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ROLE OF ENVIRONMENTAL RISK FACTORS IN PARKINSON'S DISEASE: BUCOVINA REGION CASE STUDY

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Abstract

Parkinson's disease (PD) is a neurodegenerative disease that occurs due to loss of dopamine, a neurotransmitter, and slow destruction of neurons. Brain area affected by progressive destruction of neurons is responsible for controlling movements, and patients with PD reveal rigid and uncontrollable gestures, postural instability, small handwriting and tremor. There are some risk factors that have been proven to trigger PD with a certain probability (for example insecticides exposure, and genetic and environmental factors), but still can not say for sure which are all risk factors for this terrible disease. Also, many other leads were analyzed, such as exposure to certain metals, toxins, head trauma, constipation, low intake of antioxidants, infection (chicken pox, measles, rubella, mumps), but no studies have shown clear links with them. This study's aim is to examine the association between Parkinson's disease and exposure to environmental factors such as living in the Bucovina Region (Suceava District, North of Eastern Carpathians). Exposure to metals such as lead, manganese, iron, copper and uranium have been of interest since some occupational studies focused on mining identified them as potential risk factors for PD. Moreover, we have done a statistical study based on Markov chains regarding this disease prediction and we have developed a specific screening test for early diagnosis of Parkinson's disease.

Key words: Bucovina region, environmental risk factors, non-linear signal analysis, Parkinson's disease

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1. Introduction

1.1. Clinical and pathological aspects of Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease that occurs due to loss of dopamine that is a neurotransmitter and due to a slow and inexorable destruction of neurons. Brain area affected by progressive destruction of neurons is responsible for controlling movements (Shtilbans and Henchcliffe, 2012). Parkinson's disease is a chronic, progressive, multi-lesion, neurodegenerative disease with unknown etiology characterized by the slow loss of dopaminergic neurons from the *substantia nigra*, especially from the *pars compacta* (Lees et al., 2009). Statistically, there are about 100-250 cases per 100,000 people (Dorsey et al., 2007). Reports indicated about 1.2 million cases in Europe, of which 16,000 in Austria alone (World Health Organization, WHO, 2013). The disease affected about 1 million people in 2003, representing roughly 1% of the population aged above 65. Currently, about a million Americans suffer from Parkinson's disease, with about 60,000 new cases diagnosed each year (National Parkinson Foundation, 2013). Parkinson's disease is a frequent neurological condition, with an estimated 6.4 million cases worldwide and with

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currently unknown causes and cures (Cucoș et al., 2011). Although the average age at which Parkinson's is diagnosed is 60, 1 out of 20 patients begins to exhibit symptoms even before the age of 40. These patients, diagnosed at young ages between 21 and 40, suffer from early Parkinson's onset. The data we have reveals that in 2012 there were over 75,000 patients in Romania, but this number is greater because of many undiagnosed patients due to the lack of specialists in the field but also due to the lack of a biomarker with which a screening can be performed among the population for early diagnostic of PD (Chahine and Stern, 2012).

Parkinson's disease has a group of cardinal symptoms (considered by convention "positive" and "negative") which can be seen in different combinations (National Parkinson Foundation, 2013):

1. Tremor (+);
2. Stiffness (+);
3. Bradykinesia (–);
4. Hipokinesia (–);
5. Flexed posture (+);
6. Loss of postural reflexes (–);
7. The "freezing" phenomenon (–).

According to current diagnostic criteria of the *UK Parkinson's Disease Society Brain Bank* (Parkinson's Disease, EuroPa, 2013) the presence of at least 2 of the symptoms above along with bradykinesia is mandatory for clinical diagnosis of PD. This set of well validated criteria helps the accuracy of clinical diagnosis with a specificity of 98.1% and a sensitivity of 90.4%. These criteria join medical history aspects and very useful clinical exams because since PD diagnosis is a clinical one it has an error rate of 10-20%.

Thus according to the *UK Parkinson's Disease Society Brain Bank* (Parkinson's Disease, EuroPa, 2013) the diagnostic is performed in three steps. The patient must present bradykinesia and one of the following symptoms: muscle stiffness, 4-6Hz resting tremor, postural instability that doesn't have primarily visual, vestibular, cerebellum related of proprioceptive causes.

Recently, non-motor PD symptoms were categorized as follows:

1. Neuropsychiatric symptoms (National Institute of Neurological Disorders and Stroke, 2013);
2. Sleep disorders;
3. Autonomic symptoms;
4. Gastro-intestinal symptoms;
5. Sensory symptoms;
6. Other symptoms.

