Serum Copper, Zinc and Selenium levels in Tunisian patients with Parkinson's disease

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Niveaux sériques de Cuivre, Zinc et Sélénium chez des patients tunisiens atteints de la maladie de Parkinson

LA TUNISIE MEDICALE - 2013 ; Vol 91 (n°06) : 402-405

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R É S U M É

Prérequis : La maladie de Parkinson (MP) est une maladie neurodégénérative progressive. La cause de cette maladie est encore inconnue, mais les radicaux libres ont été proposés comme cause des lésions neuronales. Les éléments trace jouent un rôle clé dans l'équilibre oxydatif intracellulaire. Cependant leur implication dans le processus dégénératif reste inconnue.

But: Evaluer les concentrations de Cu, Zn et Se sérique chez un groupe de patients parkinsoniens afin de déterminer, en comparaison avec des contrôles appariés pour l'âge, si l'altération de leurs taux pourrait être impliquée dans la MP.

Méthodes : Le taux sérique de trois oligo-éléments (Cu, Zn, et Se) a été étudié chez 48 patients atteints de MP et chez 36 témoins appariés en utilisant la spectrométrie d'absorption atomique. Nous avons comparé ces paramètres chez les patients parkinsoniens avec les contrôles, et nous avons également comparé les variations au sein du groupe des parkinsoniens selon l'âge, la durée de la maladie, le stade de la maladie et la prise ou non de la levodopa.

Résultats : Les patients atteints de MP avaient un taux significativement plus bas par rapport aux contrôles. Les taux moyens de Zn et Se ne diffèrent significativement de ceux des contrôles. Le traitement par la levodopa, l'âge, le stade et la durée de la maladie n'ont pas d'influence significative sur les paramètres mesurés.

Conclusion : Ces résultats suggèrent qu'une perturbation du taux plasmatique du Cu pourrait être un marqueur de la MP ou du moins un facteur de risque pour le développement de cette maladie. Bien que le Zn participe à la réduction du stress oxydatif et le rôle antioxydant du Se, leur implication dans l'apparition da la MP n'est pas clairement établie.

Les perspectives pour l'avenir pourraient inclure une thérapie anti-oxydante. Pour cette raison d'autres études prospectives doivent être menées sur ce sujet afin d'élucider l'implication d'éléments trace dans la MP.

M o t s - c l é s

Maladie de Parkinson, élément trace, Cuivre, Zinc, Sélénium

S U M M A R Y

Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder. The etiology of this disease is still not fully clear, but free radicals have been proposed to cause neuronal injury. Metals play a key role in the intracellular oxidative balance. However their implication in the degeneration process remains unknown.

Aim: To assess Cu, Zn and Se concentrations in serum of a group of PD patients in order to determinate, in comparison with age-matched controls, whether alteration in their levels could be involved in PD.

Methods: A serum level of 3 trace elements (Cu, Zn and Se) was investigated in 48 patients with PD and 36 matched controls using plasma atomic absorption spectrometry. We compared these parameters in PD patients with controls, and we also compared the variations within the PD group according to age, illness duration, stage of the disease and levodopa intake.

Results: Patients with PD had significantly lower Cu levels compared to controls. The mean Zn and Se levels in PD patients did not differ significantly from those of controls. Levodopa therapy, age, stage, and illness duration did not significantly influence the measured parameters.

Conclusion: These results suggest that a disturbance of the plasmatic rate of Cu could be a marker of PD or at least, a risk factor for the development of this disease.

Although zinc participates to the reduction of oxidative stress and the antioxidant role of the selenium, their implication in the onset of PD is not clearly established.

Perspectives for the future could include antioxidant therapy. For this reason, other prospective studies should be conducted on this subject to elucidate the implication of trace elements in PD.

K e y - w o r d s

Parkinson's disease, Trace element, Copper, Zinc, Selenium

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects approximately 1% of adult older than 60 years-old [1]. The etiology of PD is still not fully clear, but in recent years, there has been increasing evidence suggesting that oxidative stress plays an important role in the pathogenesis of this disease [2]. In this context, metals were believed to be involved because of their key role in the intracellular oxidative balance. Postmortem studies showed alterations in metal ion levels in substantia nigra of patients with PD [3]. Studies of elemental alteration in human fluids of living PD patients are few and conflicting.

In order to elucidate whether changes in essential trace elements may be implicated with PD, we assessed serum copper (Cu), zinc (Zn), and selenium (Se) levels in tunisian PD patients and we compared them with those of age matched controls.

PATIENTS AND METHODS

We conducted a case control study that included 48 patients with PD and 36 healthy control subjects followed in the department of neurology at the university hospital of Monastir-Tunisia. All patients and control subjects gave informed consent. All patients underwent neurological, psychiatric and physical examination. Patients with history of a severe systemic disease, chronic liver disease, gastrectomy, pancreatic disease, malabsorption, chronic renal failure or infectious condition and those taking antioxidant drugs (sélégeline, tocophérol or selenium) were excluded from both groups. Diagnosis of PD was based on the presence of at least tow cardinal symptoms of idiopathic PD according to clinical criteria [4] and a positive response to levodopa therapy.

