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STUDY AREA: BIOLOGY / BIOCHEMISTRY

PhD THESIS SUMMARY

INFLUENCE OF FISH BASED DIET ON BLOOD BIOCHEMICAL MARKERS OF DANUBE DELTA'S POPULATION

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CONTENTS

INFLUENCE OF FISH BASED DIET ON BLOOD BIOCHEMICAL MARKERS OF DANUBE DELTA'S POPULATION

OBJECTIVES AND PURPOSE OF THE STUDY

The existing data points out that the normal values of biochemical parameters vary by sex, age, diet and geographical region.

The Danube Delta is dominated by different white meat fish species, which are part of the diet of the residents living in the investigated area. Fishing was and still is the main occupation and source of income for the inhabitants of the Danube Delta.

A fish based diet is recommended for both a healthy body and for thise who aim to lose weight and keep the silhouette. The real benefits of eating fish and fish products encourage all people, regardless of geography, to bring this meat in the diet at least twice a week.

The main objective of this research was to determine the biochemical and hematological parameters leading to the establishment of new correlations between the values of determined markers and health correlations to support and contribute to the key objectives of the general theme, namely the influence of a diet based on fish, this being a major focus of nutrition and public health.

The proposed objectives were subordinated to this main objective and consist of widening the current database on the health of residents in Sulina and in the Danube Delta habitable zones. The eligible patients said they included at least two meals of fish / fish products per week.

The major objectives of our research were:

1. Determination of biochemical markers of lipid metabolism: total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total lipids, AST, ALT and correlations between the relative values considered normal for the geographical area studied.
2. Determination of biochemical markers: glucose and total protein.
3. Determination of serum electrolytes: total calcium, ionic calcium, magnesium and hemoglobin contained in red blood cells, the most commonly evaluated parameters.
4. Determination of blood group and Rh-test, necessary and vital for both mothers and new-borns and to set up a possible blood collection center for future blood transfusions.

Keywords: health, fish-based diet, metabolism, Danube Delta

PART I - STATE OF KNOWLEDGE

The most common type of fish consumed in Romania is freshwater fish (Moldoveanu, 2008).

The biodiversity of the Danube Delta biosphere reserve includes 900 plant species and over 2,800 species of fauna: molluscs (86 species), insects (2,219 species), fish (160 species), amphibians (10 species), reptiles (11 species), birds (315 species) and mammals (42 species) (Ciolac, 1995; Ciolac, 1998).

Nowadays, the interdependence between diet and health is a matter of interest both nationally and internationally. More and more research on the relationship between diet and chronic disease incidence were initiated with interest to support healthy diets (Moga, 2008).

A healthy diet must bring five advantages: adequate, balanced, calories checked, moderate, diversified.

Lifestyle factors, including diet, eating habits, physical activity levels and inactivity are often adopted during the early years of life. As childhood obesity is strongly linked to obesity in adulthood, the best time to solve the problem is childhood (Kohat et al., 2002).

First, this research highlights the nutritional aspects of the diet of the Romanian population, which must be corrected to ensure and maintain the health of people, in order to avoid nutritional imbalances that could lead to an increased risk of chronic diseases. An unbalanced nutrition can cause various affections, such as chronic and degerative diseases. Food should have an important role in nutrition to ensure better health status and play an important role in reducing the risk of certain chronic diseases. Therefore, we must pay special attention to improving nutrition and health policies, with a particular focus on developing and promoting healthy eating habits, which contributes to ensuring and maintaining the general health of the population (Constandache et al., 2013).

The minimum sets of hematology and biochemical analyzes proposed and carried out in analysis laboratories, both as routine and emergency, are:

- Complete Hemogram with differential blood count;
- Total cholesterol; HDL cholesterol; LDL cholesterol; triglycerides;
- Glucose;
- AST transaminases; ALT transaminases;

- Total protein;

- Total calcium; ionic calcium;

- Magnesium;

- Iron;

- Urea; Uric Acid; Creatinine;

- Total bilirubin;

- urine analysis with sediment.