Despite the newest medical discoveries there currently isn't a cure available for PD. The treatment's goal is to correct the symptoms, especially the motor symptoms and to minimize their consequences on the everyday life of the patient.

1.2. Risk factors and Parkinson's disease

During last decades genetic and environmental factors were mainly studied, as they might be important causes for PD development. For instance, as a part of EU-funded research project Geoparkinson "Parkinsonism and Parkinson's disease: Interaction between environmental exposures and genetic factors", European researchers have recently investigated the relation between exposure to solvent, pesticides, iron, copper and manganese and risk of PD (European Commission, 2007).

This study took place in 1989 in Scotland, Italy, Romania, Sweden and Malta and 959 patients were analyzed. Subjects completed an interview-questionnaire regarding lifetime occupational and hobby exposure to these substances (Bronstein et al., 2009). The researchers found that PD is associated with pesticide use and environmental factors exposure (Dick et al., 2007). These results suggest that relatively low intensity exposure to metals may increase risks.

Genetics is not considered a major contributing factor for PD. However, researchers of NIEHS – National Institute of Environmental Health Sciences (NIEHS, 2012) – have identified many gene variations that make people more susceptible to environmental toxins and that may trigger the development of Parkinson's disease. In the 5 to 10% of cases in which symptoms begin before the age of 50, genetics seems to play a bigger role.

Even if at least 9 mutations in different genes have been proven as risks of familial PD, the genetic substrate of most PD cases does not show a clear, Mendelian inheritance, and transmission pattern still remains unidentified. Case-control studies have shown a 2-14 fold increased incidence of the disease in close relatives of PD patients.

The cornerstone of PD research is considered to be a mutation of a gene on the 4q21-q23 chromosome, which codes alpha-synuclein (gene Park 1). This demonstrated that the mutation of a single gene could cause PD (Warner et al., 2003).

Numerous studies looked for links between diet and PD risk. Thus it has been shown that a hypocaloric diet decreased the risk of Alzheimer's disease (AD) and PD, especially if it is established around the age of 20. An even closer correlation was found between plasma levels of homocystein (normal: 5-15 μM); values over 10 μM considerably increase the risk of AD, which is a plausible hypothesis for PD. A 400 μg folic acid dietary supplement leads to a 2-5 μM decrease of homocystein, which in turn decreased the risk of AD 2-4 times. Yet, there isn't any evidence to therapeutically validate this hypothesis on the active disease (Ribicki et al., 2010; Tanner et al., 2009; Warner et al., 2003; Zorzon et al., 2002).

Studies also targeted the effect of increased intake of vitamins (E, C, carotene, etc.) and they have shown that they don't seem to decrease the risk of disease onset. The fact that a high intake of vitamin E does still decrease the risk of PD suggests that other factors (constituents) have this effect (Gorell and Rybicki, 2003).

1.3. Parkinson's disease and environmental exposure: links to exposure to metals

Exposures to metals, such as lead, iron, copper, manganese, uranium and others have been of interest since some occupational studies identified them as potential risk factors for PD. Mechanisms whereby metals may influence PD risk include increased oxidative stress and facilitation of protein aggregation. Even without excessive exposures, abnormal transport of essential metals such as copper, zinc, iron into the brain or mishandling of the metal within the brain may trigger these responses (Baldi et al., 2003; Cecchini et al., 2015; Huble et al., 1993; Priyadarshi et al., 2001).

Many meta-analyses recently done show links between environmental exposure and PD symptoms onset. In the meta-analysis "Environmental Risk factors and Parkinson's Disease: A Metaanalysis" (World Health Organization, WHO, 2013) the authors examine the association between Parkinson's disease and exposure to environmental factors such as living in a rural area, well water use, farming, exposure to farm animals or living on a farm, pesticides and metals. This study shows that people living in a rural area are at significantly increased risk of getting PD. The association of rural living with PD may be related to exposure to potential neurotoxins present in pesticides, well water or metal (Butterfield et al., 1993). Gorell et al. (2004) found a significant association of more than 20 years of occupational exposure to manganese, though caution is needed in the interpretation of the relationship, as it was driven by just three cases and one control subject with chronic manganese exposure. When considering manganese exposure as a risk factor for PD, it is important not to reject a potential association because of confusion with the severe poisoning seen in manganism, in which there is preferential affection of the *globus pallidus* rather than the subthalamic nucleus, with clinical dystonic parkinsonism produced most often (Ciccheti et al., 2009; Dhillon et al., 2008). There are studies that showed that miners can take through respiration significant quantities of manganese, and thus the risk for neurological degenerative diseases, such as PD, PD-like tremor, and other similar syndromes significantly increases (Fall et al., 1999; Hubble et al., 1993). Despite some clinical similarities, pathological studies of brain tissue after excessive manganese exposure in adulthood show that an important part of the basal ganglia known as the *globus pallidus* is the most prominently damaged, with relative sparing of the *substantia nigra* and an absence of Lewy bodies (Koller et al., 2006; Lewis and Barker, 2009).