Clinical disability of patients was ranged from I to IV according to the classification of Hoen and Yahr [5]. To estimate the effect of many parameters on the rate of metal ions, PD group was separated in different subgroups according to age (under and over then 60 years old), to be or not treated by levodopa, to the illness duration (by choosing an arbitrary limit of 5 years) and to the severity of the disease (group A comprising patients with mild PD: stage I/II of Hoen and Yahr and group B comprising patients with moderate to severe PD: stages III/IV of Hoen and Yahr). The control group included 36 age matched subjects with similarly dietary habit.

Measurements

Serum levels of Cu and the Zn were performed, by Plasma Atomic absorption spectrometry in the laboratory of Biophysics of the Medicine University of Monastir. Selenium was measured in the blood of patients with PD and control subjects in the Trace Analysis Laboratory of Lyon (France).

Statistical analysis

The statistical study was conducted by using Social Statistical Package for the Sciences (SPSS) version 13.0 of Windows with Student's t-test to compare PD patients with controls and also to compare variations within the PD groups. $P \leq 0.05$ was considered significant. Results were expressed as mean and standard deviation (SD).

RESULTS

A total of 48 PD patients as cases and 36 healthy as controls were included. The PD group was composed of 26 men and 22 women, ranging in age from 36 to 81 years with a mean age of 65.8 years (SD=10.2). The control group included 36 age matched subjects (14 men and 22 women) ranging in age from 36 to 83 years with a mean age of 59.7 years (SD=12.1). Clinical data for patients and controls are summarized in table 1. Table 2 shows plasma trace elements concentrations presented as mean values, ranges, and SD. Patients with PD had significantly lower Cu levels compared to controls (P=0.000002). The mean Zn and Se levels in PD patients did not differ significantly from those of controls (P=0.29 and P=0.45 respectively).

Table 1 : Clinical data for PD patients and control group

	PD patients (N=48)	Controls (N=36)
Male / female	26 / 22	14 / 22
Age (years)	65.8 ± 10.2	59.7 ± 12.1
Age ≤ 60 years / age > 60 years	14/34	18/18
Duration of PD (years)	6.3 ± 5.5	/
Duration of PD < 5 years/ duration of PD ≥ 5 years	26/22	/
Group A / group B	25/23	/
Patients treated with levodopa / patients not treated with levodopa	11/37	/

Group A: stages I and II of Hoehn and Yahr classification.
Group B: stages III and IV of Hoehn and Yahr classification

As can be seen in table 3, there was no significant correlation in PD patients between the plasmatic concentrations of the measured trace elements and levodopa intake. Se and Zn levels

Table 2: Trace elements levels for PD patients and control group

Elements	PD patients (N=48)		Controls (N= 36)		P
	Mean ± SD	Range	Mean ± SD	Range	
Selenium (μg/l)	98,5 / 17,6	63,5 / 139,8	95,8 / 14,4	68,8 / 125,9	0.45
Copper (μmol/l)	13,3 / 3,1	6,9 / 21,3	17,4 / 4,0	8,9 / 25,6	0.000002*
Zinc (μmol/l)	9,6 / 2,6	5,1 / 16,9	8,9 / 3,1	4,3 / 17,2	0.29

** Significantly different between PD and control groups at P≤0.05.*

SD: Standard deviation

Table 3: Selenium, copper and zinc levels in levodopa-treated and untreated PD patients

Elements	Levodopa-untreated patients (N=11)		Levodopa-treated patients (N=37)		P
	Mean	SD	Mean	SD	
Se (μg/l)	97,7	19,1	98,7	17,3	0.88
Cu (μmol/l)	13,8	3,8	13,1	2,8	0.59
Zn (μmol/l)	10,1	2,7	9,4	2,5	0.49

Selenium (Se), Copper (Cu), Zinc (Zn)

SD: Standard deviation

were not influenced by the following criteria (age, Hoehn and Yahr stage and PD duration). However a decrease but not significant of the Cu rate in patients with severe PD was noted (P=0.07). Age and PD duration did not influence Cu levels in PD patients.

DISCUSSION

Compared to controls in this study, we have found statistically significant decreased levels of plasmatic Cu, while Zn and Se serum values showed no significant changes. This result suggests a possible link between abnormal copper homoeostasis and Parkinson's disease. However it remains unknown whether this loss of Cu is implicated as cause or consequence of the neurodegenerative process. Unlike copper, involvement of Zn and Se in PD is not clearly established. In recent years there has been increasing evidence suggesting that trace elements and redox-active transition metals in particular, have been implicated in the pathogenesis of PD. the most likely mechanism being through oxidative stress [2]. The most relevant redox-active metals are Fe, Cu and Mn, and it seems likely that increased oxidative stress could lead to an imbalance in the concentration of these metals [6]. We were interested in this study in three essential trace elements, which could be related to the risk of developing PD, namely Cu, Zn and Se. These are cofactors of main antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and gluthation peroxydase (GPx).