According to research on a diet based on fish products, we have the following recommendations:

- Fish belongs to vascular protecting foods (Natea, 2008);

- In all of the schemes of diet in different conditions, it is recommended that one fish meal will be consumed by the patient;

- Improving the quality of life without degrading the environment and without compromising the resources of future generations (Brînzan et al., 2012);

- Fish and fish products help to keep under control the level of total lipids and liver metabolism (Ene et al., June 2016);

- The total carbohydrates and proteins are maintained in the normal range, because of the generally fish-based food (Ene etv al., September 2016);

- General feeling of good health (Ene et al., October 2016);

- Due to Omega 3 fatty acids, the nervous system works better (Ene et al., 2017).

- Fish provides increased levels of vitamins (A, D and B complex vitamins)

- Fish presents a high nutritional value: 200 grams of fish represents 25% of your daily protein requirement for an adult and for children and 50-75% pregnant women (Mencinicopschi, 2007);

PART II - PERSONAL CONTRIBUTIONS

MATERIALS AND METHODS

After blood samples were collected according to the sampling manual, they were put in the Rotofix 32 centrifuge for 5 minutes at 4000 rpm.

For biochemical determinations, the 350 automatic biochemistry SAPPHIRE machine was used, complying the protocols for each individual reactive.

For blood determinations, namely hemoglobin, the sample is collected in special containers (liquid EDTA tubes) and microtainers for capillary blood that work in the next 3 hours. EXCELL 2280 analyzer application determines values for measuring white blood cells, hemoglobin and particulates that help blood to clot, by purifying method of impedance measurement and classification of specialized cells.

At the basis of tests on group blood determination and Rh is agglutination. Agglutination is the aggregation of particles by antibodies. The antibodies react with the antigen (natural or artificial fixed) on the surface of their red blood cells and causes agglutination.

No patient was included twice in the study. The study was approved by the Board of Ethics of the Ovidius University, I.O.S.U.D and the medical centers with which I collaborated.

RESULTS AND DISCUSSIONS

1. The hepatic evaluation and the lipid profile in Danube Delta patients

The practical assessment of atherogenic risk lipid etiology is based on the concentration of cholesterol, triglycerides, LDL cholesterol and HDL cholesterol. For male patients (N = 97) the average amount of **total lipids M_M = 473.87 mg / dL** with a standard deviation of **SD_M = 107.04 mg / dL**. For female patients (N = 103) the average of total lipids is **M_F = 458.20 mg / dL** with a standard deviation of **SD_F = 88.21 mg / dL** (Figure 1a).

For male patients (N = 97) the average **total cholesterol is M_M = 179.89 mg / dL** with a standard deviation of **SD_M = 36.42 mg / dL.** For female patients (N = 103) the average total cholesterol is **M_F = 190.01 mg / dL** with a standard deviation of **SD_F = 33.04 mg / dL** (Figure 1b).
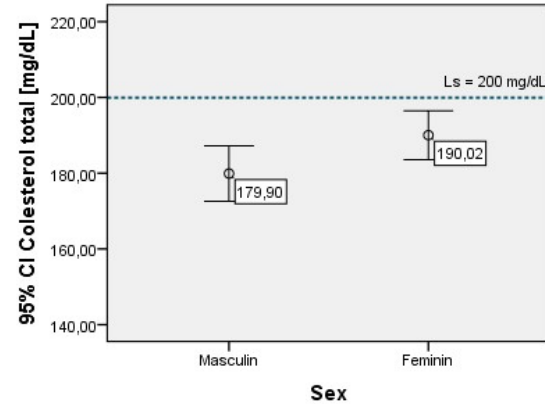
For male patients (N = 97) the mean **HDL cholesterol is M_M = 49.54 mg / dL** with a standard deviation of **SD_M = 11.99 mg / dL.** For female patients (N = 103) the mean HDL cholesterol is

$M_F = 54.82$ mg / dL with a standard deviation of $SD_F = 12.44$ mg / dL (Figure 2a).

For male patients (N = 97) the mean **LDL cholesterol is $M_M = 110.81$ mg / dL** with a standard deviation of $SD_M = 26.69$ mg / dL. For female patients (N = 103) the mean LDL cholesterol is $M_F = 123.07$ mg / dL with a standard deviation of $SD_F = 27.41$ mg / dL (Figure 2b).
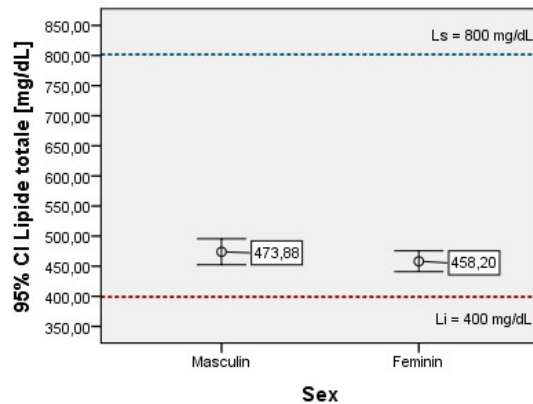
1a   1b