Studies of metal welders who may also be exposed to manganese by inhalation have been consistent with respect to risk of PD. Emission from steelmaking industries are a potential source of population-wide manganese exposure. A study in the steelmaking city of Hamilton, Ontario, reported an increased risk of PD associated with increases in manganese content in particulate air pollution (Dhillon et al., 2008).

Noonan et al. (2002) in a hospital-based case-control study in Singapore found a significant association between mercury exposure and PD. They also found a dose-response relationship when comparing blood mercury levels of subjects (Noonan et al., 2002). A potential relationship between occupational exposure to iron, copper, lead, or zinc with PD has been infrequently studied. Bronstein et al. (2009) in a study in southern Quebec, reported that, among 42 cases and 84 controls, assessed by self-report, there was a significant association between PD and occupational exposure to a combination of manganese, iron, and aluminum, particularly for more than 30 years. Gorell et al. (2004) found that greater than 20 years of occupational exposure to combinations of lead-copper, lead-iron, iron-copper were associated with PD. These combined metal exposure results showed a greater association with PD than did any metal alone. The mechanisms by which heavy metals, including lead, may increase Parkinson's disease risk include increasing oxidative stress, lipid peroxidation of cellular membranes, and abnormal folding of alpha-synuclein protein (Preux et al., 2000).

There are some risk factors that have been proven to trigger PD with a certain probability (for example insecticides exposure (BenMoyal-Segal, 2005), and genetic and environmental factors (Soreq et al., 2012), but still can not say for sure which are all risk factors for this terrible disease.

Recent studies on animals and humans subjects showed that particulate air pollution increases oxidative stress and inflammation in the brain. In this way, particulate air pollution might be a risk factor for both Alzheimer's disease and PD (Dick et al., 2007).

1.4. Parkinson's disease incidence in Moldova region

Statistical analysis of casuistry in Romania reveals an important incidence of PD among the population, with about 75000 diagnosed cases in 2012. Reports indicate an annual increase of about 1.5% of PD and secondary PD, which means about 1000 new patients per year. Considering a linear trend this will mean 12000 new cases in 2020 (Cucoș et al., 2011). Apparently, the number of new cases per year compared to a population of 21 million isn't cause for concern, but it will certainly become one and the costs to at least medicate these patients will become obvious.

PD becomes more and more widespread each year and the national healthcare system should react and come up with programs to manage this disease. The patient's background is an important element in identifying the casuistic spread because it determines a measure of the education that the patients in these environments have. In 2005, in Moldova region, PD incidence was highest in Galați County and lowest in Vaslui County. Iași County was a close second to Galați County with 30.47 cases per 100,000 people. In 2006, Galați County still had the highest incidence, with 49.84 cases per 100,000 people and Vaslui County the lowest with 14.89 cases per 100,000 people. PD incidence in age groups for patients older than 65 is greatest in Galați County (Cucoș et al., 2011). Higher values in PD incidence in age groups are a result of comparing the number of cases for the said age group. Total incidence for each county is greatly diminished by the fact that there were no reported cases between 2005 and 2012 in the 0-1 age group and in the 1-14 age group.

In 2012 the center of gravity moves towards Suceava County, which has the greatest number of reported PD cases per 100,000 people, with a value of 63.10 (Fig. 1). This high value is determined by demographics, since the total population of the county decreased by roughly 30% (mainly composed by young people) compared to previous years, thus reducing the base values. Also there was a moderate increasing of the incidence of PD patients.

Even though yearly statistics seem redundant at a first glance, they have a key role in best defining the incidence of PD in each Moldavian County. This aids prediction of the number of cases on a medium and long term, thus allowing anticipating at least the average impact on the healthcare system, which is already strained by the variety of other chronic diseases for which the government provides medication and support.

