**“OVIDIUS” UNIVERSITY CONSTANTA**

**THE DOCTORAL SCHOOL OF APPLIED SCIENCES**

**FUNDAMENTAL FIELD: BIOLOGY**

# DOCTORAL THESIS

***A STUDY OF BLOOD BIOCHEMICAL MARKERS***

***IN PATIENTS DIAGNOSED WITH SCHIZOPHRENIA***

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***A STUDY OF BLOOD BIOCHEMICAL MARKERS***

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## THE GOALS AND OBJECTIVES OF THE THESIS

Existing data in the literature show that the complex pathology of schizophrenia is a very pressing burden for the individual, the family and the society and it constitutes a major public health concern. Schizophrenia is a complex mental illness with a complex etiology, characterized by profound alterations in the totality of the mental functions, by the reduction of the reality testing capacity and by a deeply negative influence on the social inclusion of the affected individual.

Schizophrenia is a major psychiatric disorder affecting the core of personality, causing severe problems of perception, cognition, affectivity and social behavior. Although schizophrenia is generally considered a functional disorder-order, it is strongly associated with neurochemical and neuroanatomical abnormalities. There is also evidence pointing to te existence of some strong genetic factors.

Schizophrenia is a severe mental disorder with the onset usually during adolescence or youth. It is a chronic psychotic syndrome, which causes fluctuating or relatively stable disturbances of behavior, thought and perception. Characteristic symptoms of schizophrenia include positive symptoms (delusions, hallucinations, disorganized speech, disorganized or catatonic behavior) and negative symptoms (affective flattening, alogia, avolition, social withdrawal).

The main objective of this research theme has been the investigation of some biochemical parameters in patients with schizophrenia which could lead to the observation of new potential relations between the neurochemical substrate and the appearance and severity of schizophrenia symptoms, relations which could provide also useful knowledge for the main objectives of the therapeutic strategy in the treatment of schizophrenia represented by:

- Improvement of symptoms.
- Restoring the patient in terms of social, vocational, and interpersonal functioning.
- Preventing suicide.

The other objectives were subordinated to this primary objective and will help to broaden the current database of biochemical imbalances caused by schizophrenia.

Thus, our research focused on six major objectives, namely:

**1)** The analysis of the levels of some enzyme biomolecules(G-SH, GR, TAS, SOD, GSH-Px), and non-enzyme biomolecules (bilirubin, uric acid, serum albumin) involved in the body defenses against the oxidative stress based - on one hand – on the idea that stress leads to increased free radicals which cause cell death or brain atrophy with cognitive impairment and memory changes and - on the other hand - the idea that some of these biomolecules are involved in the metabolism of neurotransmitters.

**2)** The analysis of some $NH_3$ related biomolecules levels. Ammonia is toxic to the body, especially the central nervous system. Consequently, the body benefits from certain biochemical possibilities which produce ammonia detoxification:

- NH3 elimination via the kidneys in the form of urea, a non-toxic compound,
- NH3 uptake by glutamic acid to form glutamine, which is retained in the body and used in other metabolic processes,
- Formation of creatine, creatine phosphate and creatinine,
- Formation of $NH_4Cl$.

In our study we analyzed the values of urea, creatine, creatinine creatine index and creatine phosphokinase.

3) The analysis of biochemical parameters of lipid metabolism - serum total lipids, total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerols (TAG), AST, ALT, LDH, GGT – with the aim to identify possible relations between quantitative changes of these parameters and some of schizophrenia's symptoms.

4) The investigation of other blood parameters, namely glucose and total proteins, biochemical markers that could be influenced by schizophrenia.

**5)** The analysis of concentrations of electrolytes: sodium, potassium, calcium, magnesium, iron, phosphorus, ionic calcium, knowing that their deficit could be involved in a number of signs and symptoms of neuropsychiatric diseases and that some psychotropic drugs can affect the concentrations of these elements.

**6)** **The identification of some statistical correlations between biochemical markers analyzed in patients diagnosed with schizophrenia,** that could cause the elaboration of some assessements, comments and conclusions on the subject of the changes of blood biochemical markers in patients diagnosed with schizophrenia.

## MATERIAL AND METHODS

For the biochemical determinations were used mainly the automated Beckman Coulter Synchron analyzers. The measurements of blood biochemical markers on these automated analyzers were performed in the clinical laboratory of The Emergency Military Hospital Constanta, following the work protocols provided by the companies producing equipment and reagents .

However, the reduced glutathione and the total creatine and creatinine were determined for some of the research included in this paper also in the biochemistry laboratory of the Faculty of Medicine, "Ovidius" University**,** in accordance with the methods described by Rosoiu and Serban, 2002; Rosoiu et al., 2005; Rosoiu, 2010.

The patients diagnosed with schizophrenia, which were 79 in number, and on ocassion divided into groups with medication and without medication, were selected from the Department of Psychiatry at "Palazu Mare" of the Constanta County Emergency Hospital in collaboration with health care professionals of that medical facility.

For the statistical processing of data resulting from measurements of biochemical markers analyzed, we used the SPSS software, namely SPSS 17.0 and SPSS 19.0.

Of the statistical tests that the software allowed us to use, we used the following:

**1. In order to test the normality of the distribution of scores** we used the statistical tests **Kolmogorov-Smirnov** and **Shapiro-Wilk**.

**2. In order to assess the equality/inequality of variances** when we were using more than one group of participants at a time – a preliminary condition for the application of other statistical tests – we used **the Levene test.**

**3. To determine the existence or non-existence of a statistically significant difference** between the results obtained for the group of participants analyzed and the average normal values known in clinical practice we used the **One-sample t-test (Student test**) .

-the application of this parametric test has as a prerequisite the existence of a normal distribution of scores obtained for the analyzed group; if there was a distribution different from the normal distribution, we used instead the nonparametric **Wilcoxon Signed Rank Test** which compares the median values of the scores obtained with the median of the normal range of scores.

**4. To measure the statistical effect size for the researches in which we used only one sample of subjects we calculated Cohen's d** coefficient as the ratio of the difference of the means(the mean for the analyzed group and the clinically normal mean value) and the standard deviation obtained. The values considered in this case to have significance as "thresholds" for statistically small, medium and respectively high effects are 0.2, 0.5 and 0.8.

**5. To determine the existence/non-existence of statistically significant differences (if the means differ significantly between them) when we used results for three groups at the same time – if were satisfied the preconditions of the existence of a normal distribution of scores in the three cases and that of homogeneity of variances - was used the One-way ANOVA parametric test.**

- **after applying the ANOVA test, a post hoc Tukey test** was also used for making all possible comparisons between the means of the three samples taken two at a time to form all possible pairs;

**-** in case of failure of the preconditions mentioned above was used **as an alternative to the ANOVA test a nonparametric test, namely the Kruskal-Wallis test.**

**6. To measure the statistic effect size after the ANOVA test previous applications for three samples we calculated the η2 coefficient as the ratio of the sum of the intergroup variances squares and the sum of total variation squares. We used as reference values/threshold values for the existence of small, medium and large statistical effects exist the values 0.001, 0.059 and 0.138 respectively.**

**7. To determine the correlations that exists between different markers studied through the reaearches we calculated the Spearman's rank correlation coefficient(Spearman rho),** which has the advantages that it is a nonparametric statistical test(so it does not require a normal distribution of scores for the groups analyzed ) and is less affected by the presence of scores that deviate strongly from the rest of the same group scores than other similar statistical tests (for example the Pearson coefficient calculation).

## RESULTS OBTAINED

### 1. Oxidative stress and antioxidant defenses in schizophrenia

a) In a first research, we investigated the values of reduced glutathione (G-SH) for a group of 12 male schizophrenic patients aged between 20 and 55 years and who received antipsychotic medication prior to the investigation.[9]

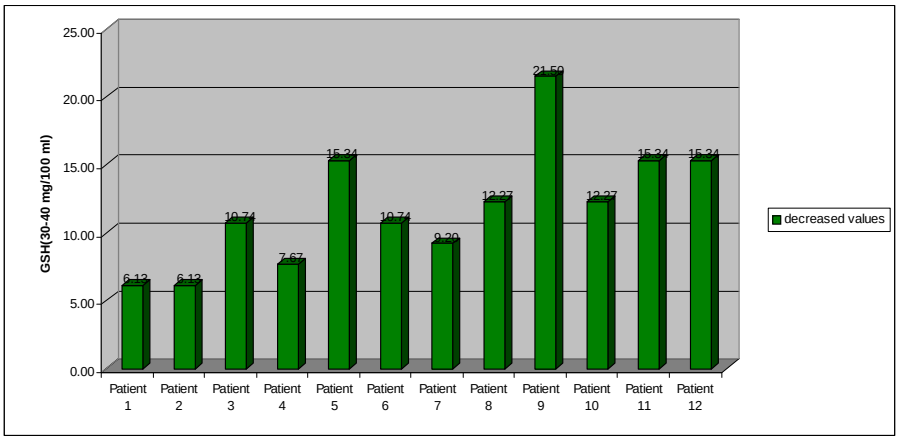



**Fig. 1 – Individual values obtained for GSH**

The statistic data obtained for GSH were: mean 11.8892, standard deviation 4.49639, $p<0.01$, $t=-17.805$, 11 degrees of freedom, $d=5.14>0.8$;

- the clinically normal mean value: 35 mg/100 ml.

**b)** In a second research devoted to this aim we selected three samples of subjects: a group of 15 schizophrenic patients who received medication, a group of 15 schizophrenic patients who did not receive medication and a control group of 15 participants. The two patient groups were composed of men and women aged between 22 and 55 years. The selected biochemical parameter for investigation in order to determine the action of the oxidative stress was in this case the reduced glutathione (G-SH).