<u>Figure 1a:</u> Average values of total lipids in men and women

<u>Figure 1b</u>: Average values of total cholesterol in men and women
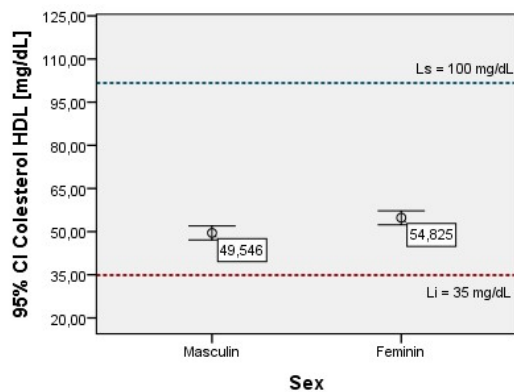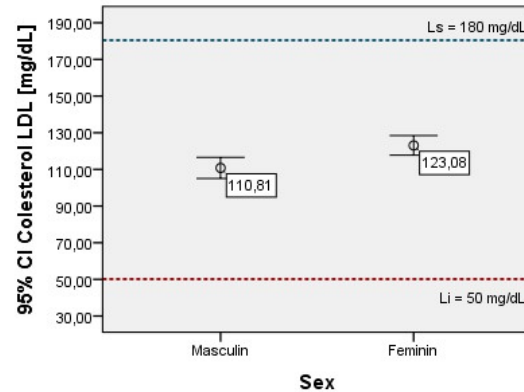
2a   2b

<u>Figure 2a:</u> Average values of HDL cholesterol in men and women

<u>Figure 2b</u>: Average values LDL cholesterol in men and women

For male patients (N = 97) average value of **triglycerides is $M_M = 123.65$ mg / dL** with a standard deviation of $SD_M = 52.28$ mg / dL. For female patients (N = 103) the average value of triglycerides is $M_F = 100.40$ mg / dL with a standard deviation of $SD_F = 52.83$ mg / dL (Figure 3).

Figure 3: Average values of triglycerides in men and women

For male patients (N = 97) average value of **AST** is M_M = **27.91 IU / L** with a standard deviation of SD_M = **7.73 IU / L.** For female patients (N = 103) the average value of AST is M_F = **24.33 IU / L** with a standard deviation of SD_F = **6.98 IU / L** (Figure 4a).

For male patients (N = 97) mean **ALT** value is M_M = **29.24 IU / L** with a standard deviation of SD_M = **8.35 IU / L**. For female patients (N = 103) the average value of ALT is M_F = **24.20 IU / L** with a standard deviation of SD_F = **7.98 IU / L** (Figure 4b).

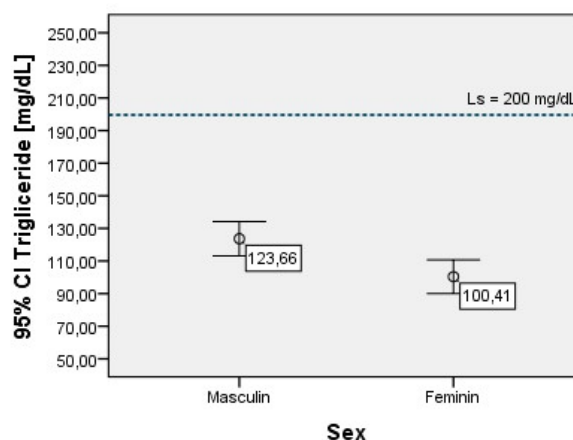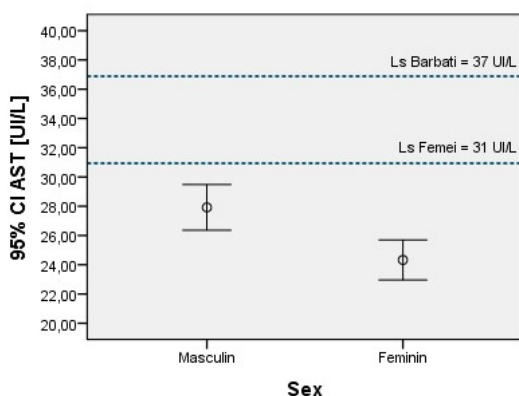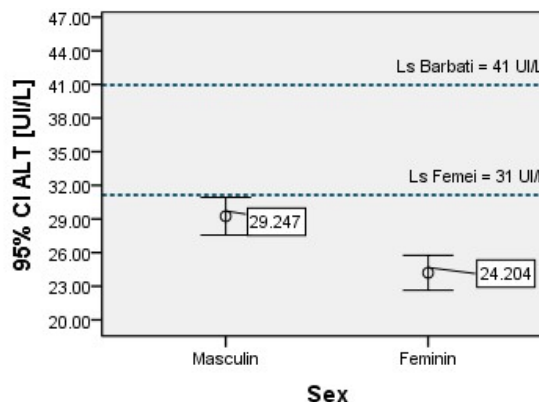4a   4b