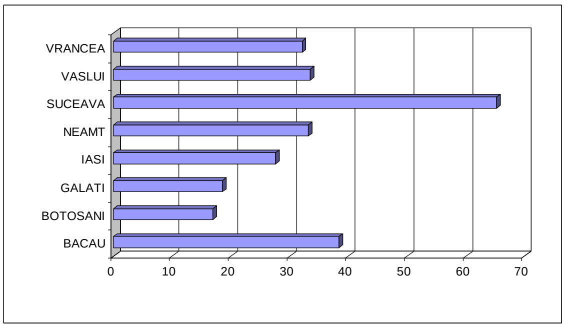

Fig. 1. PD incidence in Moldova region in 2012 (per 100,000 people)

As a partial conclusion, average PD incidence is slightly higher in rural areas. One of the future goals is to determine the exact stage (early, mid-stage, and advanced) in which any new case was identified. This would mean identifying causes of diagnosis at certain stages influenced by social and economic factors, education and access to education of the patient, etc.

We also have calculated PD incidence per gender using data from the same source, the Public Health Board, for Moldavian Counties (Bacău, Botoşani, Galaţi, Iaşi, Neamţ, Suceava, Vaslui and Vrancea). The highest number of male patients per 100,000 people in 2012 was observed in Suceava County, followed closely by Bacău County with 37.88 cases per 100,000 people (Fig. 2). The lowest numbers were reported in Botoşani and Galaţi. Female incidence of PD was similar with some differences in Botoşani, Galaţi and Neamţ. In 2008, Suceava had the most male PD patients and was a close second to Neamţ regarding female PD patients (Cucoş et al., 2011).

Distribution between male and female PD patients is approximately balanced in 2012, with slightly more male PD patients in some counties and slightly more female PD patients in others (Fig. 3). Suceava County reports 66.28 male PD patients and 63.45 female patients per 100,000 people. At the opposite pole, Galaţi County reported 11.28 male patients and just 9.45 female patients per 100,000 people.

On a national level, males who live in rural areas present a higher incidence of PD than those who live in urban areas. There is a growing trend, with an approximate 5% yearly increase for both gender groups and for both rural and urban areas.

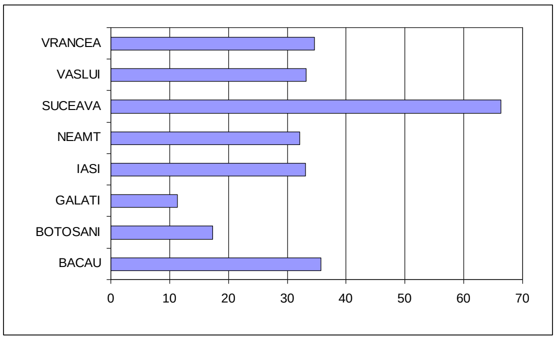

Fig. 2. Male PD incidence, 2012 (Suceava County reported 66.28 male PD patients per 100,000 people)

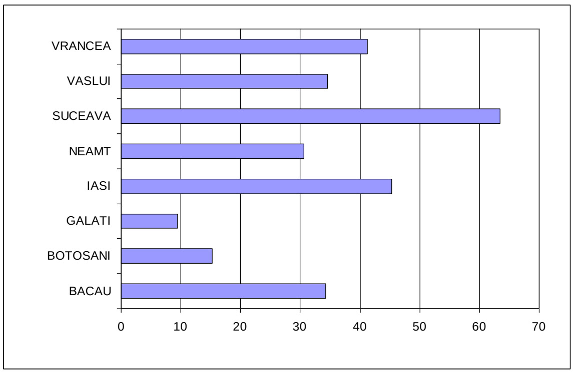

Fig. 3. Female PD incidence, 2012 (Suceava County reports 63.45 female patients per 100,000 people)

2. Material and methods

2.1. Case study – Suceava County

The studied area corresponds geo-structurally to the crystalline-Mesozoic area of the Eastern Carpathians. The earliest documents attesting mining activities in the region date back to 1770. True prospecting of the region can be considered only after 1777, as a consequence of Bucovina being annexed by the Austro-Hungarian Empire. Thus iron deposits have been found at Arșița and Terezia (Vatra Dornei), galena ore at Fluturica Cârlibaba, copper ore at Dealul Negru-Fundu Moldovei, manganese ore at Orata, Colacu, Șaru Dornei and magnetite at Runcu (Iacobeni), Bretila and Rusaia (Cârlibaba). These ores were processed at Cârlibaba, Iacobeni and Pojorâta (Popescu and Popescu, 2007).

Manganese ore presents itself under a lenticular shape, with variable dimensions due to successive deformational events. The Dadu-Iacobeni site was closed, but its effects on the environment are still present. Manganese extraction changed the quality of the environment, especially by altering the topography of the area because of mine dumps and mine water, ore transport and due to increased incidence of PD in the area and in its vicinity. At Dadu, mine dumps cover roughly 20000 m^2, and soil samples from the area show manganese concentrations 2 to 3 times higher than normal values. At Tolovanu, mine dumps cover about 5500 m^2.

