Study of Urine Organic Sulfate Changes in Schizophrenia by Colorimetry

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Objective: Finding a suitable laboratory test that can diagnose schizophrenia in its early stages could be very important. According to the hypothesis of lack of noradrenalin balance in the brain, it is illustrated that the disorder severity has a negative correlation with the amount of urine noradrenalin metabolite [3-methoxy-4-hydroxy phenyl glycol (MHPG) sulfate]. In this research, instead of measuring 24-hour urine MHPG sulfate level by standard expensive HPLC method, 24-hour urine organic sulfate mixtures were measured and compared between schizophrenic patients and control group by colorimetry.

Methods: Forty schizophrenic patients (20 males and 20 females) diagnosed by two psychiatrists according to DSM-IV-TR criteria and 40 controls (20 females and 20 males) with nearly the same diet and physical activity levels were included. After primary laboratory tests and ruling out general medical conditions in both groups, all medications in schizophrenic patients were tapered. For all subjects, 24-hour urine samples were collected and organic sulfate was measured by colorimetry method.

Results: Mean 24-hour urine organic sulfate in case and control groups were 0.465 ± 0.03 and 0.475 ± 0.04, respectively (p= 0.219). Mean 24-hour urine organic sulfate in case females was 0.46 ± 0.028 g/dl. In control females, this amount was 0.47 ± 0.044 g/dl (p= 0.393). Mean 24-hour urine organic sulfate in case males was 0.47 ± 0.031 g/dl. In control group, it was 0.48 ± 0.039 g/dl (p= 0.382).

Conclusion: Measuring organic sulfate by colorimetry method cannot help to distinguish schizophrenic patients from normal individuals.

Declaration of interest: None.

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Introduction

Schizophrenia is a chronic clinical disorder with severe destructive variable signs that affects thought, sensorium, emotion and one's other behaviors. Although this disease has been known for a long time and we have obtained various therapeutic methods, yet the usage of psychoactive drugs (old and new generation drugs) and electroconvulsive therapy can comparatively recover about 60% of patients, while in the remaining, there is progressive deterioration of function. Therefore, it is regarded as the most expensive illness in the world. In addition, it directly or indirectly imposes a considerable expense for patients, families and society (1,2).

The duration of primary signs to diagnose this disorder is nearly two years. Moreover, it creates a poor prognosis by passage of time. It should be noted that it is better to diagnose this disorder at an early stage, thus, identifying and controlling its symptoms will achieve a more successful outcome. Thus, nowadays, it is considered to recognize this disorder even when there are no complete clinical signs and symptoms (2,3).

It is anticipated to use lab tests for diagnosing psychiatric disorders in DSM-V (4). There is a hypothesis regarding lack of dopamine balance as a reason for schizophrenia.

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There are also other hypotheses based on the existing lack of noradrenalin balance in the brain of schizophrenic patients (1,3,5-11). Thus, it is illustrated that brain noradrenalin and its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) in cerebrospinal fluid (CSF) are decreased in these patients. This metabolite is conjugated to sulfate in the blood and excretes in the urine; therefore, treatment with antipsychotics will increase the MHPG (12-15).

Although it is confirmed that MHPG sulfate decreases significantly in the urine of patients in comparison to control group using high-performance liquid chromatography(HPLC) (8,12,16-21), due to high cost and complex techniques, HPLC is not a suitable method for widespread use for screening schizophrenic patients .

Therefore, in this research, instead of measuring 24-hour urine MHPG sulfate level by HPLC method, 24-hour urine organic sulfate mixtures were measured and compared between schizophrenic patients and control group by colorimetry (22-24). It should be noted that organic sulfate (which cannot be measured by colorimetry) is obtained by decreasing inorganic sulfate from total urine sulfate. Moreover, studies show that foods containing organic or inorganic sulfur equal with 60g daily meat, and usual physical activities do not have affect on all or inorganic sulfate amounts of urine (25,26). So we compared urine results of patients with healthy controls who had the same diet and physical activities.

Materials and Methods

Forty patients (20 men and 20 women) with schizophrenia (diagnosis was confirmed with DSM-IV-TR criteria by two psychiatrists) who were admitted in Zare Hospital (Sari-Mazandaran, north of Iran) were randomly selected after written informed consent, and primary laboratory tests (blood cells counting, hemoglobin, haematocrit, fasting blood glucose, urea, creatinine, urine analysis, liver function tests, morphine test, cannabis test, pregnancy test, sodium , and potasium) were performed for them. The patients had not taken any medication from their last consumption of drugs for seven half lives. In case of the existence of irritable behavior, lorazepam was administered 3-5mg daily. At the end of seven half lives from the last administration of antipsychotic medications, 24-hour urine samples were collected and transferred to the laboratory. Control group consisted of 40 healthy people selected from Zare Hospital personnel with same physical activity and similar diets who had no schizophrenic first degree relatives. Their 24-hour urine samples were also collected and transferred to the laboratory.