Figure 4a: Average values of AST in men and women

Figure 4b: Average values of ALT in men and women

For male patients (N = 97) the mean **LDL cholesterol / HDL cholesterol** is M_M = **2.27** with a standard deviation of SD_M = **0.53.** For female patients (N = 103) the mean LDL

cholesterol / HDL cholesterol is $M_F = 2.29$ with a standard deviation of $SD_F = 0.51$ (Figure 5a).

For male patients (N = 97) the average **total cholesterol / HDL cholesterol** is $M_M = 3.70$ with a standard deviation of $SD_M = 0.69$. For female patients 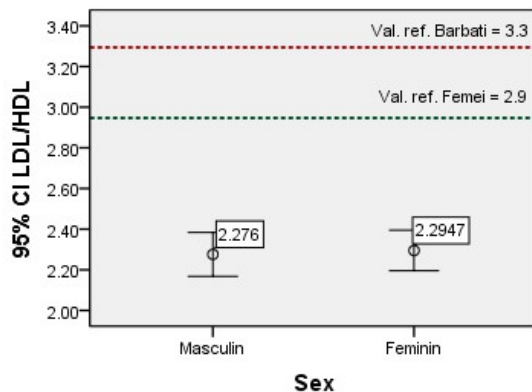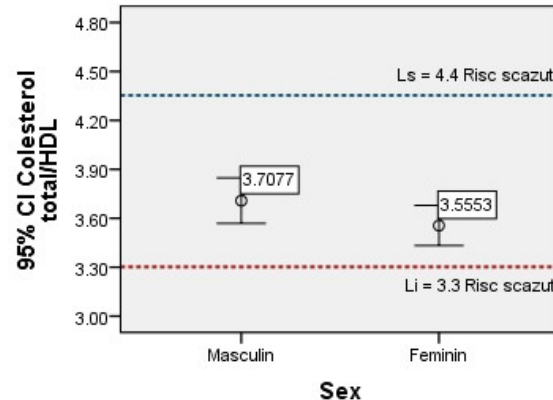(N = 103) the average total cholesterol / HDL cholesterol is $M_F = 3.55$ with a standard deviation of $SD_F = 0.62$ (Figure 5b).

5a   5b

Figure 5a: Average values of LDL Cholesterol / HDL cholesterol in men and women

Figure 5b: Average values of Total Cholesterol / HDL cholesterol in men and women

Ritis ratio is the ratio AST / ALT and in normal physiological conditions must not be greater than 1.33.

For male patients (N = 97) average value of **AST / ALT is $M_M = 1.00$** with a standard deviation of **$SD_M = 0.32$.** For female patients (N = 103) the average value of **AST / ALT is $M_F = 1.09$** with a standard deviation of **$SD_F = 0.42$** (Figure 6).

2. **Determination of biochemical markers: blood sugar and total protein**

For male patients (N = 100) the mean **serum glucose is $M_M = 102.6$ mg / dL.** For female patients (N = 130) the mean serum glucose is **$M_F = 97.56$ mg / dL** (Figure 7a).

