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DETERMINATION OF TOTAL GLUTATHIONE LEVEL AND TOTAL PROTEIN IN THE SERUM OF MADONNA UNIVERSITY STUDENTS.

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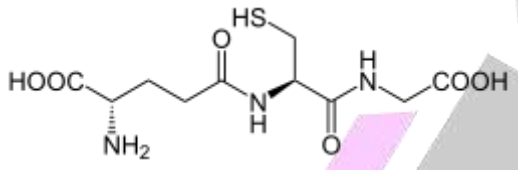
Abstract

The study was carried to investigate the total glutathione level and total protein of Madonna university students, Elele campus of Rivers State. Elele is a town in Rivers state which is located at the tropics of south-south of Nigeria.The population of the study consists of 40 students within the ages of 16-27 years, both male and female. The questionnaires for this study were distributed to individuals to be filled base on gender, age, active and less active individuals, drug and non-drug dependent individual and individual in different sporting activities. Blood was collected by venipuncture and serum prepared by centrifugation, serum was collected using micropipette and stored into aliquot at -2^{0}C in a fridge. Ellman reagent was used for glutathione determination while Biuret reagent was used for protein assay using biochemical standard. The results showed that the male students have higher level of glutathione concentration (0.0277 ± 0.001) when compared to female students (0.0259 ± 0.009). However, there was no significant difference (P>0.05) in glutathione concentration of male and female students. The result also showed that female students have higher level of total protein (5.21 ± 0.56) when compared to the male students(5.02 ± 0.87) .Therefore, there was no significant different (P>0.05). The total protein and glutathione level were not age dependent, there was no significant difference(P>0.05) in glutathione level and total protein of individuals aged 16-21 years when compared to those aged 22–27 years. Table 3.3 showed that the glutathione level and total protein were not activity dependent. In conclusion, the individuals have their total glutathione and total protein level within the normal range. These showed that the individuals are free from some of the diseases caused by the accumulation of free radical and foreign substances in the body because glutathione is an antioxidant.

Keyword: Glutathione level, Antioxiant, Protein, Serum, determination, Tripeptide compound.

Introduction

Glutathione is a tripeptide compound composed of amino acids glutamic acid, cysteine and glycine .This is found in all cells in the body including the bile ,the epithelial lining fluid of the lungs and at much smaller concentrations in the blood(Gross *et al*.,1993).



Structure Of Glutathione

The highest concentration of glutathione is found in the liver, making it critically important in the detoxification and elimination of free radicals from the body. Accumulation of these dangerous compounds can result to oxidative stress which occurs when the generation of free radicals in the body exceeds the body's ability to neutralize and eliminate them (Chow *et al.*, 2007).Glutathione is the body's most powerful antioxidant and it is also called the master antioxidant. It is a tripeptide found inside every single cell in the body. Antioxidants are crucial in eliminating free radicals from the body while free radicals are basically very reactive particles or reactive oxygen species that bounce all around the cell damaging everything they touch (Bounous, 1991; Gold, 1991).

Most of the free radicals are produced during the process of metabolism but they can also arise from exposure to toxins, irradiation, and toxic metals (Clementi, *et al.*, 1999).

Because free radicals are so destructive, cells have a network of defences designed to neutralize them. This antioxidant network is composed of numerous components that include vitamins, minerals and special chemicals called thiols (glutathione and alpha-lipoic acid).They can also enter the body through the environment (Chow et al., 2007).

Several powerful oxidants are produced during the course of metabolism in both blood cells and most other cells of the body. These include superoxide (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and hydroxyl radicals (OH^-) are referred to as reactive oxygen species (Meyer *et al*., 2007).

Glutathione is different from other antioxidants in that it is intracellular. It has

the unique ability of maximizing the activity of all the other antioxidants, including vitamins C and E, CoQ10, alpha lipoic acid, and the fresh veggies and fruits you (hopefully) eat every day. It removes toxins from your cells and protects you from the damaging effects of radiation, chemicals, and environmental pollutants (Gross, *et al*., 1993).

Protein

Proteins are essential nutrients for the human body. They are one of the building blocks of body tissue, and can also serve as a fuel source. As fuel, proteins contain 4 kcal per gram, just like carbohydrates and unlike lipids, which contain 9 kcal per gram (Bairoch, 2000).

Proteins are polymer chains made of amino-acids linked together by peptide bonds. In nutrition, proteins are broken down in the stomach during digestion by enzymes known as proteases into smaller polypeptides to provide amino acids for the body, including the essential amino acids that cannot be biosynthesized by the body itself (Grey *et al*.,2003).

Amino acids can be divided into three categories: essential amino acids, non-essential amino acids and conditional amino acids. Essential amino acids cannot be made by the body, and must be supplied by food. Non-essential amino acids are made by the body from essential amino acids or in the normal breakdown of proteins. Conditional amino acids are usually not essential, except in times of illness, stress or for someone challenged with a lifelong medical condition.(Grey *et al*.,2003).