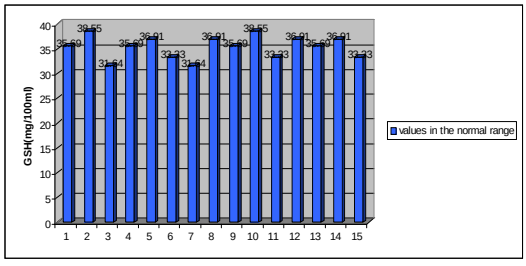



**Fig. 2 – GSH individual values – control group**

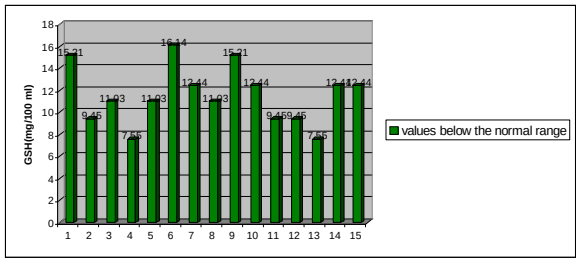



**Fig. 3 – GSH individual values - pacients without medication**

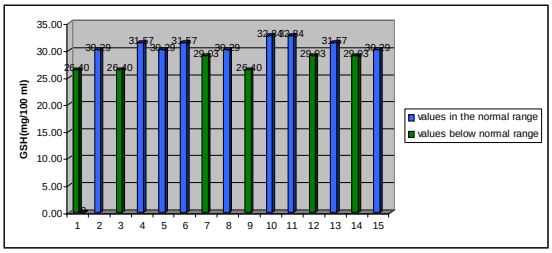



**Fig. 4 – GSH individual values - pacients with medication**

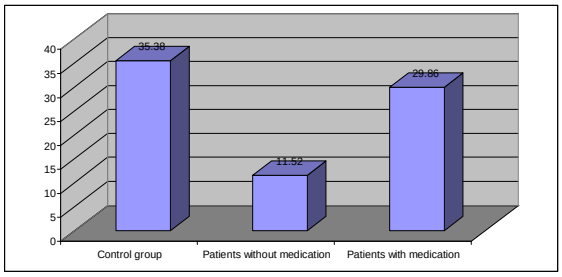



**Fig. 5 – GSH average values for the three groups**

**After applying the One-Way ANOVA test and the Tukey post hoc test, the results thus obtained revealed the fact that there were statistically significant differences between the results for the three groups and for all the possible pairs formed by these groups ($p<0.01$).**

The statistic data obtained for the three groups: mean of the control group 35.38, standard deviation 2.24; mean pacienți for the patients without medication 11.52, standard deviation 2.63, η2=0.95>0.138.

- mean for the patients with medication 29.86, standard deviation 2.16.

**c)** We chose for the following research a group of 16 male patients with schizophrenia aged between 27 and 56 years and who have received specific antipsychotic medication being treated in the Clinic of Psychiatry at Palazu Mare, Constanta County Emergency Hospital. In what regards the subject of this chapter, in the experiment were investigated in the blood values of reduced glutathione (G-SH), glutathione reductase (GR) and total antioxidant status (TAS). The Blood samples were analyzed using the automatic biochemical analyzer Beckman Coulter Synchron CX7.

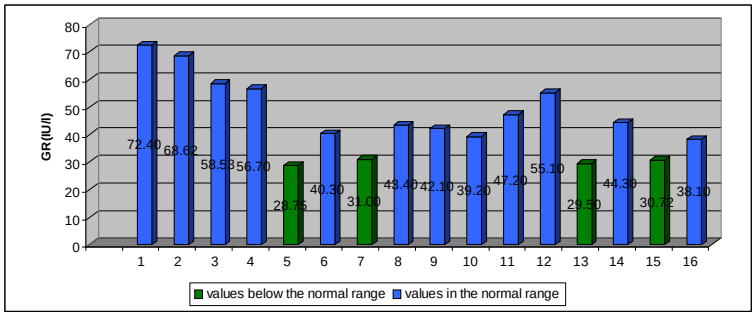



**Fig. 6 – GR individual values**

The statistic data obtained for GR: mean 45.37, standard deviation 13.547, p<0.05, t=-2.253, 15 degrees of freedom, d=0.56>0.5;

- the clinically normal mean value : 53 IU/l.

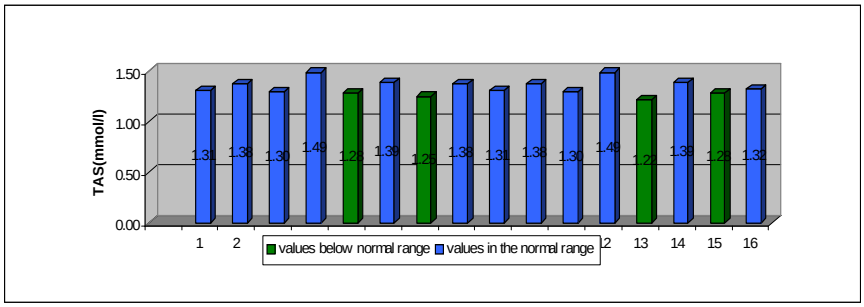



**Fig. 7 – TAS individual values**

The statistic data obtained for TAS: mean 1.3419, standard deviation 0.07739, p<0.01, t=-10.24, 15 degrees of freedom d=2.56>0.8;

- the clinically normal mean value: 1.54 mmol/l.

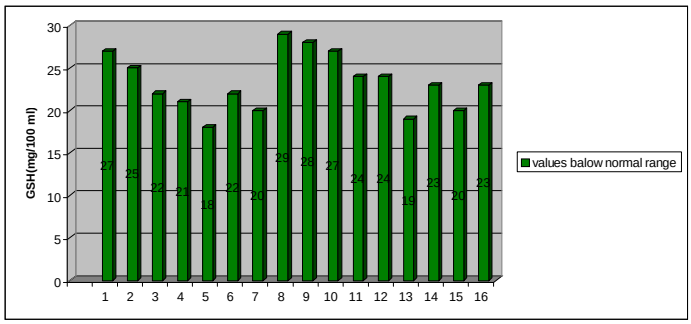



**Fig. 8 – GSH individual values**

The statistic data obtained for GSH: mean 23.25, standard deviation 3.296, $p<0.01$, $t=-14.258$, 15 degrees of freedom, $d=3.56>0.8$;

- the clinically normal mean value: 35 mg/100 ml.

d) The fourth research during which was determined the influence of oxidative stress was conducted with the participation of a group of subjects that included 5 men and 3 women aged between 40 and 68 years, all with medication in the past. The parameters chosen for this case study were total antioxidant status (TAS), uric acid, albumin (ALB), total bilirubin, direct bilirubin and indirect bilirubin.

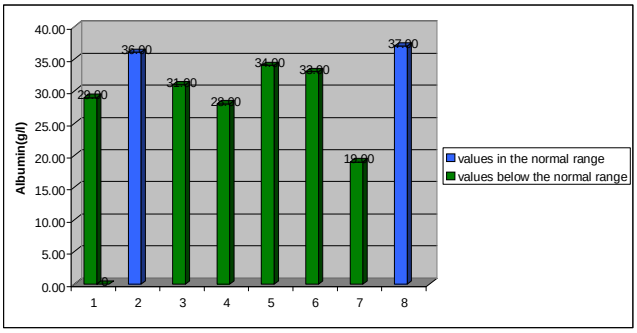



**Fig. 9 – Albumin individual values**

The statistic data obtained for albumin: mean 30.88, standard deviation 5.743, $p<0.01$, $t=-5.725$, 7 degrees of freedom, $d=2.02>0.8$;

- the clinically normal mean value: 42.5 g/l.

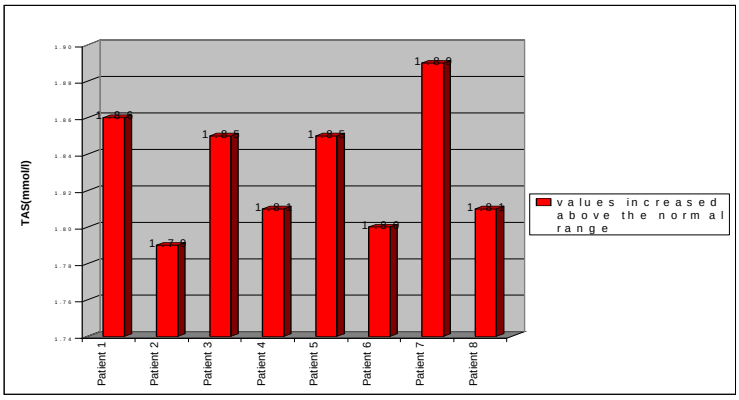



**Fig. 10 – TAS individual values**

The statistic data obtained for TAS: mean 1.8325, standard deviation 0.03495, $p<0.01$, $t=23.672$, 7 degrees of freedom $d=8.37>0.8$;

- the clinically normal mean value: 1.54 mmol/l .

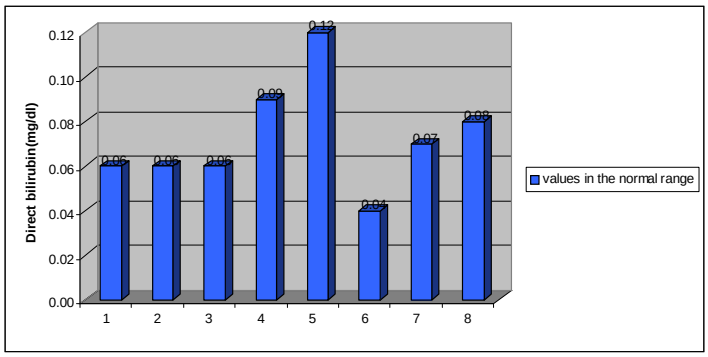



**Fig. 11 – Direct bilirubin individual values**

The statistic data obtained for direct bilirubin: mean 0.0725, standard deviation 0.02435, $p<0.05$, $t=-3.194$, 7 degrees of freedom, $d=1.13>0.8$;

- the clinically normal mean value: 0.1 mg/d.

e) In this research we investigated the possible existence of changes in the levels of two enzymes with important roles in the defensive antioxidant mechanisms, namely glutathione peroxidase and superoxide dismutase. For this purpose we selected in the case of GSH-Px a group of 11 patients, 7 women and 4 men aged between 28 and 72 years, and in the case of SOD a group of 9 patients, 5 women and 4 men aged between 21 and 72 years. Seven patients, 4 women and 3 men were part of the both groups of participants mentioned previously and in their specific case we could also investigate the existence of possible correlations between the values of the two biochemical markers(this investigation didn't found a statistically significant correlation). Patients in both groups received specific medication prior to the analyzes included in this research.

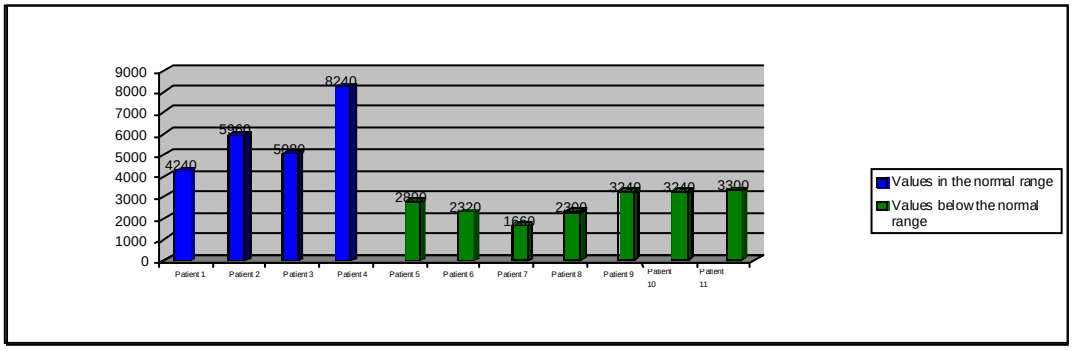



**Figura 12. GSH-Px individual values**

The statistic data obtained for GSH-Px: mean 3852.73, standard deviation, , 1925.63, p<0.001, t=-6.327, 10 degrees of freedom, d=1.91.
- the clinically normal mean value: 7526 U/l.