After collection of 24-hour urine samples for measuring total and inorganic sulfates, the following processes were performed (8,16,23):

1. Due to the interfering phosphate in the measurement of urine sulfate, first we added 4 ml of urinal acetate 0.4% to 1 ml urine and separated precipitate of phosphate mixtures and the remaining solution was used for measuring total and inorganic sulfate.
2. For measuring inorganic sulfate we added 2 ml of tri-chloride acetic acid 10% to 1 ml of above solution which removed the phosphate. After centrifuging and separating basic solution from precipitates, we added aluminum chloride. After centrifuging, basic solution was separated from precipitates and 2 ml chloroacetic acid in acetone 5% was added up to 3.5 ml, and then 6 ml Benzedrine solved in Acetone was added to the above solution. The solution was held in 0°C for two hours and after centrifuging these obtained precipitates was made into a solution with 0.2 NHCL in a hot bath. We then added 0.5 ml Gum Arabic and 1 ml phosphotungstic acid to this solution and this obtained solved inorganic sulfate was measured by 5010V5+ photometer in 546 nm wave.
3. For measuring 24-hour total urine sulfate we mixed and heated 0.5 ml of urine solution in which obtained in part 1 with 3 ml distilled water and 0.5 ml NHCL until dried. Then, we added 6 ml distilled water, 0.5 ml chloride 2.5 % and 5 ml ammoniac 20 %. After centrifuging, we held 3.5ml of this solution with 2 ml tri-chloroacetic acid and 6 ml Benzedrine 1.5 percent solved in

acetate in 0°C for 2 hours. After re-centrifuging (2000 rpm), obtained precipitate was separated and made it as a solution with 0.2 NHCL in a hot place to this solution. We then added 0.5 ml Gum Arabic and 1 ml phosphotungstic acid and this obtained solution containing total sulfate was measured by 5O1OV5+ Photometers in 546 nm wave. Twenty four-hour urine sulfate was calculated by deducting total sulfates from inorganic sulfate.

Data were analyzed by SPSS software (version 16.0), using independent T test.

Results

Twenty men with schizophrenia (mean age of 40 years), 20 men in control group (mean age of 39 years), 20 women with schizophrenia (mean age of 42 years), and 20 women in control group (mean age of 39 years) participated in this study (Table 1).

Table 1. Comparing age(range and mean) in schizophrenic and control groups

	Case Group		Control Group	
	Female	Male	Female	Male
Age range	26-60	28-59	28-48	27-56
Mean	42	40	39	39

Mean 24-hour urine organic sulfate in case females was 0.46 ± 0.028 g/dl. In control females, this amount was 0.47 ± 0.044 g/dl (p= 0.393) (Table 2).

Table 2. Comparing Mean and standard deviation of 24-hour organic urine sulfate in female schizophrenic and control groups

	Control		Case		df	p
	Mean (gr/dl)	SD	Mean (gr/dl)	SD		
24-hour organic urine sulfate	0.47	0.044	0.46	0.028	38	0.393

Mean 24-hour urine organic sulfate in case males was 0.47 ± 0.031 g/dl. In control group, it was 0.48 ± 0.039 g/dl (p= 0.382) (Table 3). Mean 24-hour urine organic sulfate in case and control groups were 0.465 ± 0.03 and 0.475 ± 0.04, respectively (p=0.219)(Table 4).

Table 3. Comparing mean and standard deviation of 24-hour organic urine sulfate in male schizophrenic and control groups

	Control		Case		df	p
	Mean (gr/dl)	SD	Mean (gr/dl)	SD		
24-hour organic urine sulfate	0.48	0.039	0.47	0.031	38	0.382

Table 4. Comparing mean and standard deviation of 24hour organic urine sulfate in total schizophrenic and control groups

	Control		Case		df	p
	Mean (gr/dl)	SD	Mean (gr/dl)	SD		
24-hours organic urine sulfate	0.475	0.04	0.465	0.03	78	0.219

Discussion

In this study 24-hour organic urine sulfate levels were measured and compared among men and women with schizophrenia and control group by colorimetry, which is less expensive and an available method compared to HPLC (7,20,27). Due to lack of similar investigations regarding measuring of urine organic sulfate in schizophrenic patients, comparison of the current findings with former reports is not possible. By this investigation, our results indicated that the amount of measured organic sulfate within 24-hour urine in patients with schizophrenia by colorimetry did not show a significant difference between case and control groups. In fact, this laboratory test was not useful to distinguish schizophrenic patients from normal subjects.

Previous studies have confirmed a significant decrease in the amount of urine and CSF MHPG sulfate (8,12,14,16,27). In our study we did not find any significant difference between urine organic sulfate of schizophrenic patients and control group. So it seems that organic sulfates other than MHPG sulfate may act as important confounding variables. However, in other studies, reduction of MHPG sulfate in CSF was not confirmed (7), and our findings are in line with theirs.

The majority of our patients had residual (15 patients) and disorganized (12 patients) subtypes of schizophrenia. This sampling bias is another limitation of our study. Perhaps the

organic sulfate changes in other subtypes of schizophrenia may show different results. Thus, we recommend further research with larger sample sizes, studying different subtypes of schizophrenia.

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