Objective of the Study

This study is aimed at determining the total glutathione level and total protein in Madonna university students by:

1. Knowing the gender that has higher total glutathione and total protein level.

2. Knowing whether total glutathione and total protein are age dependent.

3. Knowing whether total glutathione and total protein are activity dependent

4. Knowing whether total glutathione and total protein are drug dependent

5. Knowing whether different sporting activities affect total glutathione and total protein.

Materials And Methods

The work was carried out using samples from students in Madonna University, Elele

campus of Rivers state. The population of this study consist of 40 students within the ages of 16-21 years both male and female.

Equipment

Equipment used were calibrated and in good working state. Some equipment used for the analysis include: weighing balance, centrifuge, Refrigerator, photometer and UV-Spectrophotometer.

Reagents

The chemicals and reagents used are of analytical grade and were obtained from major distributors.

Collection of blood Samples

The blood samples were collected through venipuncture from the students/patients. The patients were positioned in a chair and a suitable site for venipuncture were selected, by placing th tourniquet 3 or 4 inches above the selected site on the patients. When a vein was selected, the area was cleansed with cotton wool and methylated spirit in a circular motion to sterilize the site before venipuncture was performed. The patient was asked to make a fist and needle was swiftly inserted through the skin into the lumen of the vein through which blood was drawn. The blood drawn was transferred into an EDTA bottle.

Preparation of Reagents

0.2M of Phosphate-EDTA (pH 8.2)

0.020mol ⟶ 1000ml

X ⟶ 200ml

x=0.002 x 200

100

=0.004mol

Converting to gram

x X molecular weight

0.004 x 292.25 =1.169

=1.17g

1.17g of Ethylene Diamine Tetraacetate (EDTA) was weighed and dissolved in 200ml of phosphate buffer

Buffer Preparation

500ml of water was used to dissolve the 2 required buffer which are

Sodium phosphate buffer Na2HpO4

Potassium dihydrogen phosphate buffer or phosphate buffer.

Both buffer concentration used was 0.1m at a pH of 7.4.

Sodium phosphate salt Na2 HPO4

0.1m ⟶ 1000

X ⟶ 500

Cross multiplication:

X= 0.1x5=0.05mol
10

m. w = 141.96 =0.05mole x141.96
= 7.0g

After the calculations, I dissolved in 500ml of water ie 7.0g in 500ml of water and 6.80g in 500ml of water.

Potassium dihydrogen phosphate KH_2PO_4

m.w = 136.09

0.1m ⟶ 1000
x ⟶ 500
x = 0.05mole x m.w (136.09)
= 6.80g

20mn EDTA (pH 4.7)

0.020mol ⟶ 1000ml
X ⟶ 200ml
x =0.020 x 200
1000 =0.004mol

Converting to gram

x X molecular weight

0.004 x 292.25 =1.169
= 1.17g

1.17g 0f EDTA was weighed and dissolved into 200ml of distilled water

20Mm of Ellman Reagent (10Mm 2,2-dithio-5.5'-dibezoic acid(DTNB)

0.2M 1000ml

X 2ml

0.2X2/1000 = 0.0004

Converting to gram

x X molecular weight

0.0004 x 396.35 =0.158
=0.16g

0-16g of DTNB was weighed and dissolved in 2ml of methanol

Assay of serum glutathione.

Tube	Blank
Serum (µl)	/
0.2 M Tris–EDTA buffer pH 8.2 (µl)	1000
20 mM EDTA, pH 4.7 (µl)	1000
Ellman Reagent (µl)	20
Mix and incubate at room **temperature for 1 hour**	
Read the Optical Density (**DO) at 412nm** against blank	

Sodium chloride 0.9% w/v in µl	500	400	300	200	0	
Biuret reagent in ml	5	5	5	5	5	5
Mix and incubate at ambient temperature for 30 min						
Then read the OD against the blank at 545 nm						
OD						

Reagents For Total Protein Assay

1.5g of $CuSO_4.5H_2O$

6g of Tartrate Na, K

QSP 500ml H2O

1.5g of $CuSO_4.H_2O$ and 6g of Tartrate Na,K were weighed and dissolved in 500ml distilled water.(sol.A)

30g of NaOH

QSP 500ml H2O

30g of NaOH was weighed and dissolved in 500ml of distilled water (Sol.B)

The two solutions (A &B) were mixed very well to give the Biuret Reagent.

5mg/ml of Egg albumin5 1ml

X ⟶ 100ml

5x100= 500 i.e 0.50g

0.50g was weighed and dissolved in 50ml of distilled water

Assay of Total protein in serum

	0	1	2	3	4
egg albumin(5mg/ml) in µl	0	100	200	300	500
mples in µl					

Results

Serum Total Glutathione and Protein Levels in Male and Female students in Madonna University.