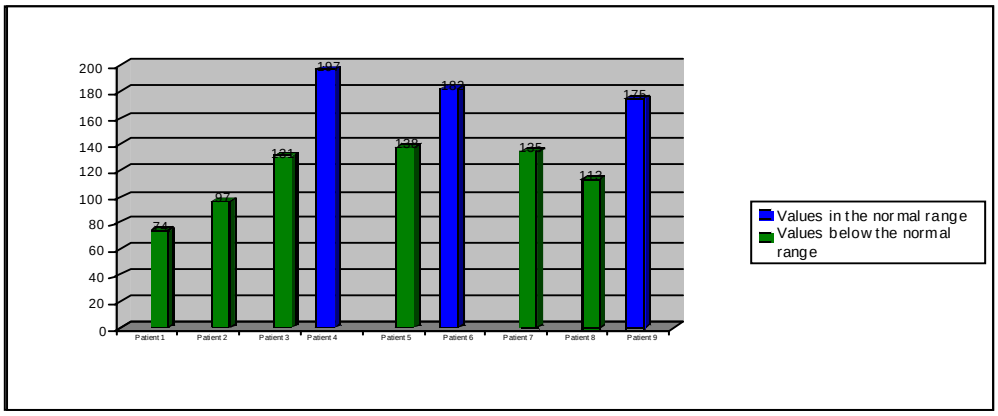



**Figura 13. SOD individual values**

The statistic data obtained for SOD: mean 138 , standard deviation 40.691, p=0.002, t=-4.718, 8 degrees of freedom, d=1.57;

- the clinically normal mean value: 202 U/l.

## 2. Ammonia metabolism in schizophrenia

a) In a first research, we investigated creatine, creatinine and creatine index for a group of 12 patients with schizophrenia, all males aged between 20 and 55 years and who received antipsychotic medication.

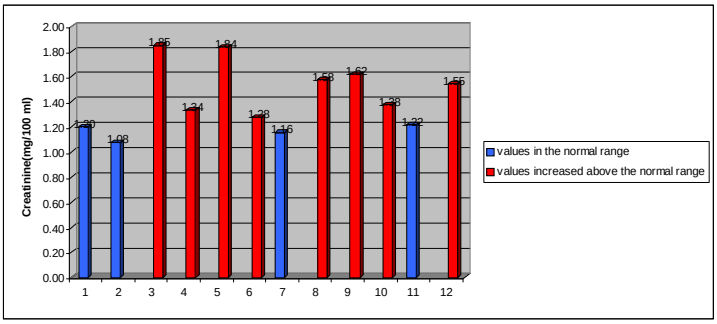



**Fig. 14 – Creatinine individual values**

The statistic data obtained for creatinine: mean 1.4212, standard deviation 0.26054, $p<0.01$, t 7.595, 11 degrees of freedom, $d=2.19>0.8$;

- the clinically normal mean value: 0.85 mg/100 ml.

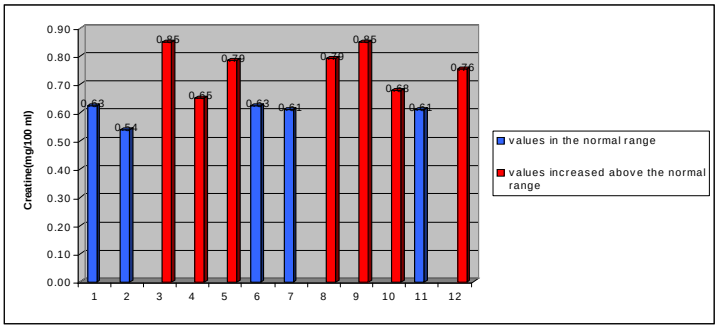



**Fig. 15 – Creatine individual values**

The statistic data obtained for creatine: mean 0.6975, standard deviation 0.10411, $p<0.01$, t=9.899, 11 degrees of freedom, $d=2.86>0.8$;

- the clinically normal mean value: 0.4 mg/ml.

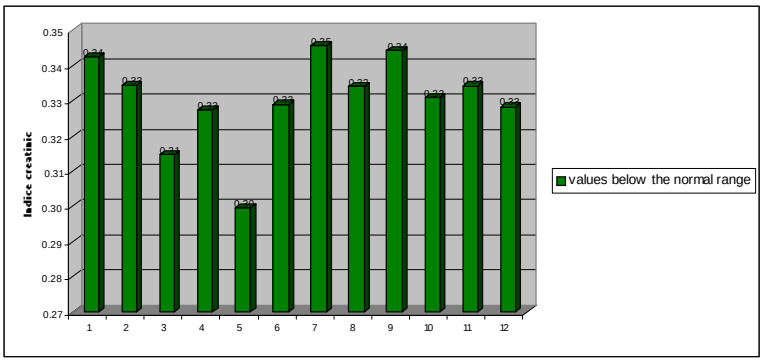



**Fig. 16 – Creatinic index individual values**

The statistic data obtained for the creatinic index(Ic): mean 0.3304, standard deviation 0.01285, $p<0.01$, t=-45.734, 11 degrees of freedom, $d=13.2>0.8$;

- the clinically normal mean value: 0.5.

b) In a second research devoted to this aim we selected three samples of subjects: a group of 15 patients with schizophrenia who received medication, a group of 15 patients with schizophrenia who did not receive medication and a control group of 15 participants . The two groups of patients were composed of men and women aged between 22 and 55 years. The biochemical parameter selected in this case for the investigation of ammonia metabolism was the creatine index (Ic).

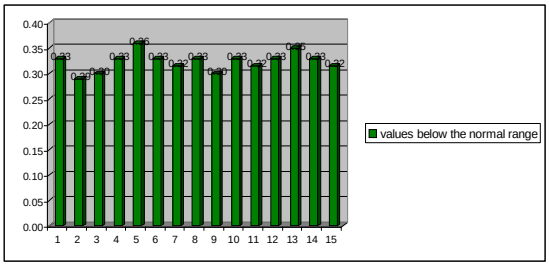



**Fig. 17 – Creatinic index individual values**

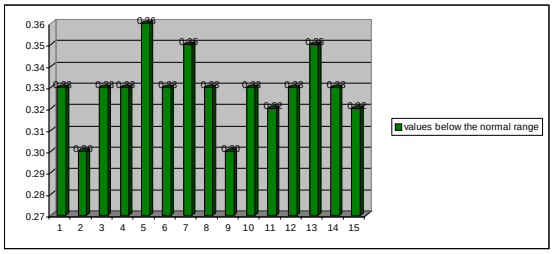



**Fig. 18 – Creatinic index individual values – patients with medication**

**– patients without medication**

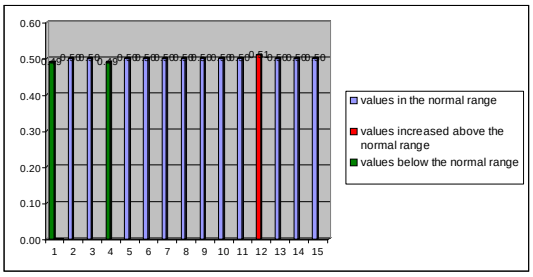



**Fig. 19 – Creatinic index individual values - control group**

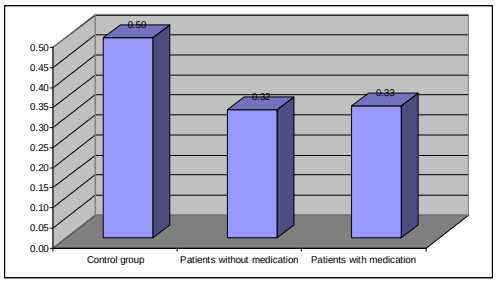



**Fig. 20 – Creatinic index mean values for the three groups**

It was chosen in this case the non-parametric Kruskal-Wallis test as an alternative to ANOVA test. The result is the existence of significant differences between the results of the three groups (H (2)=31.487, p <0.01) with an average rank of 38 for the control group, one of 14.1 for the group of patients without medication and one of 16.9 for the patients with medication.
The statistic data obtained for the three groups: the mean of the control group 0.5, standard deviation 0.005; the mean for the patients without medication 0.3237 standard deviation 0.018; the mean for the patients with medication 0.3293, standard deviation 0.016.

**c)** We chose for the following research a group of 16 male patients with schizophrenia aged between 27 and 56 years and who have received specific antipsychotic medication while being treated in the Clinic of Psychiatry at Palazu Mare, Constanta County Emergency Hospital. In what regards the the objective approached here, in that experiment were investigated in the blood values of creatine, creatinine, creatine phosphokinase and creatinic index.

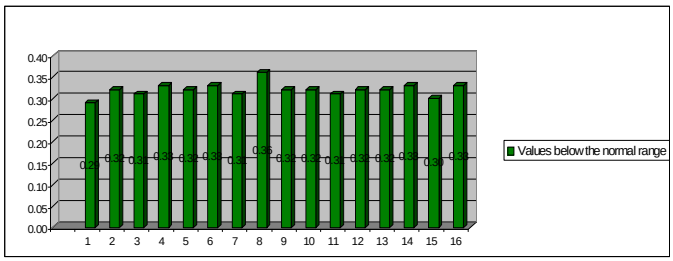



**Fig. 21 – Creatinic index individual values**

The statistic data obtained for the creatinic index: mean 0.32, standard deviation 0.01549, p<0.01, t=-46.476, 15 degrees of freedom, d=11.62>0.8;

- the clinically normal mean value: 0.5.

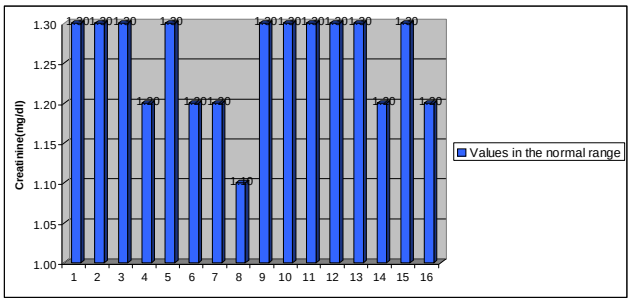



**Fig. 22 – Creatinine individual values**

The statistic data obtained for creatinine: mean 1.256, standard deviation 0.629, $p<0.05$(One Sample Wilcoxon Signed Rank Test);

- the clinically normal mean value: 0.95 mg/dl.