They are generally stable, except one dump that contains waste from the manganese ore roasting oven. At Oița, mine dumps cover roughly 40200 m^2. The chemical composition of mine waters from the mine shafts of the three sites at Oița-Colacu have Cu, Pb, Zn, Ca, Mg, NO_2 ions and organic CBO_5 concentrations right at the maximum allowed limits (Popescu and Popescu, 2007).

The Dadu, Oița and Tolovanu sites lead to a series of important environmental changes. The opening, exploitation and closing of these sites altered the area's topology and destroyed natural habitats. Air pollution results from dust from mine dumps and gases from blasting, transport, grinding and initial processing of the ore. Water pollution is done by draining mine water, which is slightly acidic and dumping it in surface water and by material coming from mine dumps.

Soil pollution is mainly a result of contaminating the soil with fuel. Manganese influence coming from mine dumps is usually materialized in secondary dispersion halos, which are usually fan shaped, with the center located in the emission source area (Popescu and Popescu, 2007). Because of its particularities, manganese has reduced mobility in soils with neutral pH and its mobility slightly increasing in soils with an acidic pH.

Another important aspect of the studied area is the existence of radioactive metal mining sites, which were closed without taking many measures destined to protect the environment. The only active uranium mining site in Romania is in Crucea, but authorities consider closing it.

Suceava County now has 3 active mining sites: manganese is extracted at Dorna Arini, uranium at Crucea and copper at Iacobeni, with two mine shafts (Fig. 4). These sites have 10 active dumps, which cover 18.56 ha and hold a total of 3857000 m^3 of waste.

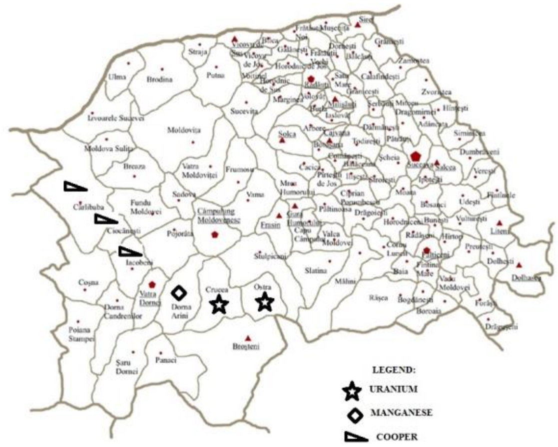

Fig. 4. Repartition of Suceava County mining sites

These active mine dumps are the target of measurements that insure their stability. Waste management programs have been elaborated, according to Order number 2024/Nov. 22nd, 2010, regarding the approval of the extracting industries' waste management plans. The four waste management plans were approved by the National Mineral Resource Agency in Bucharest and the Environment Protection Agency in Suceava.

Suceava County had 1700 PD patients in 2012. Public data from the National Statistics Institute show that Suceava County is in the top 7 most populated counties in Romania, with a population of 614451 in 2012. Statistical data shows a population of 708714 in 2006, and by the end of October 2010 Suceava County had a population of 710142. Reports from the Public Health Board show on average roughly 250 new cases each year, which isn't a large enough number to be a cause for concern but is still large enough to cause issues related to resources needed for long term management of this disease.

Epidemiologic studies done in Moldova, a Romanian region which Suceava County is part of, report varying numbers for primary and secondary PD year to year and county to county. For example, in 2012 Galați still has an incidence of primary PD of 48.89, followed by Iași with 46.24 and Suceava with 40.58, Vaslui County still having the lowest incidence of 13.64. In what regards PD incidence in age groups, Suceava has the largest incidence for the 60+ age group. Epidemiological studies on a nation-wide level show that the incidence has a yearly growing trend.

For a growth of 9.4 over a 5 year period we estimate, considering a linear approximation, that in

10 years' time, in 2019, PD incidence in Romania will be about 50-53, which will cause a 50% higher strain on the healthcare system in what regards the need for medical attention that must be given to patients in this category.

2.2. PD prediction with Markov chains

In mathematics, a Markov process is a stochastic process, which has the property that, given its present state, the future states are independent of the past. This property is called the Markov property (Beck and Pauker, 1983). In a Markov process, the system can change or keep its state, according to a certain probability distribution. Changes of its state are called transitions. A random experiment that consists of a series of random sub-experiments is called a stochastic process. Such a special class of these processes is the Markov chain (Jackson et al., 2003; Magni et al., 2000).