The result showed that female students had higher levels of total proteins (5.21 ± 0.56) when compared to males (5.02±0.87), however the mean difference was not statistically significant (p>0.05). Also there was no significant difference (p>0.05) in the mean glutathione concentration of the males and females.

Serum Total Protein and Glutathione Levels in Male and Female students

	Each serum sample duplicate	Glutathione Concentration (nmol/L)	Total Protein (g/dL)
Females	100	0.0259 ± 0.009	5.21 ± 0.56

Males	0.0277 ± 0.001	5.02 ± 0.87

Data represented as Mean ± SEM

Serum Total Glutathione and Protein Levels in Students of Different Age Bracket in Madonna University.

The table below showed that there were no significant differences ($p>0.05$) in the levels of glutathione levels and total protein of students aged 16 – 21 years compared to those aged 22 – 27 years.

Serum Total Glutathione and Protein Level in Male and Female students of different Age bracket.

Age	**Glutathione Concentration (nmol/L)**	**Total Protein (g/dL)**
16 - 21 yrs	0.0260 ± 0.007	5.20 ± 0.28
22 – 27 yrs	0.276 ± 0.001	5.04 ± 0.34

Serum Total Glutathione and Protein Levels in Active and Less Active Students in Madonna University.

The total glutathione and protein level was not activity dependent. There were no significant differences ($p>0.05$) in the level of glutathione and total proteins of students who walked less than 30 minutes daily

compared to those who walked more than 30 minutes daily.

Serum Total Glutathione and protein Levels in Active and Less Active Students in Madonna University.

WalkTime	Glutathione Level (nmol/L)	Total Protein (g/dL)
< 30 mins walk	435.51 ± 209.76	4.51 ± 0.52
> 30 mins walk	483.30 ± 73.4	5.31 ± 0.23

Serum Total Glutathione and Protein Levels in students on Medication and Those not on Medication in Madonna University.

The results on the table below showed that the level of total glutathione and protein were drug dependent. There were significant differences ($p<0.05$) in the total glutathione and protein level of students on medicaion compared to those not on medication.

Serum Total Protein and Glutathione Levels

Medication	Glutathione Level (nmol/L)	Total Protein (g/dL)
No – Medication	488.62 ± 77.49	5.19 ± 0.22
Yes – Medication	339.91 ± 76.59	4.83 ± 0.90

Serum Total Glutathione and Protein Level in students in different Sporting Exercises in Madonna University.

As shown in table below, type of sporting exercise did not affect the levels of total glutathione and protein There were no significant differences ($p>0.05$) in the level of glutathione and total proteins of students involved in the different sporting exercises.

Serum Total Protein and Glutathione Levels in students in Different Sporting Exercises in Madonna University

	Glutathione Level (nmol/L)	Total Proteins (g/dL)
Badminton	0.0310 ± 0.000	5.84 ± 0.00

Football	0.0263 ± 0.001	4.90 ±0.48
Jogging	0.0254 ±0.004	5.12 ±0.38
Tennis	0.0280 ± 0.002	5.51 ±0.23
Volleyball	0.0300 ± 0.004	5.07 ±0.82

Discussion

The most common function of glutathione is in detoxification and elimination of free radicals in the body. Protein helps in the production of antibodies which help to fight against foreign substances in the body. It also helps in the production of haemoglobin. In the quantitative analysis carried out in Madonna University students on total glutathione and total protein concentration. The results showed that total glutathione and total protein level in male and female are within the normal range. The result showed that total glutathione and total protein level in serum were not age dependent because the students are still growing, as glutathione level drops with age. Hence, when one is growing older, it reduces.. The result showed that total glutathione and total protein level in serum was not activity dependent because there was no significant difference on students that walked less than 30 minutes when compared to students that walked walked more than 30 minutes daily. The result showed that the total glutathione and total protein level in serum can be affected by medications, because they(medicine/ drugs) are foreign substances in the body, therefore, can alter biochemical processes and metabolism of the body.

Conclusion

Conclusively, it was observed that the determination of total glutathione and total protein level showed that all the individuals have their total glutathione and total protein level within the normal ranges, which means that they are free from some of the disease caused by the accumulation of free radicals and foreign substances in the body. They have strong immunities.

Recommendation

This study recommends that Consume sulfur-rich foods should be eaten to boost glutathione level. Sulphur is an important mineral that occurs naturally in some plant and protein. Sulphur is found in two amino acids in food which are methionine and cysteine such as beef, fish and poultry, vegetables like broccoli, cauliflower, kale, watercress, Brussels sprout and mustard green.

Also, steady exercise is recommended, as exercise boosts glutathione level and thereby helps boost the immune system, improve detoxification and enhance your body's own antioxidant defence.

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