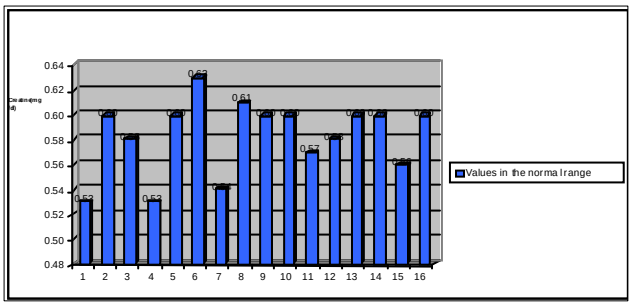



**Fig. 23 – Creatine individual values**

The statistic data obtained for creatine: mean 0.5831, standard deviation 0.0296, $p<0.05$(One Sample Wilcoxon Signed Rank Test);

- the clinically normal mean value: 0.42 mg/dl.

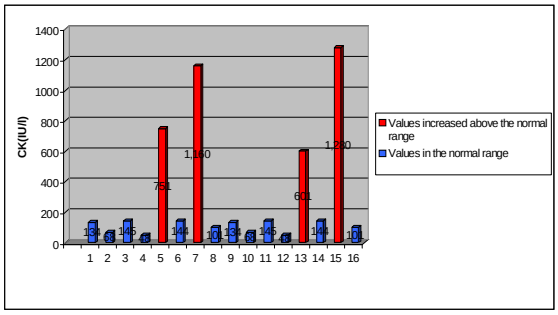



**Fig. 24 –Creatine phosphokinase individual values**

The statistical data obtained for creatine phosphokinase: the mean 317 IU / l, standard deviation 404.563, $p> 0.05$ (it was not obtained in this case a statistically significant difference from the normal average value). In this particular case the statistics were strongly influenced (the standard deviation was even higher than the mean) by the existence of four individual cases characterized by very high levels of creatine, this biochemical marker being known as presenting big increases especially in cases of adverse reactions to the antipsychotic medication . [2][14]

d) The fourth research in which was determined the influence of the ammonia metabolism was conducted with the participation of a group of subjects that included 5 men and 3 women aged between 40 and 68 years, all with medication. The parameters for study chosen in this case were creatine phosphokinase, creatinine and urea.

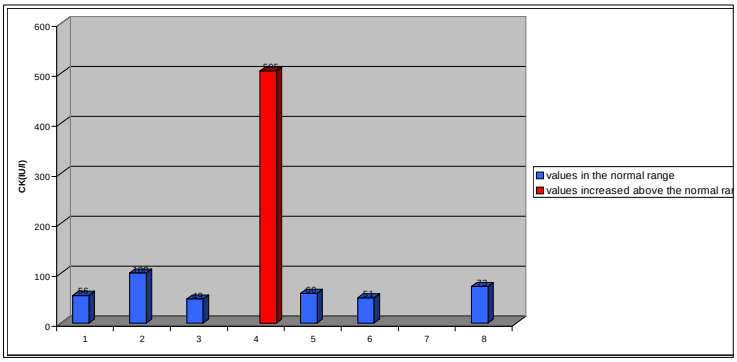



**Fig. 25 - Creatine phosphokinase individual values**

The statistical data obtained for creatine phosphokinase were: the mean 114.25 IU / l, standard deviation 159.493, $p > 0.05$. (it was not observed in this case a statistically significant difference from the normal mean value). And in this particular case the statistical data were strongly influenced (the standard deviation being higher than the mean) by the existence of an individual case characterized by a very high level of creatine, which is known to be a possible indicator of an adverse reaction to the antipsychotic medication .

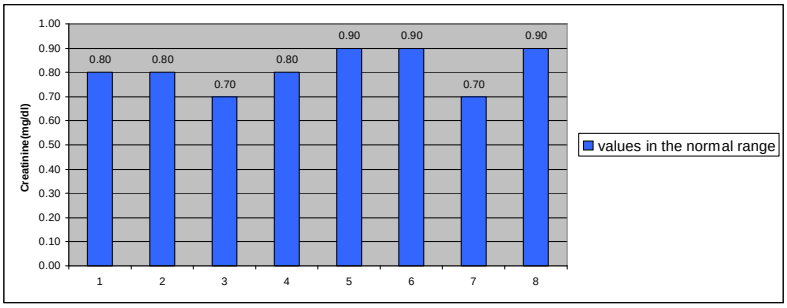



**Fig. 26 – Creatinine individual values**

The statistic data obtained for creatinine were: mean 0.8125, standard deviation 0.08345, $p<0.01$, $t=-4.66$, 7 degrees of freedom, $d=1.65>0.8$;

- the clinically normal mean value: 0.95 mg/dl.

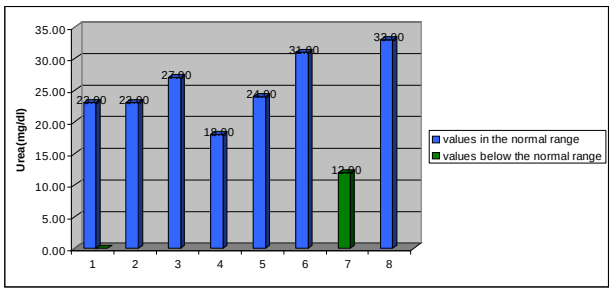



**Fig. 27 – Urea individual values**

The statistic data obtained for urea were: mean 23.8750, standard deviation 6.77047, $p<0.01$, $t=-3.603$, 7 degrees of freedom, $d=1.27>0.8$;

- the clinically normal mean value: 32.5 mg/dl.

### 3. Lipid profile and potential liver damage in schizophrenia

a) The first research which aimed to determine the values of markers of the liver activity was carried out with the participation of a group of 16 patients suffering from schizophrenia, all males aged between 27 and 56 years who have received specific antipsychotic medication while being treated in the Clinic of Psychiatry at Palazu Mare, Constanta County Emergency Hospital. In what regards the objective approached here, in that experiment were investigated the blood values of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and gamma glutamyl transpeptidase (GGT). The blood samples were analyzed using the automatic biochemical analyzer Beckman Coulter Synchron CX7.

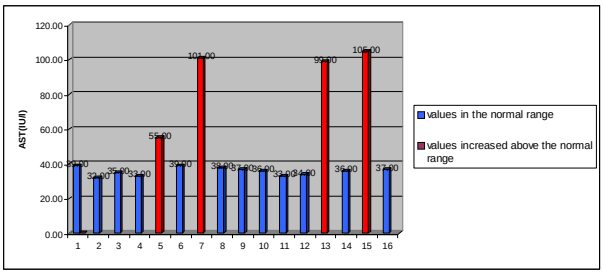



**Fig. 28 – AST individual values**

The statistic data obtained for AST were: mean 49.31, standard deviation 26.512, p<0.01(Wilcoxon Signed Rank Test), d=0.97>0.8;

- the clinically normal mean value: 23.5 IU/l.

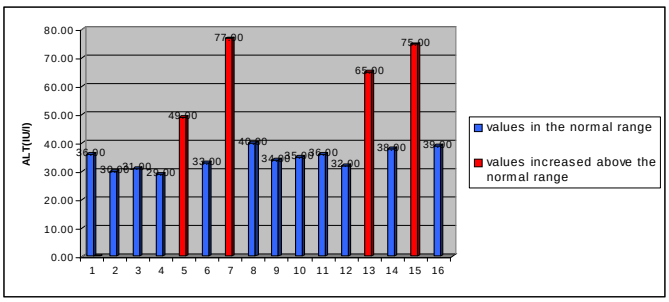



**Fig. 29 – ALT individual values**

The statistic data obtained for ALT were: mean 42.44, standard deviation 15.744, p<0.01(Wilcoxon Signed Rank Test), d=1.27>0.8;

- the clinically normal mean value: 22.5 IU/l.

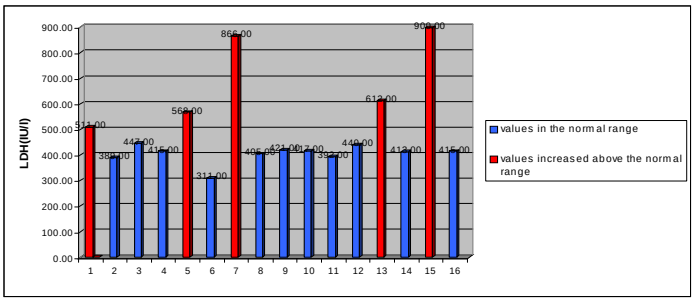



**Fig. 30 – LDH individual values**

The statistic data obtained for LDH were: mean 495.13, standard deviation 167.562, p<0.01(Wilcoxon Signed Rank Test), d=0.67>0.5;

- the clinically normal mean value: 383 IU/l.

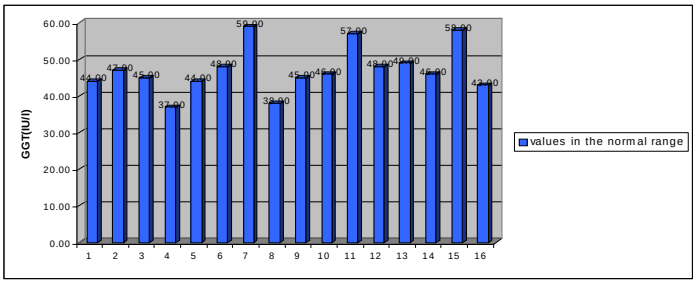



**Fig. 31 – GGT individual values**

The statistic data obtained for GGT were: mean 47.13, standard deviation 6.302, $p<0.01$, $t=7.378$, 15 degrees of freedom, $d= 1.85>0.8$;

- the clinically normal mean value: 35.5 IU/l.

There were also calculated the values of the De Ritis ratio(AST / ALT) for the patients participating in the study, two patients having increased values of this ratio.

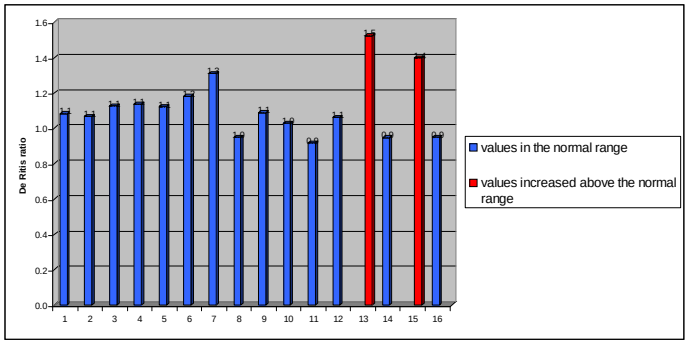



**Fig. 32 – De Ritis ratio individual values**

b) The second research in which were studied the markers of hepatic activity and also the lipid profile was conducted with the participation of a group of subjects that included 5 men and 3 women aged between 40 and 68 years, all with medication. The parameters chosen for this study were aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and total serum lipids. [11]

In what regards total cholesterol, triglycerides, total lipids and LDH, there were not obtained statistically significant differences from the normal mean values.