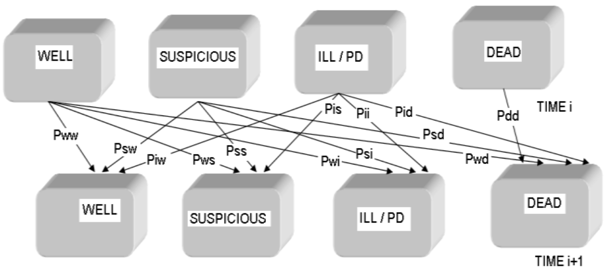

Fig. 5. Three-state Markov Model for Parkinson's disease stage (well, suspicious, ill/PD, dead)

The evolution of a Markov process can be described by a transition matrix. We can consider the evolution of the health status of a patient as a Markov process that passes through the following states: Well, Suspicious, Ill (PD), or Dead, as it is illustrated in Fig. 5. Using Markov chain is a pretty good way to find the vector of features for an individual patient at a given time, used to predict and identify a stage of Parkinson' disease. So, the physician can choose a treatment, based on this forecast with the appropriate level of medication impaired by Parkinson's disease patient.

PD prediction done with Markov chains was described in detail in the paper (Geman and Costin, 2013). Having data on the number of PD patients distributed across years from areas with risk factors we can use these Markov chains to assess the number of PD patients in the future.

We proposed to predict the state of a patient using Markov chains. In this analysis the state vector is defined as: $S = [X_{min}, X_{med}, X_{high}, Y_{min}, Y_{med}, Y_{high}, Z_{min}, Z_{med}, Z_{high}, C_{Mn\text{-}blood}, C_{Mn\text{-}urine}, C_{Cu\text{-}blood}, C_{Cu\text{-}urine}]$, where *Cs* are the concentrations of manganese and copper in blood and urine, respectively, for all tested subjects who are referred in Section 3.

By means of a Wii™ Remote device (see Section 3) we defined the following linguistic variables (for instance for X axis), by using methodology from (Geman and Costin, 2013):

- If x is between -0.10 mm and -1 mm then x is minimum (X_{min});
- If x is between -0.10 mm and 0.10 mm then x is medium (X_{med});
- If x is between 0.10 mm and 1 mm then x is maximum (X_{max}).

We counted the number of spikes for each interval, and we used these values to describe the state vector.

We also may note the very good prediction power of this method, as the features vector elements for the predicted tremor signal after 24 months from the first recording are very similar with the same vector elements, but acquired and measured by means of Wii™ Remote device and the appropriate software. The maximum error between prediction and measured values was 1.33%.

Similar judgement was used and corresponding good results concerning the prediction of disease evolution were obtained in the case of "suspicious PD" patients, for whom some early signs were found, such as increasing the concentration of heavy metals in blood and urine, insomnia, constipation, loss of smell, equilibrium and postural impairment, tremor symptom or speech difficulties and they had to be attentively monitored. Also, another remark may be made related to the similarity between features vectors measured for "suspicious PD" patients and "diagnosed PD" patients, when using the same Markov chains for status prediction, using concentrations of heavy metals in blood and urine, and also from the environment they live in these patients (from soil, air, and water).

3. Experimental results

In recent researches conducted by the authors of this paper (Geman, 2011; Geman et al., 2013; Pohoață et al., 2012) the physiological information and the time series parameters measured from gait and tremor have been combined in developing an automatic diagnostic system for PD monitoring by means of non-linear dynamics and analysis.

We demonstrated that nonlinear dynamics parameters of PD gait or tremor signals can be used in knowledge discovery to better assess the PD.

Our database contains gait and tremor measures from 32 patients with PD (from Suceava Hospital, Neurology Clinic), 58 healthy subjects and 14 "suspicious" PD subjects (who live near uranium mining site of Crucea-Botușana or work in manganese, copper or uranium mining sites of Suceava county). Young adults (n = 42; ages: 20-35 yrs, 28 males and 14 females) and older adults (n = 62; ages: 65-87 yrs, 41 males and 21 females) participated in this study.

Fig. 6 describes the block diagram of "i-Parkin Screening System" mainly developed by first author of this paper.