For male patients (N = 100) the **average total protein is $M_M = 6.89$ g / dL.** For female patients (N = 130) the average total protein is **$M_F = 6.68$ g / dL** (Figure 7b).
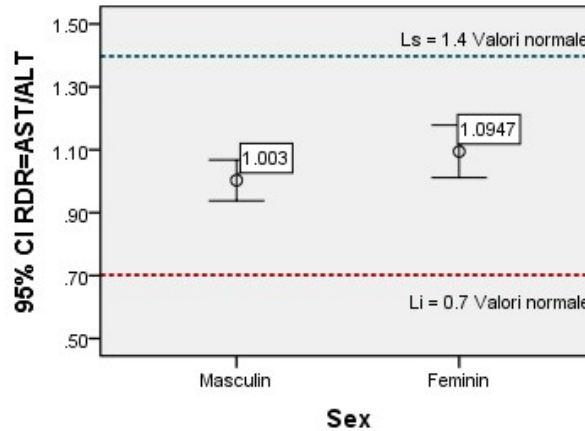
Figure 6: "De ritis" average values AST / ALT in men and women

7a

7b
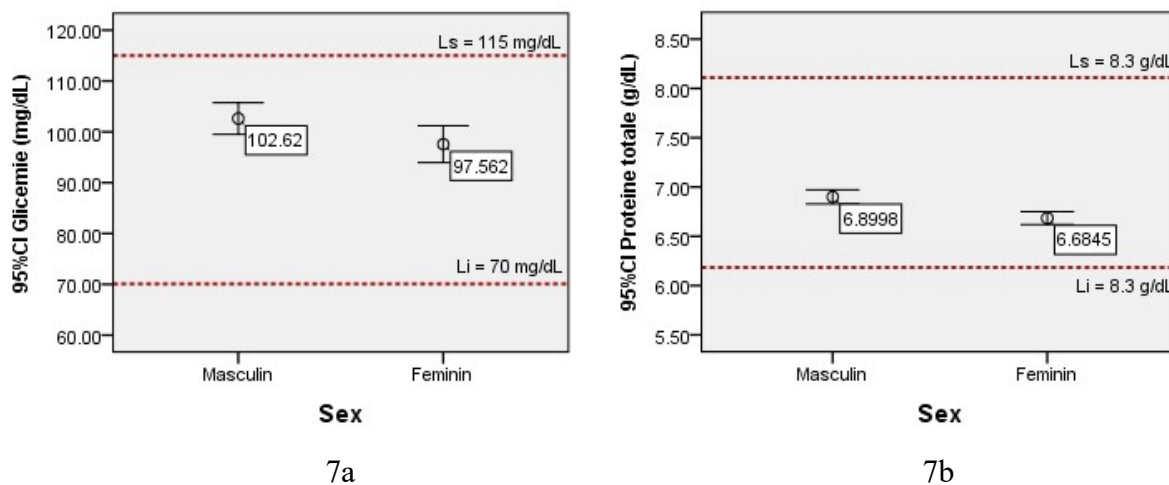
Figure 7: Average values of blood glucose in men and women

Figure 7b: Average values of total protein in men and women

3. **Determination of serum electrolytes: total calcium, ionic calcium, magnesium and hemoglobin contained in red blood cells, the most commonly evaluated parameters in routine medical tests**

For male patients (N = 90) the mean of **total calcium** is M_M = **9.10 mg / dL.** For female patients (N = 170) the mean of total calcium is M_F = **9.17 mg / dL** (Figure 8a).

For male patients (N = 90) the mean **ionic calcium** is M_M = **4.11 mg / dL.** For female patients (N = 170) the mean ionic calcium is M_F = **4.12 mg / dL** (Figure 8b).
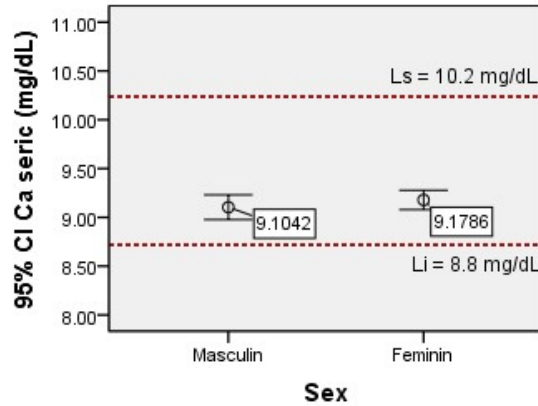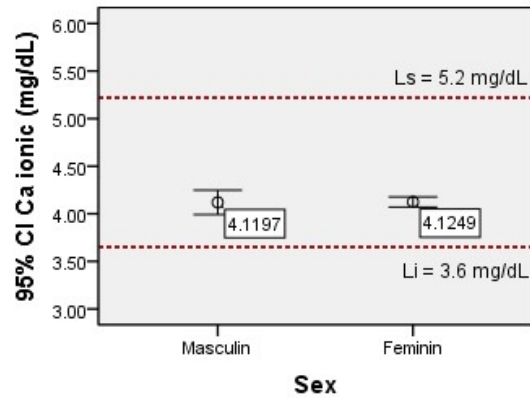
8a   8b