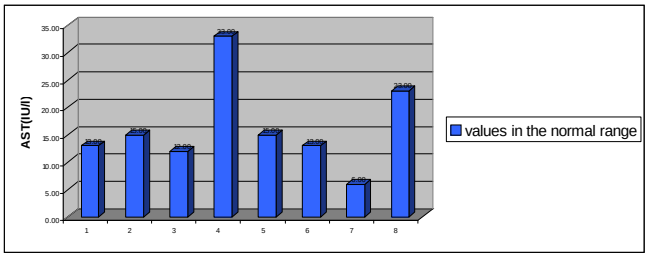



**Fig. 33 – AST individual values**

The statistic data obtained for AST were: mean 16.25, standard deviation 8.22453, $p<0.05$(testul Wilcoxon), $d=0.88>0.8$;

- the clinically normal mean value: 23.5.

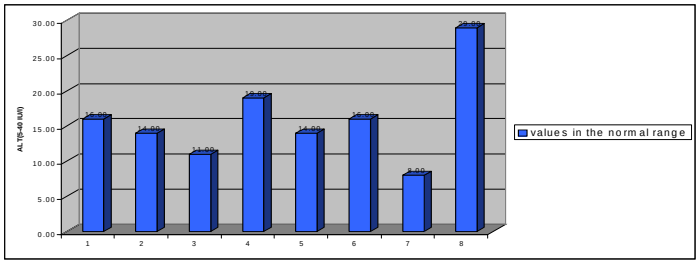



**Fig. 34 – ALT individual values**

The statistic data obtained for ALT were: mean 15.8750, standard deviation 6.26641, t=-2.99, 7 degrees of freedom, p<0.05, d=1.06>0.8;

- the clinically normal mean value: 22.5.

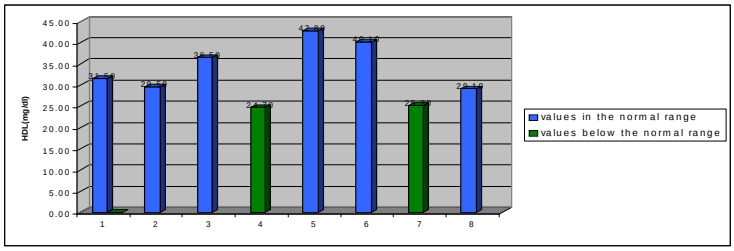



**Fig. 35 – HDL individual values**

The statistic data obtained for HDL were: mean 32.4250, standard deviation 6.71347, p<0.01, t=-7.404, 7 degrees of freedom, d=2.61>0.8;

- the clinically normal mean value: 50 mg/dl.

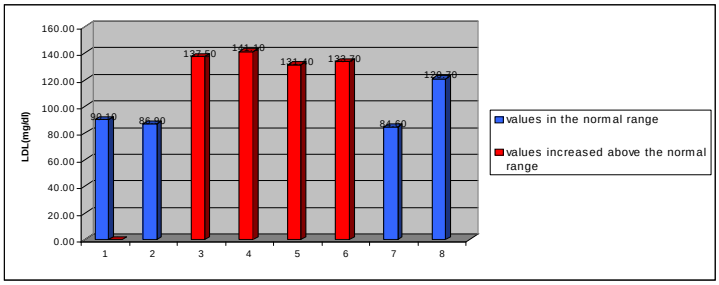



**Fig. 36 – LDL individual values**

The statistic data obtained for LDL were: mean 115.75, standard deviation 24.40105, p<0.05(Wilcoxon test),d=1.75>0.8 ;

- the clinically normal mean value: 75 mg/dl.

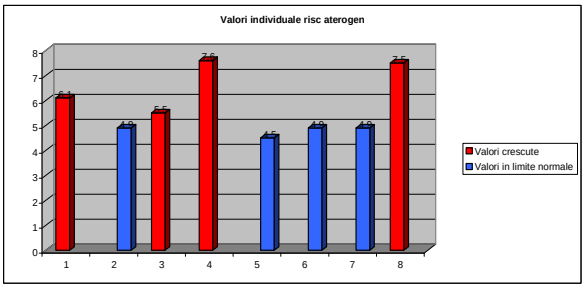



**Fig. 37 – Atherogenic risk individual values**

The statistic data obtained for the atherogenic risk were: mean 5.7375, standard deviation 1.21883, p<0.01, t=7.513, 7 degrees of freedom;

- normal range of values: 0-5.

There were also calculated the values of the De Ritis ratio(AST / ALT) for the 8 patients and it was obtained a level higher than normal for one of these patients:

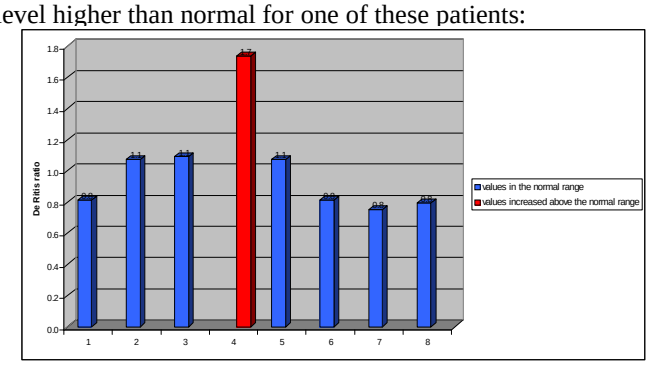



**Fig. 38 – De Ritis ratio individual values**

## 4. Other blood biochemical markers in patients with schizophrenia

We thought to be useful also the performance of a blood glucose a total protein determinations, in order to investigate if these biochemical markers could show some changes in this disease.
a) In a first research, we investigated the blood glucose levels for a group of 12 patients with schizophrenia, all males aged between 20 and 55 years and who received medication.

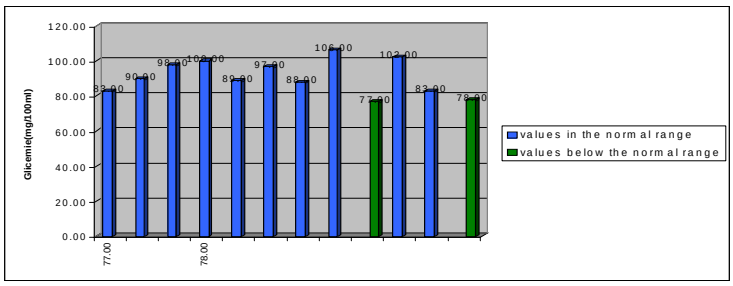



**Fig. 39 – Glycemia individual values**

The statistic data obtained for glicemia were: mean 90.9167, standard deviation 9.62439, $p<0.01$, $t=-3.269$, 11 grade, $d=0.94>0.8$;

- the clinically normal mean value: 100 mg/100 ml.

b) In a research in which participated a group of subjects that included 5 men and 3 women aged 40 to 68 years, among the biochemical parameters investigated on this occasion was also included the total serum protein value.

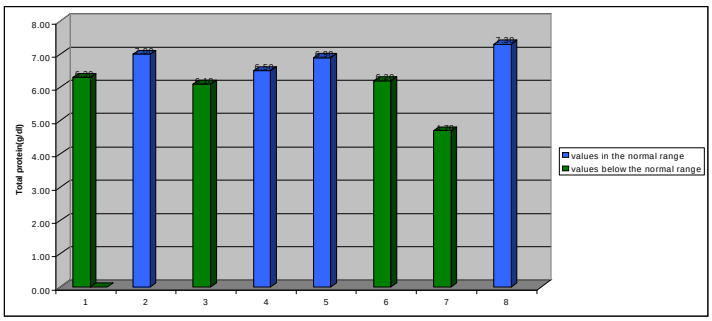



**Fig. 40 – Total proteins individual values**

The statistic data obtained for total proteins were: mean 6.3750, standard deviation 0.79776, $p<0.05$, $t=-3.457$, 7 degrees of freedom, $d=1.22>0.8$;

- the clinically normal mean value: 7.35 g/dl.

## 5. Evaluation of blood electrolytes(Na, K, Ca, ionic Ca, Mg, Fe, $PO_4^{3-}$) in schizophrenia

We conducted an evaluation of the blood electrolytes during a research carried out with the participation of a group of 8 patients with schizophrenia, 5 men and 3 women aged between 40 and 68 years and who received specific antipsychotic medication while being treated in the Clinic of Psychiatry at Palazu Mare, Constanta County Emergency Hospital. The electrolytes investigated on this occasion were serum sodium, serum potassium, serum calcium, serum ionized calcium, serum magnesium, serum iron(sideremia) and serum phosphate ($PO_4^{3-}$). The blood samples were analyzed using the automatic biochemical analyzer Beckman Coulter Synchron CX7. [12]

The serum phosphate results obtained were not significantly different from normal mean values.

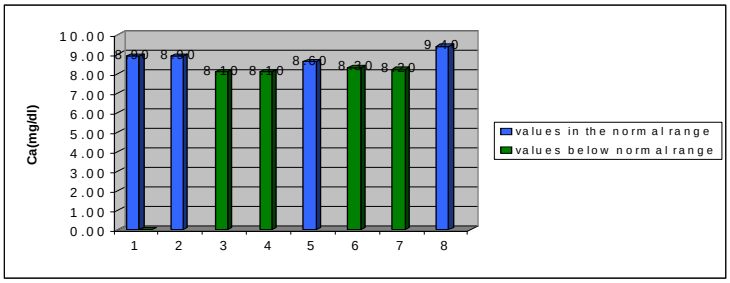



**Fig. 41 – Serum calcium individual values**

The statistic data obtained for serum calcium were: mean 8.563, standard deviation 0.4719, $p<0.01$, t=-5.320, 7 degrees of freedom, d=1.88>0.8;

- the clinically normal mean value: 9.45 mg/dl.

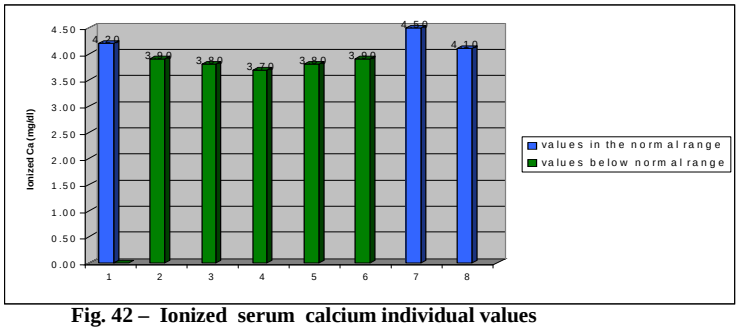



**Fig. 42 – Ionized serum calcium individual values**

The statistic data obtained for ionized serum calcium were: mean 3.988, standard deviation 0.2642, $p<0.01$, t=-6.556, 7 degrees of freedom, d=2.32>0.8;

- the clinically normal mean value: 4.6 mg/dl.