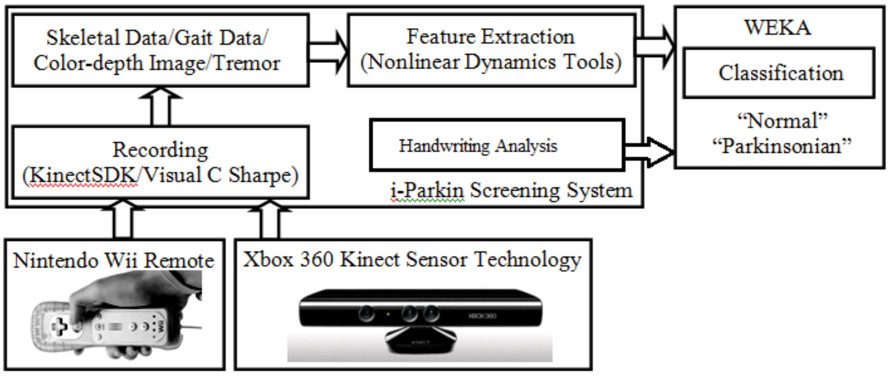

Fig. 6. The workflow of this research and i-Parkin Screening System blocks

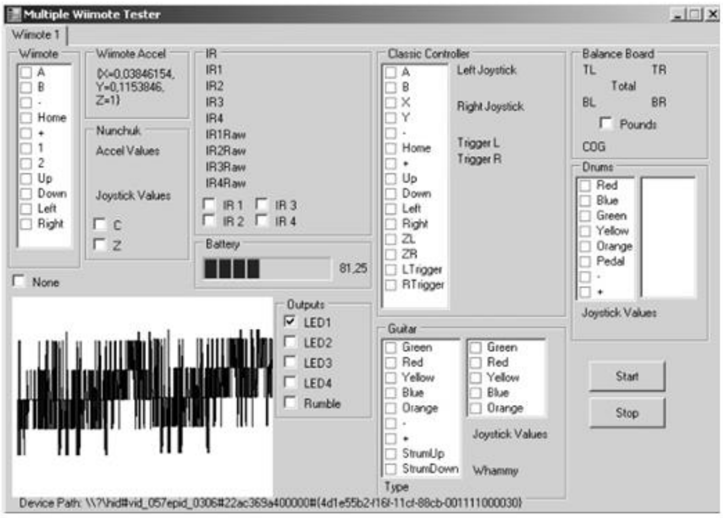

Fig. 7. Interactive GUI using Wii™ Remote (tremor application) (Pohoață et al., 2011)

Our Screening System (i-Parkin Screening System) consists of four components: the first component records the skeletal information, gait parameters and tremor information, using Kinect™ and Wii Remote™ devices (Nintendo Wii™, 2013). These information are then analyzed using linear dynamics (Chaos Data Analyzer, 2013), nonlinear dynamics (Nlyzer, 2013) and statistical tools – the second component. The third and fourth steps consist in feature extraction and classification, respectively. For the last stage of our research, we use K-means algorithm – one of the simplest but efficient unsupervised learning algorithms to identify a "normal" or a "Parkinsonian" subjects.

A complementary method to assess and predict PD is based on the automatic processing of the image of the handwritten script belonging to a candidate of PD, a method tested with good results by one of the authors (Costin and Rotariu, 2001). In order to extract geometric features used for PD evaluation, the following steps are necessary: scanning of handwritten text; image binarization; image filtering; thinning of the handwritten text; segmentation of words for end points, fork points and loops detection; removal of entities extracted, i.e. segments achieving; feature extraction for each segment; statistical calculus for each detected feature.

Now coming back to a Wii™ Remote device, this system is capable to analyze frequency and to estimate the amplitude of tremor between 3-15 Hz (normal tremor is between 5-12 Hz, and PD tremor is between 4-6 Hz) (Lemoyne, 2010). The Wii™ Remote and PC are connected by Bluetooth - Human Interface Device Profile. The tremor analysis program was developed by present authors using Visual C 2010 Professional (see Fig. 7).

As shown in Fig. 8a and Fig. 8b, the considered indexes have different trajectories for Parkinson's disease tremor. Significant differences appear between the "normal" (N), "Parkinsonian" (P) or "suspicious" PD (S) tremor, respectively. The three nonlinear dynamic parameters are represented in detail in Figs. 8a-c (Geman, 2011). We intend to use the values of the correlation dimension to develop a knowledge-discovery module for the Parkinsonian tremor. Analyzing the data, we observed that the fractal dimension allows us to recognize with good accuracy the "normal" tremor class, and Lyapunov exponent helps in recognizing with good accuracy "suspicious" or "Parkinsonian" tremor.

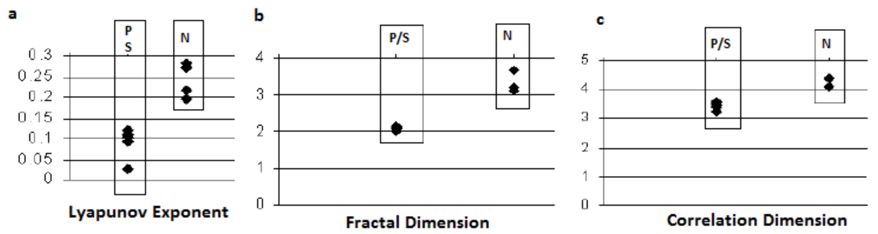

Fig. 8. Graphical values representations for Lyapunov Exponent (a), Fractal Dimension (b) and Correlation Dimension (c) for "normal" (N), "Parkinsonian" (P), "suspicious" (S).