Figure 8a: Average values of total calcium in men and women
Figure 8b: Average values of ionic calcium in men and women

For male patients (N = 90) average value of **magnesium is M_M = 2.45 mg / dL.** For female patients (N = 170) the average value of magnesium is M_F = **2.34 mg / dL** (Figure 9a).

For male patients (N = 90) the mean **hemoglobin is M_M = 15.04 mg / dL.** For female patients (N = 170) the mean hemoglobin is M_F = **13.91 mg / dL** (Figure 9 b).
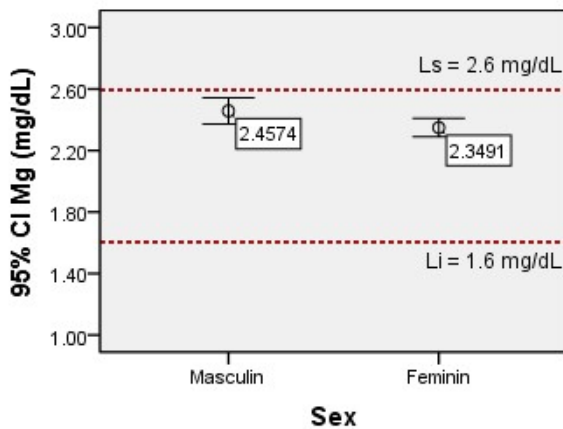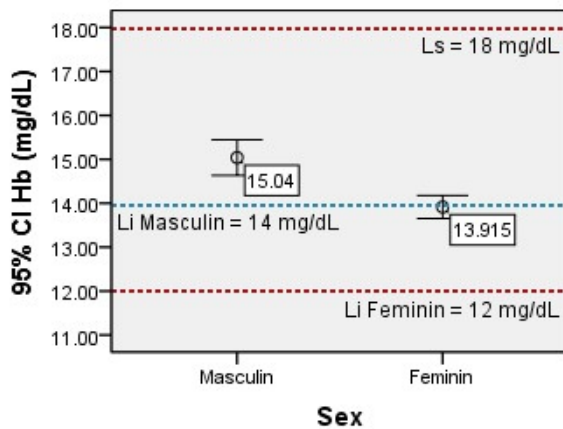
9a   9b

Figure 9a: Average values of magnesium in men and women

Figure 9b: Average values of hemoglobin in men and women

4. Determining the OAB blood group and Rh factor

Immuno-haematology tests were performed on 100 patients (60 women and 40 men), through the direct haemagglutination plate method using Anti-A, Anti-B, anti-AB and anti-D reagents. The results obtained were: 33 patients had blood group O_I, with 25 Rh positive (+) and 8 Rh negative (-); 42 patients presented A_{II} group, of which 36 showed Rh (+) and Rh 6 (-); 19 patients had B_{III} group, with 12 Rh (+) and 7 Rh (-); 6 patients had AB_{IV} group, with 4 Rh (+) and Rh 2 (-) (Figure 10).
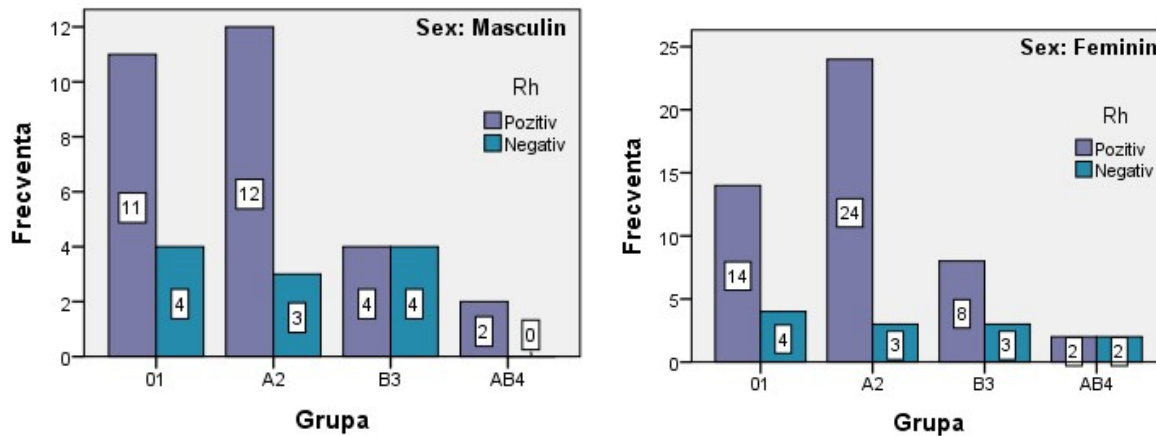
Figure 10: Blood type and Rh the male and female patients