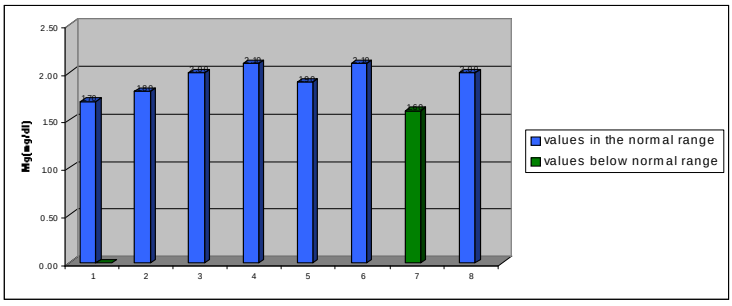



**Fig. 43 – Serum magnesium individual values**

The statistic data obtained for serum magnesium were: mean 1.9, standard deviation 0.1852, $p<0.01$, t=-5.346, 7 degrees of freedom, d=1.89>0.8;

- the clinically normal mean value: 2.25 mg/dl.

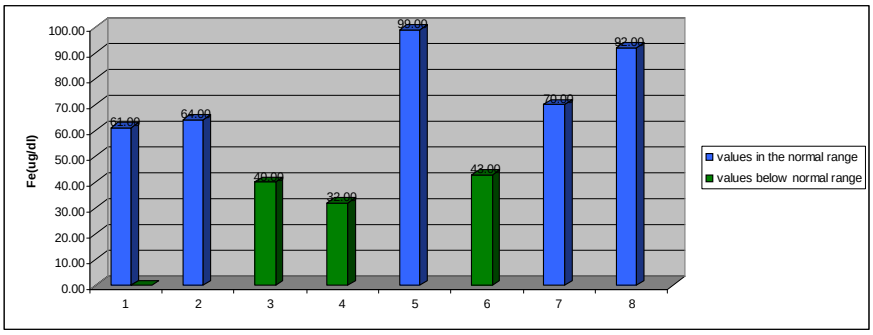



**Fig. 44 – Serum iron individual values**

The statistic data obtained for serum iron: mean 62.63, standard deviation 24.142, $p<0.01$, $t=-5.843$, 7 degrees of freedom, $d=2.07>0.8$;

- the clinically normal mean value: 112.5 µg/dl.

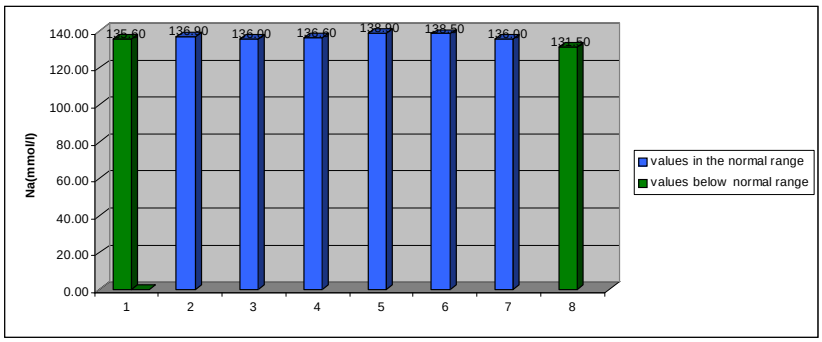



**Fig. 45 – Serum sodium individual values**

The statistic data obtained for serum sodium: mean 136.2, standard deviation 2.2596, $p<0.01$, $t=-5.320$, 7 degrees of freedom, $d=1.88>0.8$;

- the clinically normal mean value: 140.5 mmol/l.

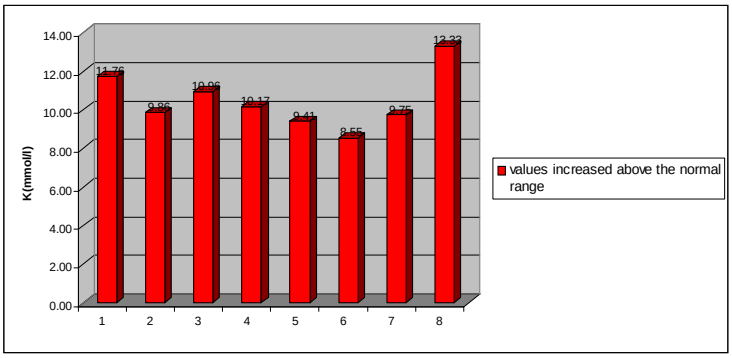



**Fig. 46 – Serum potassium individual values**

The statistic data obtained for serum potassium: mean 10.4737, standard deviation 1.50590, $p<0.01$, $t=11.596$, 7 degrees of freedom, $d=4.1>0.8$;

- the clinically normal mean value: 4.3 mmol/l.

**6. Correlations between biochemical markers evaluated in patients diagnosed with schizophrenia**

In order to determine the existence of possible correlations between different markers that were addressed until now in this study separately under each particular objective, correlations that could provide useful information, we have chosen that the statistical test which was to be applied to the scores obtained for the groups analyzed Spearman rank correlation coefficient(rho), a nonparametric test that could be applied also in those cases where a particular marker distribution of scores obtained differed significantly from the normal distribution or when there were individual scores with values much different from most of the values for the same indicator in the same group .

a) In one of the researches conducted to develop this thesis we got the participation of a group of 16 male patients with schizophrenia aged between 27 and 56 years who have received specific antipsychotic medication while being treated in the Clinic of Psychiatry at Palazu Mare, Constanta County Emergency Hospital. We analyzed on this occasion a broader range of biochemical markers of antioxidant defense mechanisms, of ammonia metabolism and of the liver functions(all of these presented in detail previously for which category in turn), and we also investigated the existence of possible correlations between the markers belonging to the different categories listed.

A particular case was that of the scores obtained for serum creatine phosphokinase (CK) and of the correlations that we obtained for this marker with two markers related to the antioxidant defense mechanisms, namely Total antioxidant status (TAS) and reduced glutathione(G -SH). In these two cases we obtained values of Spearman coefficient of -0.830 at $p < 0.01$ and -0.618 respectively at $p < 0.05$. If these values are taken into account alone, this would indicate a significant negative correlation between serum creatine phosphokinase on one hand and TAS and, respectively, reduced glutathione on the other hand.

In the scatter plots, however, it can be seen that in each of these two cases in the group of patients are present actually two different subgroups, one of which consists of four patients who had very high creatine phosphokinase(possibly as an effect of the medication) and the other of patients with quite different TAS and GSH values but without too big differences in the CK levels .

We applied the Spearman correlation test also for TAS and the creatinic index. The result is a value of the Spearman coefficient of 0.622 at $p < 0.05$. In this case from the scatter plot was not visible the existence of two very different populations within the same group as in the cases mentioned previously, but the correlation obtained didn't appear to be quite linear.

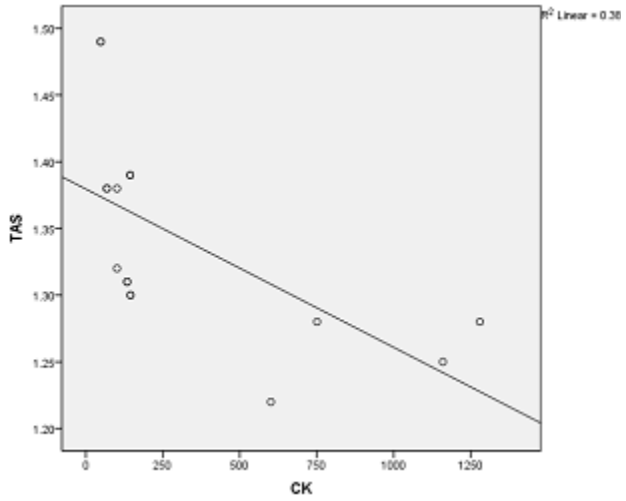



**Fig. 47 –CK-TAS correlation**

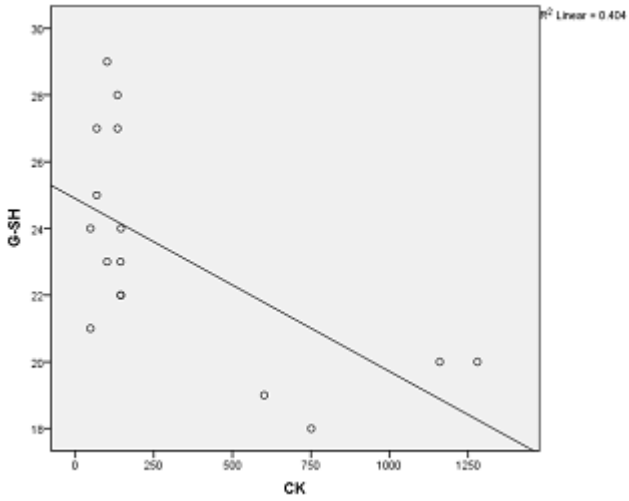



**Fig. 48–CK – GSH correlation**

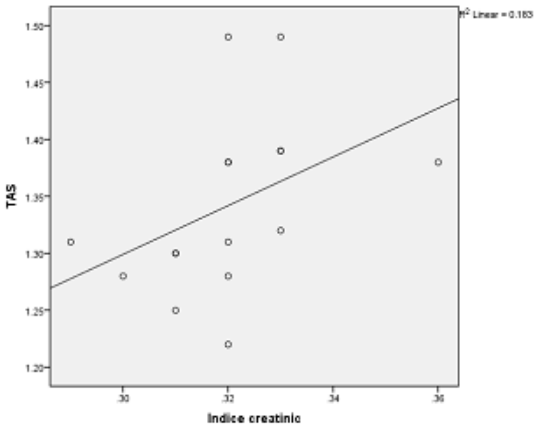



**Fig. 49 –TAS- Creatinic index correlation**

We also calculated the Spearman coefficient for creatinic index and LDH, in order to investigate the existence of possible correlations between ammonia metabolism and potential tissue damage(including hepatic damage) in patients suffering from schizophrenia, resulting a rho coefficient value of -0.610 at $p < 0.05$ (which would indicate a negative correlation between LDH level and the creatinic index, sugesting that any potential liver injury, was associated with decreases in creatinine index, index that was much lower than normal in all patients investigated by us). The scatter plot also showed that the correlation found was not a clearly linear one.