Cu is present throughout the brain and is most prominent in the basal ganglia, hippocampus, cerebellum, numerous synaptic membranes and in the cell bodies of cortical pyramidal and cerebella granular neurons [7]. It has a functional role in many enzymes that require redox-reactions. Imbalance in Cu homeostasis might lead to increased free radical production and this may be involved in the neuropathology of disorders such as PD [8]. Cu is fixed to the active site of several enzymes such as the SOD which plays an important role as antioxidant. In the present study a significant decrease of the plasmatic concentrations of Cu in PD patients compared to matched controls was noted. Cu levels did not correlate with age, duration of PD, and levodopa intake. Whereas we found a decrease, but not significant of Cu rates in advanced stages of PD. These results suggest a disturbance in Cu homeostasis in the late stage of the disease. The decreased serum level of Cu suggests the possible involvement of this trace element in PD. Literature on serum Cu in PD is conflicting: Bocca et al in 2006 [9], and Forte et al in 2004 [10], reported decrease serum Cu levels. But Hegde et al in 2004 [11], reported an increase of serum Cu levels and Jiménez-Jiménez et al in 1998 [12], showed no significant difference in serum Cu levels in PD compared with controls. Our results are in agreement with the histological studies which showed a decrease of the rate of Cu of 34 % in the substantia nigra versus controls of the same age [3]. This decrease was selective, they did not note disturbance of the rate of Cu in sustantia nigra in patients affected by supranuclear palsy or by multisystemic atrophy. Besides, Pall and al in 1987 [13], reported an increase of the Cu rate in the cerebrospinal fluid (CSF) of PD patients but this increase was not confirmed by other studies [9].

Zn plays a key role in several brain functions like preserving the structure of the SOD and inhibing the nitric oxide synthetase to reduce oxidative stress. It participates also in moduling the response of both excitatory and inhibitory receptor [14]. We did not show significant difference of the plasmatic rates of Zn between PD patients and matched controls. The age, the sex, the illness duration, the severity of disease and the levodopa intake have no significant effect on the results. Thus, the majority of studies support our results showing no difference in plasmatic Zn levels of patients with PD compared with controls [10, 12, 15, 16]. On the other hand Hegde in 2004 [11], found a significant decrease of the plasmatic concentrations in the severely affected patients. Besides, Dexter et al. [3], showed a selective increase of the rate of the Zn in the substantia nigra of patients with PD. They concluded that Zn can be then a sign of the existence of excessive oxidative stress in the substancia nigra especially if this increase joins to an increase of the activity of the SOD [17]. Jiménez-Jiménez and al in 1998 [18], found a significant decrease of the rate of Zn in the CSF compared to matched controls suggesting that this decrease could be related to the risk of development of the PD.

Se is a well known anti oxidant and there is some indication from animal models that Se may be protective in PD [19]. It is a cofactor of the GPx, which constitutes the second line of defence, against oxidative stress. So it has a crucial role in the prevention of the damage for cells and particularly for dopaminergic neurones. Our study showed no significant decrease of the plasmatic rate of Se in PD patients versus controls. We did not find significant variation according to the age, to the sex and to the severity of the disease. To our knowledge few studies were interested to measure Se in the

blood and in the CSF of PD patients. Gellein et al in 2008 [8], showed a decrease of plasmatic Se rate. Aguilar and coll., 1998 [20], did not revealed significant difference of Se in the blood and in the CSF, between the PD patients and matched controls. On the other hand they showed a significant difference between the treated patients and untreated by levodopa, with a significantly higher CSF rate in the treated group. In contrast with this previous study, we did not observe significant differences in plasma Se rates in patients with or without levodopa. But the weak sample of patients let us not conclude. In normal subject, the rate of Se in the brain is particularly higher in the substancia nigra however, this content is less important in PD group [21].

In their prospective study, Gellein et al in 2008 [8], showed that except a small differences in Hg, no significant differences were found in trace elements in serum collected before diagnosis from PD patients versus controls, in contrast significant changes were found for several traces elements in serum collected from PD patients 4 to 12 years after they were diagnosed compared with pre-diagnosis. They concluded that the illness could introduce a change in trace elements homeostasis rather then a trace element being a causative factor in PD. Wile some authors [22] concluded that specific metals like Cr, Fe, and Pb were the most suitable metals to distinguish PD patients from healthy group.

In conclusion the implication of trace element is clearly established according to the majority of the studies particularly in the pathologies where oxidative stress is incriminated in particular the PD. Decrease of Cu plasmatic rate could be viewed as peripheral marker for PD, but such markers will have no diagnostic values in PD because they are disturbed in some other neurodegenerative diseases. Many strangers concerning the role of trace elements in the neurodegenerative diseases remain to clarify. So other prospective studies concerning a more important number of patients must be conducted to be able to establish definitive conclusions and throw of the therapeutic behaviours to know a supplementation of some trace elements.

Acknowledgement

We would like to thank Ben Salah J, Chekir A, Ammar Y, and Boukottaya S for their excellent technical assistance.

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