CONCLUSIONS

After the analysis of data obtained from the four major goals of our study, we offer the following feedback, comments and conclusions:

1. **Determination of biochemical markers on lipid metabolism:** total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total lipids, AST, ALT and correlations between relative values considered normal for the geographic limits are within limits.

2. **Determination of biochemical markers: blood glucose and total protein,** they are disrupted daily in relation to diet, but they were also within the limits. It is well known that diabetes is one of the diseases of the century and numerous studies on screening, the diagnostic, treatment throughout the patient's life are carried out in all research centers in order to improve patients' lives and relieve its symptoms.

3. **Determination of serum electrolytes:** total calcium, ionic calcium, magnesium and hemoglobin contained in red blood cells are the most commonly evaluated parameters.

Given the studied area, the Danube Delta natural environment free of pollutants (with higher levels of oxygen), we chose to study the level of hemoglobin in patients instead of sideremia (iron plasma), which is disrupted by diet prior to measuring biochemical erythrocytes having a lifespan of 120 days. I considered that hemoglobin level is a more accurate index terms of the amount of iron in the body.

From the statistical analysis of these patients, with undetected disease symptoms, clinically healthy, we have demonstrated that they do not have disturbances in terms of electrolyte metabolism.

4. **Determining the OAB blood group and Rh factor -** tests necessary and vital for both mothers and new-borns and to set up a possible blood collection center for future blood transfusions. Because in Sulina, in the Danube Delta, there is no such unit, building a center to the blood transfusion is a good perspective.

Our research on the general health of the inhabitants of Sulina, Danube Delta, is an innovation, since previous studies related to the Danube Delta have focused on the bio-ecological area (the natural reserve of the biosphere research focused on introducing the concept of protected area, the study of vertebrate and invertebrate animals, studies related to fauna and flora, different types of resources in the area etc.).

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2. **Romanian Academy of Scientists , Spring Scientific Session, May, Bucharest, (2016) - Oral presentation**

3. **Romanian Academy of Scientists , Autumn Scientific Session, Durău - Neamț, (2016) - Oral presentation**

4. **Romanian Academy of Scientists , Spring Scientific Session, March, Bucharest, (2017) - Oral presentation**

5. **3rd International Conference "WATER RESOURCES AND WETLANDS" 8-10 sept. 2016, Tulcea, Romania, poster presentation**

6. **16th International Conference of Physical Chemistry - ROMPHYSCHEM, 21-24 Sept. 2016, Galați, Romania, poster presentation**

7. **19th International symposium "ENVIRONMENT AND INDUSTRY", 13-14 Oct. 2016 , Bucharest, poster presentation**

8. **6th International Conference "PROTECTION OF NATURAL RESOURCES AND ENVIRONMENTAL MANAGEMENT: THE MAIN TOOLS FOR SUSTAINABILITY", 11-13 Nov. 2016, Bucharest, poster presentation**

AWARDS

Special mention for the best presenting poster (section 7) at **6th International Conference "PROTECTION OF NATURAL RESOURCES AND ENVIRONMENTAL MANAGEMENT: THE MAIN TOOLS FOR SUSTAINABILITY", 11-13 Nov. 2016, Bucharest,** for original paper **"Comparative Hepatic Evaluation and the Lipid Profile in Danube Delta Patients with Fish-based Diets", Georgiana Ene,** Cristian Lucian Petcu, Magda-Ioana Nenciu, Natalia Roșoiu.