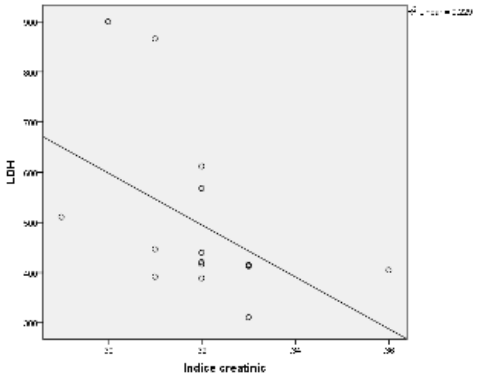
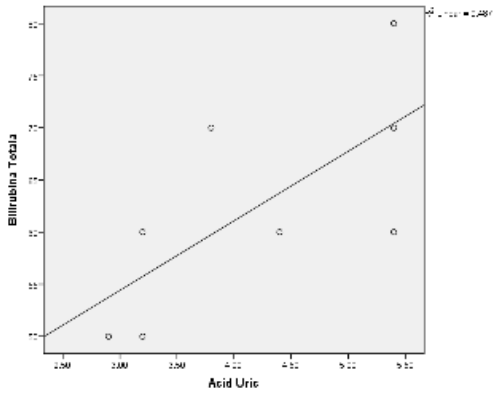



**Fig. 50 –LDH-Creatinic index correlation**

From the results presented above we could conclude that it might be useful to further investigatie in patients with schizophrenia the existence of such correlations between the antioxidant defense mechanisms and the ammonia metabolism in one hand, and between the ammonia metabolism and the indicators of the liver functionality on the other hand, but using some patient groups much larger than those used in the researches presented in this thesis.

**b)** In order to investigate the existence of possible and not yet properly investigated links between oxidative stress, lipid metabolism and the protein metabolism, we determined the values of a wider range of blood biochemical compounds for a group of 8 patients with schizophrenia(the results for each category of the compounds were presented previously). The group of subjects included 5 men and 3 women aged 40 to 68 years old, all with medication.

Firstly, some strong positive correlations were observed between non-enzymatic antioxidant factors. Thus, for indirect bilirubin and albumin was obtained a Spearman coefficient value of 0.932 at $p < 0.01$, and for uric acid and total bilirubin a Spearman coefficient of 0.732 at $p < 0.05$. The scatter plots did not show the presence of very linear correlations, but these presented though a tendency close to linearity. Such positive correlations between specific markers of the same overall broader antioxidant defense mechanisms may have some relevance on the overall condition of that set of mechanisms and may indicate that the entire antioxidant defense system is affected in schizophrenia.



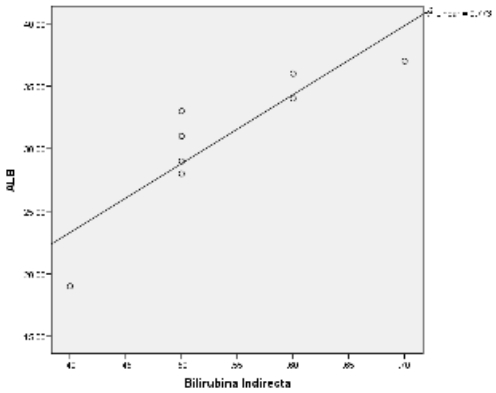




**Fig. 51 – Uric Acid-Total Bilirubin correlation** **Fig. 52 – Albumin-Indirect Bilirubin correlation**

We also investigated the possible existence of correlations between ammonia metabolism and energy metabolism on the one hand and protein metabolism, on the other hand, by calculating the Spearman correlation coefficient for total serum proteins and creatine phosphokinase. The result was a 0.833 value of the rho coefficient for $p < 0.05$. After completing the scatter plot was visible a correlation close to a linear one with the exception of a single case where a patient presented with a very big increase of creatine phosphokinase level(as mentioned previously above, such large increases can occur in patients with schizophrenia as a result of adverse reactions to the antipsychotic medication taken). The observed relationship might indicate some increases in protein synthesis associated if an increase of the available energy as a result of increases in creatine phosphokinase levels is present, and vice versa.

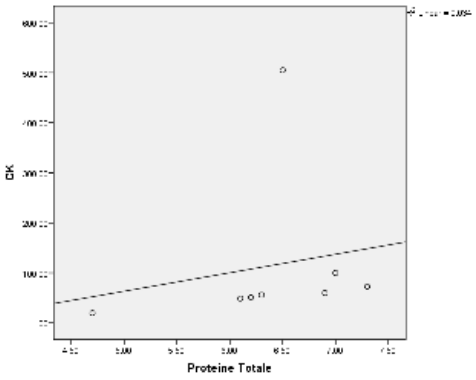



**Fig. 53 – Total Proteins – CK correlation**

We obtained also some interesting results on the correlations between the lipid metabolism and the blood levels of some electrolytes in the group of patients with schizophrenia investigated. Thus was obtained a Spearman coefficient value of 0.738 for HDL cholesterol and serum phosphate at $p < 0.05$.The scatter plot indicates a positive correlation pretty close to a linear one between the two indicators mentioned. For LDL cholesterol and serum magnesium was obtained a Spearman coefficient value of 0.88 at $p < 0.01$, the scatter plot indicating the existence of a positive correlation close to a linear one. For calcium ion and LDL was obtained a negative correlation indicated by a Spearman coefficient value of -0.843 at $p < 0.01$ and the scatter plot showed a correlation pretty close to a linear one.

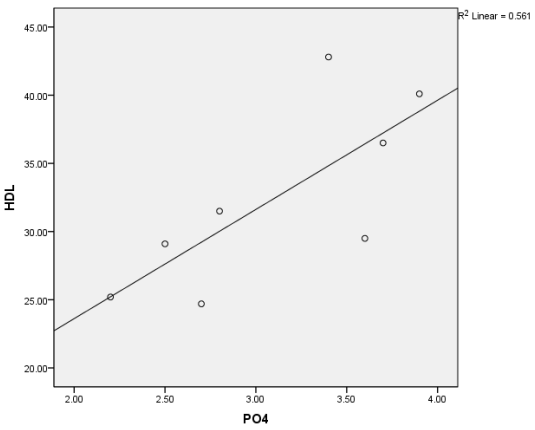



**Fig. 54 –HDL-$PO_4$ correlation**

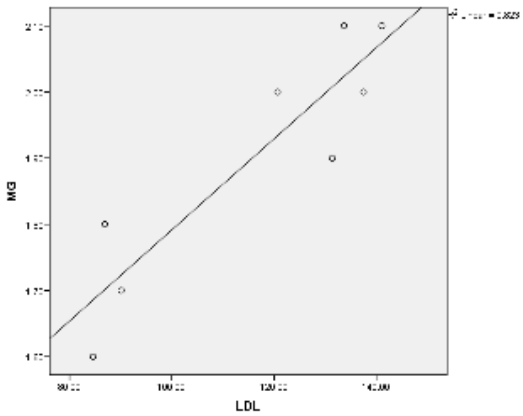



**Fig. 55 –Mg-LDL correlation**

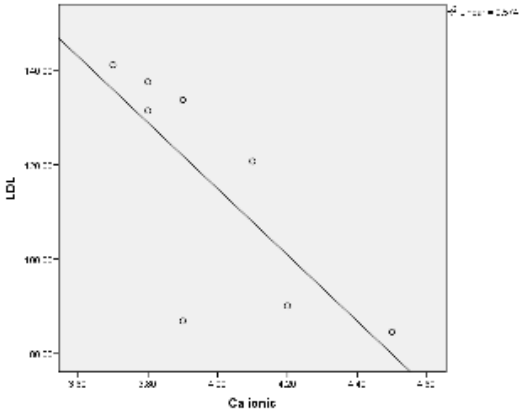



**Fig. 56 – LDL-Ca ionic correlation**

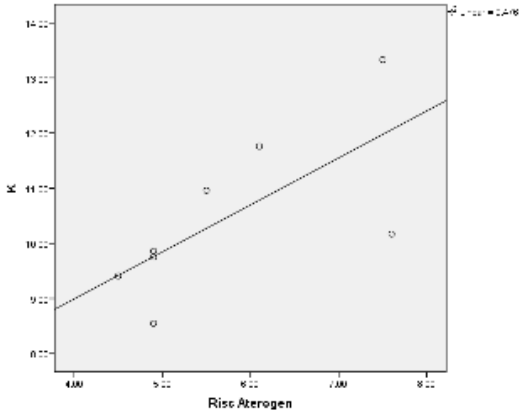



**Fig. 57 - K-Atherogenic risk correlation**

We calculated the Spearman coefficient for serum potassium and the value calculated for the atherogenic risk. The result is a Spearman coefficient value of 0.781 at $p < 0.05$ and the scatter cloud shows a positive correlation pretty close of a linear one.

## *DISCUSSION AND CONCLUSIONS*

From the analysis of data obtained in the 6 major objectives of our study, we can draw the following assessments, comments and conclusions:

**1) The oxidative stress and antioxidant defense in schizophrenia**

Regarding the oxidative stress and the fight against it, the people with schizophrenia presents particular big magnitude dysfunctions of those antioxidant defense mechanisms that use reduced glutathione as a cofactor. This implies an increased vulnerability to both the harmful activity of $H_2O_2$ and to the risks of the lipid peroxidation, the effects of the latter being observed in schizophrenia[8]. And this is particularly important in the case of such a disease for which the functionality of the neuronal membranes plays a crucial role, these membranes being very rich in lipids containing unsaturated fatty acids that are very vulnerable to peroxidation.

The very low level of glutathione observed in patients suffering from schizophrenia that participated in in three of the researches conducted by us indicate the presence of major disturbances in the mechanisms that the body uses to neutralize the negative impact of free radicals/reactive oxygen species and in particular that of $H_2O_2$, compounds that are formed during normal or accidental metabolic processes, primarily in the mitochondria and that lead to the emergence of the "oxidative stress" and accelerate degenerative morpho-functional processes[3] that can speed up the aging process with negative consequences at the brain level.

Since reduced glutathione is the main non-protein antioxidant and plays a critical role in protecting the cell from damage caused by reactive oxygen species at all levels, reduced glutathione deficiency could lead inclusively to neural membranes peroxidation and the emergence of lesions around the dopaminergic terminals microlesions around, resulting a loss of synaptic connectivity.

Our research results, however, seem to indicate that the specific pharmacological treatment applied to patients with schizophrenia may be associated at least in some cases with an improvement of the situation of reduced glutathione and a return to near-normal values. The very significant decrease in reduced glutathione observed repeatedly our researches contrasts to some extent with a more moderate decrease of glutathione reductase observed in one of the research, a situation which suggests that the decrease of glutathione seems to be less the result of a possible deficitary activity of oxidized glutathione reduction and rather seems to be the effect of an accelerated oxidation due to free radicals that are in excess.