Table 1. Analyses for heavy metals

Analyses for heavy metals identification	*Reference values*	*Average values for patients with PD in our database (at the time of their diagnosis)*
Manganese in blood	0.3 - 0.9 μg/L	1.2 - 2.9 μg/L
Manganese in urine	< 2.9 μg/L Occupational exposure: LBT = 10 μg/L; LBT = biological tolerable limit.	10.6 - 12.3 μg/L
Copper in blood	Adults - F: 76-152 μg/dL - M: 70-140 μg/dL	Adults - F: 155-214 μg/dL - M: 144-210 μg/dL
Copper in urine	2-80 μg/L; 10-60 μg/24h; <50 μg/g_creatinine	100-110 μg/L; 65-93 μg/24h; <50 μg/g_creatinine

Patients, many of them being former miners, were analyzed in terms of the toxicological profile due to exposure to heavy metals. Heavy metal toxicity is a medical situation less common, but still clinically significant. If not recognized and treated properly, it can cause significant morbidity and mortality. Some heavy metals are essential in various biochemical processes (for example Zn, Cu, Cr, Fe, Mn - necessary for the body in small amounts, but becomes toxic for the body in large amounts). The most common metals involved in acute and / or chronic toxicity are Pb, Mn, As and Hg. Metals may enter the body via: oral (through food, water), inhalation or skin absorption. Almost all organs are involved in the toxicity of heavy metals (Rusu, 2015).

Most affected organs are the central nervous system, peripheral nervous system, gastro-intestinal system, hematopoietic, renal and cardiovascular systems. To a lesser extent, Pb toxicity involves musculoskeletal and reproductive systems. Type of organ affected and severity of damage vary with the metal involved, the individual's age and level of toxicity (Soghoian, 2006).

Patients diagnosed with PD in our database have presented general symptoms of heavy metal poisoning. In their medical history patients experienced headache, mental confusion, pain in muscles and joints, gastro-intestinal disorders, allergies, vision problems or chronic fatigue.

For instance, for the 32 investigated patients with PD who live near uranium mining site of Crucea-Botușana or work in manganese, copper or uranium mining sites of Suceava county blood figures are as indicated in Table 1. We found elevated concentrations of the two heavy metals in blood and urine for patients analyzed.

4. Conclusions

The toxicity of manganese was acknowledged since the early 20th century (employees in manganese mines frequently exhibited a neurological syndrome dominated by tremor, hypokinesia and stiffness). These symptoms could be reproduced in lab animals by exposure to manganese. In almost every former mine site in Suceava County, including former uranium mines, there still is waste material that must be isolated using clay rocks or with synthetic or composite materials. Moreover, Crucea uranium mine is still active. If the mine dumps aren't cleared there is a risk of (radioactive) pollution, which would have a high impact on the environment and on the people and would manifest itself over a long period of time. There was also an increased number of patients with neurological conditions (Alzheimer's / Parkinson's disease, multiple sclerosis) in the studied area, but also many cases of malignant tumors (breast, ovary and thyroid tumors in women and prostate tumors in men) at people living near Crucea uranium mine area.

The main added value of this paper beyond the state-of-the-art, besides the statistical study of the PD incidence and its link with the mining sites in Suceava region, is that we have done a study based on Markov chains regarding disease prediction and we have developed a screening test for early diagnosis of the disease, which takes into consideration exposure to noxious materials of people living in Suceava county.

We have investigated patients with Parkinson's disease in mining areas such as Botuşana, Crucea and Argestru, where the Charge - Transport Railway Station where uranium ore is situated. Mining activities and processing of radioactive ores in the environment causes the elimination of wastes from these activities that may cause further radioactive emissions to the natural environment. Mode of disposal and storage of radioactive waste from mining may affect natural features, if not taken legal measures for radiation protection and environmental protection as a whole.

Uranium mining increases environmental natural radioactivity in the mines and have a significant radiological impact in their proximity.

Environmental degradation by air pollution (especially particulate matter and ozone), noise, chemicals, decreased water quality and loss of natural areas, combined with changes in lifestyle can contribute to increases in rates of obesity, diabetes mellitus, cardiovascular and nervous systems diseases and cancer - all of which are major public health problems for people worldwide.

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