatic group and the asymptomatic carriers of HTLV1. The ANOVA test was used to compare mean 8 OHdG values (p=0.989). *SD* standard deviation, *NS* not significant. *Group A* Defined HAM/TSP, *Group B* Probable /oligosymptomatic, *Group C* Positive carrier, *Group D* Negative. The Pearson's correlation coefficient was not significant, p value = 0.493, correlation (r) = 0.08.

DISCUSSION

In the present study, most symptomatic patients belong to the group of probable or oligosymptomatic patients, whose importance of being individualized was emphasized in the study by (Koyama, 2018) due to the evolutionary feature of the disease, since they are patients who do not fulfill paraparesis criteria defined tropical spasticity, however, are not asymptomatic. This corroborates the use of the Castro-Costa[3] classification in this paper, which better defines these patients clinically rather than the WHO (1988) classification (WHO, 1988). Regarding the epidemiological profile, we found similar to the literature (Gotuzzo, 2004 and Champs, 2010), the majority of women, with a mean age of 47,9years old with no statistical difference between groups.Infection time was statistical longer in the defined group than in the asymptomatic group, collaborating the thesis of disease progression over time, as demonstrated by Tanajura et al. (2015). The neurological manifestations of all defined patients had signs of pyramidal release with weakness, spasticity, hyperreflexia in the lower limbs, clonus and Babinski sign. In those more likely, the most frequent alteration was the hyperreflexia (100% of cases) similar to the study by Koyama et al (2018). Tropical spastic paraparesis is an immunomediated disease, with an exaggerated proinflammatory response of the organism against the virus. There is also the expression of TAX viral oncoprotein that alters the expression of cellular genes involved in controlling viral replication and apoptosis, causing high oxidative stress with DNA damage in host cells, which can be measured by 8 OHdG (Araujo, 2015 and Kinjo, 2010). The 8 hydroxydeoxyguanosine (8 OHdG) is produced by oxidation of cellular DNA due to free radical attack, is associated with individual factors such as age, gender and diseases such as kidney disease (Rahimpour, 2018), cancer (Valavanidis, 2009) diabetes (Stein, 2018 and Xiao, , 2018). Martinez-Moral and Kanan (Martinez-Moral, 2016) in an observational study of urinary DNA oxidative stress biomarkers in healthy individuals, concluded that 8OHdG was the most suitable biomarker for this purpose, as well as others authors (Miwa, 2004), for this reason was chosen in the present study. There is extensive literature demonstrating the predictive value of 8OHdG in cancer patients, as well as in degenerative diseases such as Parkinson's, Alzheimer's disease and type 2 diabetes. In investigating HPLC urinary 8 OHdG oxidative damage in viral diseases Paul et al found no difference between HIV patients and normal controls. Mahmound et al[28]found a higher value of 8OHdG in patients with hepatits C and hepatitis C with carcinoma than controls. OLIA et al [29]used the elisa methods in the investigation of urinary 8 OHdG, found an increase de 8 OHdG in human papillomavirus patients compare to healthy controls, proving DNA damage in this pathology. There is no evidence in the literature on the research of urinary 8OHdG in patients with HTLV1 tropical spastic paraparesis, this being the first study to this purpose. Diabetic, renal, liver disease and patients with a history of rheumatologic disease and cancer were excluded in order to analyze the value of 8OHdG without these biases. Our results showed in healthy control patients an average of 8 OHdG below the literature (2.5ng/mL) compared to the study by

Martinez – Moral and Kanan[19] (3.65ng/mL) performed by the same method, demonstrating the low DNA damage in healthy population studied. In the present study in relation to age and gender, there was no difference in healthy patients, as in the study by Bogdanov et al (Bogdanov, 1999), and there was no difference between sex and age in symptomatic and asymptomatic HTLV1. Miwa et al found in healthy Japanese the decrease in urinary 8OHdG level with increasing age, but in the present study it was probably not observed due to the homogeneity of the sample.No statistical differences were found between the sick group and the healthy controls, as well as no difference between the symptomatic group and the asymptomatic carriers of HTLV1, despite the literature demonstration in the pathogenesis oxidative DNA damage by HTLV1 TAX oncoprotein. Therefore, in HTLV1 infection in patients with tropical spastic paraparesis and carriers, no oxidative DNA damage was demonstrated by the 8OHdG urinary method, in this study. This may be due, as demonstrated in histopathological studies such as Iwasaki (Iwasaki, 1990) and neuropathological studies according to Hollisberg (Hollsberg, 1997). Overstimulation of the immune system with expression of inflammatory cytokines and antibodies against cellular elements, predominantly lesion in the thoracic medullary white matter (myelin) with little neuronal injury, consequently with little DNA damage in tropical spastic paraparesis. The study was limited by the small number of samples, especially in the symptomatic ones, but it is a rare disease and all patients treated during the research period were included.

Conclusion

The epidemiological clinical findings of this study are consistent with those reported in the literature. The 8OHdG concentration found in the symptomatic, asymptomatic and ealthy controls groups are in the same order of magnitude as those of healthy controls reported in the literature. 8 OHdG also showed no difference between patients and healthy controls suggesting that oxidative DNA damage is not the most likely mechanism in the pathogenesis of tropical spastic paraparesis. Further studies in this line of research should be encouraged.

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