The results obtained for TAS (total antioxidant status) in two of our investigations were contradictory, not allowing us to draw a conclusion in this case.

In what regards other two important enzymes involved in the antioxidant defense mechanisms, namely glutathione peroxidase and superoxide dismutase, our results during one of the researches presented in this paper show very marked decreases in the levels of both these enzymes, similar results being obtained by other researchers[1] (there are also other researches in which it was observed an increase of the superoxide dismutase related to the positive symptoms of schizophrenia[6]). This makes it quite evident the existence of significant decreases in the defense capacity of the body against the harmful action of free radicals.

The results obtained for albumin and direct bilirubin are part of the same pattern of a marked decrease of the concentrations of the antioxidant compounds, which may indicate an exhaustion of the anti-oxidant defense mechanisms after strong activity of the free radicals.

Thus, the data obtained reveal the fact that **in schizophrenia is visible a marked decrease of some key components of the antioxidant defense system**. Although the results for TAS were contradictory, the repeated results indicating a strongly reduced glutathione as also those obtained for glutathione reductase, glutathione peroxidase, superoxide dismutase, albumin and direct bilirubin, indicate **that the schizophrenia sufferers antioxidant defenses are clearly deficient**. We can conclude that, quite clearly, **oxidative stress show a strong enough aggravation in people with schizophrenia.**

**2) The ammonia metabolism in schizophrenia**

The imbalance observed between creatine and creatinine and indicated by **the low levels of creatinic index** in the schizophrenic patients (both in patients suffering from schizophrenia who received medication as well as of those without medication – the creatine index value seems to be very little influenced by specific medication) could indicate the fact that in people with schizophrenia creatine dehydrate quickly, producing creatinine faster by cyclization at the expense of the the normal conversion to creatine phosphate. This leads to the idea of the existence

of both possible disturbances of energy metabolism and also to the possible existence of major imbalances in ammonia detoxification processes of the body (ammonia being a result of the nitrogen metabolism). These disturbances can lead to damage of brain tissue, the brain being one of the organs most affected in case of a possible state of hyperammonemia.

The very elevated creatine phosphokinase values observed in the case of some of the patients contrast sharply with the values for the rest of the patients in the groups to which they were part, and this may be a potential indicator of some particular individual adverse reactions to specific medications(that this can happen in some cases is a fact long known and mentioned inclusively in DSMIV). Even statistically was apparent that in this case there were differences inside the groups between individual patients with regard to the this major biochemical marker.

**Urea decreased activity can mean a reduction of one normal pathways for detoxification of ammonia** through the synthesis of urea as a final product.

**3) Lipid profile and liver disfunctions in schizophrenia**

In the two studies in which we analyzed markers of the hepatic activity(AST, ALT, LDH, GGT) we can see that they have led to contradictory results, so we can not draw a clear conclusion about a possible impaired liver function in patients suffering from schizophrenia.

The data obtained in one of the studies in what regards the lipid profile indicated results that do not differ statistically significantly from normal mean values for a range of indicators such as total cholesterol, triglycerides and total lipids. **But there were also obtained results that indicated a statistically significant decrease of HDL cholesterol from normal mean values, an increase in LDL cholesterol and an increase in atherogenic risk** as compared to normal mean values, but the average age of the patients in this particular group of participants was 54 years, being high enough so that age could have also constitute an important factor in determining such changes. However, we believe that this aspect deserves further study in the future.

**4) Other blood biochemical markers in patients with schizophrenia**

In two of the studies conducted we studied blood glucose and serum total protein. The results indicate some disturbance of the metabolism of carbohydrates and protein, but at least for glucose we think that a possible factor could be not so much the disease itself as a lifestyle disruption caused by it. People with schizophrenia usually have strong functional socio-economic disturbances with effects which may include poor personal hygiene and poor nutrition.

All the patients in the two studies mentioned above received specific medication, antipsychotic medication (especially including atypical antipsychotics) being sometimes accused that under certain conditions may favor the development of diabetes. Our results for glucose appear to indicate that the existence of such complications could had been present in the patients who participated in these studies.

**5) The evaluation of blood electrolytes (Na, K, Ca, ionized Ca, Mg, Fe, $PO_4^{3-}$) in schizophrenia**

In one of our researches we have included the analysis of the electrolyte concentrations mentioned above. We chose to study these markers also beacause we were aware of the existence of a previous body of research whose authors studied these markers at the blood level in relation with the general functioning of the nervous system and with other neuropsychiatric disorders. [4][5][7][13]

Our results include **marked decreases in serum concentrations of electrolytes such as serum calcium, serum ionic calcium, serum magnesium, serum iron and serum sodium, concomitant with marked increases in serum potassium. Changes in the opposite direction of sodium and potassium levels observed in this case may indicate the presence of possible disturbances in the functioning of the sodium-potassium ATP-dependent pump with the role of regulating intra-and extracellular levels of both these electrolytes.**

The calcium ion plays a crucial role in neuronal activity, especially in the process of neurotransmitter release at the synaptic knob. Magnesium in ionic form in neurons can sometimes have the opposite action to calcium, making ionic links with water different than those made by the calcium ion and therefore blocking the activity of some membrane receptors (eg NMDA receptors of glutamate) which normally works by opening of ion channels that allow passage of calcium ions. The research made by us resulting in **low serum levels of both calcium and magnesium, could indicate that further research is needed to investigate this issue further.**

**Decreased serum iron levels may indicate the presence of some possible problems in the process of oxygen transport by hemoglobin, possible disfunctions involving myoglobin and/or possible dysfunctions in the cytochromes and hemenzymes activity(catalase, peroxidase).** The brain is the largest oxygen consumer of all the body's organs and thus is sensitive to any possible reduction of oxygen supply.

We investigated the concentration of these electrolytes in the blood in a single study in a small number of patients suffering from schizophrenia. **But we believe that the results indicate the usefulness of further research to explore this issue in depth with the participation of a larger number of subjects and also of subjects with**

**and without previous use of antipsychotic medication in order to observe the possible effects of the medication in this case.**

6) **Correlations between biochemical markers investigated in patients diagnosed with schizophrenia**

In two of the research conducted, during which we investigated several markers of different categories that we have addressed in this paper in separate chapters, we obtained some interesting statistical correlations between blood biochemical markers belonging to different categories.

Thus, **strong positive correlations were observed between the different nonenzymatic antioxidant factors.** In the case of **indirect bilirubin and albumin was obtained a Spearman coefficient value of 0.932 at p <0.01, and for uric acid and bilirubin a Spearman coefficient of 0.732 at p <0.05**. Such positive correlations between specific markers of the same overall broader antioxidant defense mechanisms may have some relevance on the global condition of that set of mechanisms, the respective values of the two pairs of markers analyzed simultaneously increasing or decreasing eventually (within each pair in part) in relation with the size of the action of some oxidizing factors that both members of the pair try to neutralize at the same time.

During one research we also investigated the possible existence of correlations in people with schizophrenia that participated in the study between ammonia and energy metabolism on the one hand and protein metabolism, on the other hand, by calculating **the Spearman correlation coefficient for creatine and total blood protein. The results indicated a positive correlation value of that coefficient of 0.833 for p <0.05. The observed relation might indicate the existence of some increase in protein synthesis associated with an increase of available energy as a result of increases in creatine phosphokinase levels and vice versa, a decrease of protein synthesis when the available energy is low(such can be the case if the creatine conversion to creatine phosphate mechanism is affected and instead the cyclization of creatine is accentuated).**

After statistical processing have been obtained some interesting results on the correlation between lipid metabolism and blood levels of some electrolytes in the group of patients with schizophrenia investigated**.** Thus was obtained **a Spearman coefficient value of 0.738 for HDL cholesterol and serum phosphate at p <0.05. For LDL cholesterol and serum magnesium was obtained a Spearman coefficient value of 0.88 at p <0.01. For calcium ion and LDL was obtained a negative correlation Spearman coefficient value of -0.843 at p <0.01. There also resulted a positive correlation Spearman coefficient value of 0.781 at p <0.05 between serum potassium and atherogenic risk.** Given the positive role in the functioning of the cardiovascular system that is assigned to HDL cholesterol and respectively the LDL cholesterol assigned negative role, the results above obtained for electrolytes and other biochemical markers shows that **higher levels of serum phosphate and an increased calcium ion may be associated with better functioning of the cardiovascular system in this case(due to the associated increase in HDL cholesterol in the first case and the related decline in LDL cholesterol in the second), while an increase in serum magnesium and a serum potassium may be associated with that problems for that system(being associated with increased LDL and atherogenic risk respectively).**

In what regards the correlations of some ammonia metabolism markers (creatine phosphokinase and creatine index) with markers of oxidative stress (TAS and GSH), there were obtained significant values of the Spearman coefficient, but for creatine phosphokinase the scatter plot reveales the fact that in this case is involved a big heterogeneity of the group analyzed (which is basically divided into two subgroups showing different relations between CK on the one hand and TAS, respectively GSH on the other side).

In the case of the creatinic index and TAS has resulted a value of the Spearman coefficient of 0.622 at p <0.05. Upon completion of the scatter plot in this case was not visible the existence of two very different populations like in the previous cases involving creatine phosphokinase, and the correlation resulted to be non-linear. The correlation obtained is positive, indicating that **an eventual increase in TAS, which is an overall indicator of defensive antioxidant activity, is associated with an increasing of the creatine index, whose value was found to be decreased in all patients diagnosed with schizophrenia.** It must however be noted that in what regards TAS, in another study presented in this thesis we have obtained some results that go in the opposite direction, so this correlation between TAS and the creatinic index should be treated with some caution.

In order to investigate the existence of possible correlations between ammonia metabolism and a degree of potential liver disfunctions in patients suffering from schizophrenia **was calculated the Spearman coefficient for the creatinic index and LDH. The result is a value of the Spearman coefficient of -0.610 at p <0.05 (which indicates a reverse correlation between the LDH and creatine index, any increases in LDH, usually manifested in cases of liver injury and also in other cases of tissue injury, was associated with decreases in creatine index, index was much lower than normal in all patients investigated by us).**

**In conclusion, we feel that the data that we obtained contribute to increase of the knowledge base regarding the biochemical imbalances in schizophrenia and outlines new motives to deepen further research in some directions that we have approached. Some biochemical disturbances seem to be visible in schizophrenia not only purely at cerebral level(where the diagnosis tools access is more difficult due to the**

**increased sensitivity of the brain to the eventual exogenous disturbance and to the complexity of the technological means of investigation used at this level) but also at the blood level, a level that can permit the utilization of investigation methods less complex and costly. So that the identification of markers with potential diagnostic role in schizophrenia in the blood could be in the future a significant step towards identifying useful methods and techniques of clinical laboratory more accessible and less expensive for this condition.**

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