Glutathione level estimation in obese individuals

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Abstract

This is a comparative study carried out on three cohorts, normal weight volunteers, overweight and obese subjects. The aim was to find out the effect of obesity on glutathione(GSH) concentration as a master antioxidant. GSH concentration was measured in whole blood sample of each volunteer. The procedure was done according the principle that Elman's reagent. The present study did not find any significant differences in GSH concentration between normal weight volunteers and overweight subjects and between normal weight volunteers and obese subjects. These inconsistent results with the results of many previous studies, may be attributed to many factors which affect the glutathione level rather than obesity and oxidative stress associated diseases.

Key words: Glutathione, obesity and oxidative stress.

ألخلاصه

هذه دراسة مقارنه تم تنفيذها على ثلاث مجاميع من الأشخاص:ذوي الوزن الاعتيادي ذوي الوزن الزائد وذوي البدانة.كان الهدف من الدراسة هو إيجاد تأثير السمنه على الكلوتاثيون(GSH) كمضاد أكسده رئيسي بالجسم .تم قياس تركيز GSH في عينه دم كل متبرع واعتمد مبدئ العمل على استخدام كاشف ELLMAN لم تظهر الدراسة الحالية أي فروقات معنوية بين مجموعه الوزن الطبيعي ومجموعه الوزن الزائد او بين مجموعه الوزن الطبيعي و مجموعه ذوي البدانة. إن هذه النتائج الغير متوافقة مع نتائج دراسات سابقه امكن تفسيرها بعده عوامل يمكن ان تؤثر على مستوى GSH غير البدانة وغير الأمراض المتعلقة بالجهد التاكسدي.

مفتاح الكلمات: الكلوتاثيون ,السمنة ,الجهد التاكسدي

Introduction

An obese person has accumulated so much body fat leading to a negative effect on the body health .If the person's body weight is at least 20% higher than it should be ,the person is considered obese.[1] Obesity is a complex multi factorial chronic disorder that develops from interaction of genotype and environment. Both overweight and obesity must be regarded as serious problems since its associated with many diseases and co-morbidity .[2] Many studies have revealed that obese individuals are at high risk for hyperinsulinaemia ,insulin resistance, diabetes mellitus, cardiovascular diseases and many diseases induced oxidative stress, which is imbalance between production of reactive oxygen species(ROS) and degeneration of these ROS. [3,4] Obesity is a principle causative factor in the development of metabolic syndrome. Others have reported that increased oxidative stress in accumulated fat is an important pathogenic mechanism of obesity –associated metabolic syndrome. [4]

glutathione (GSH) is a tripeptide with a gama peptide linkage between the amino acid group of cysteine and carboxyl group of the glutamate side chain.[5] It's is a master antioxidant cellular defense ,prevent damage to the important components caused by ROS such as free radicals and peroxide[6,7] .It dysregulation represents one of the main factors responsible for overproduction of ROS in diabetes mellitus and many other obesity associated diseases. [7]

This study aims to investigate the effect of obesity and overweight in apparently health subjects who are free of any oxidative stress associated diseases on the activity of antioxidants such as GSH.

Material and method

Patients

The study included three cohorts of subjects: Obese subjects who have body mass index(BMI) is over 30, (n=50),overweight subjects who have BMI 25-29,(n=53) and normal volunteers(n=50),who have BMI is less than 25. [1] All subjects involved in the study are within age range (45-65) years old .All subjects are apparently healthy and free of any disease that may alter the oxidants and antioxidants balance. All the subjects are nonsmokers.

Method

One ml of whole blood is collected from each volunteers and kept in vial containing ethylenediamintetraacetic acid (EDTA)anticoagulant. Measurement of GSH concentration was done according to the principle that Elman's reagent, the 5,5-dithiobis-2-nitrobenzoic acid (DTNB) react with sulfhydral group of GSH to yield a colored product, and in a sample comparing with the standard curve of GSH. Sulfhyral group can be assessed using the extinction coeffient of TNB at 412nm. [8]

GSH standard curve was established by preparing different concentration (5,10,15,20,30,40)mg/dl of GSH standard.100 mg GSH is dissolved in 100 ml of distilled water(DW).A working solution was prepared from each concentration by adding 0.2ml of the concentration ,1.8 ml of DW

and 3 ml of precipitating solution (1.67g of glycial metaphosphoric acid ,0.2g EDTA and 30g of sodium chloride are dissolved in 100 ml DW. The cuvetts were constructed as in the Table (1).

The procedure to measure GSH is done by placing 0.2ml of whole blood in 10 ml test tube and adding of 1.8ml of DW .3ml of precipitating g solution was added. The mixture was filtered by coarse grade filter paper .In two clean cuvetts, the previous reagents were placed, as in Table(2).The absorbance was measured by spectrophotometerat 412 nm.[9]

Table (1): Reagents for measuring GSH standard curve.

	Blank (ml)	Assay (ml)
GSH standard solution	/	2
Precipitating reagents	1.2	/
H_2O	0.8	/
Phosphate buffer	8	8
DTNB	1	1

Table (2): Reagents for measuring GSH concentration.

Reagent	Blank (ml)	Assay (ml)
Filtrate	/	2
Precipitating reagents	1.2	/
DW	0.8	/
Phosphate buffer	8	8
DTNB	1	1

Statistical analysis

Statistical analysis of the data was done using pooled t-test; statistical package SPSS (Statistical Packages for Social Science).Data expressed by mean ±SD .The comparison between the studied groups was tested at the level of 0.05 of significance. [10]

Results and discussion

Analysis of data showed no significant differences in GSH concentration between normal weight subjects and obese subjects (12.50±3.71 *VS.* 10.30±4.20), p>0.05, as in a Table (3).It's also not a significant difference between normal weight subjects and overweight subjects (12.50±3.71*VS.* 11.17±4.9), P>0.05, as shown in Table(4).

Table (3): GSH Concentration comparison between normal weight subjects and obese subjects .

Group / Parameter	Normal weight subjects	Obese subjects
GSH concentration (µg/dl) ±SD	12.50±3.71	10.30±4.20
No	50	50
p.Value	0.15	

Table (4): GSH Concentration comparison between normal weight subjects and overweight subject .

Group / Parameter	Normal weight subjects	Overweight subjects
GSH concentration (µg/dl) ±SD	12.50±3.71	11.17±4.9
No	50	53
p.Value	0.73	

Among adults ,the prevalence of obesity has doubled in the past 20 years .[2]Although it has been argue that the independent effect of obesity on cardiovascular diseases risk is small, obesity promote cluster of risk factors that greatly increase early cardiovascular diseases. The idea that obesity is a state of chronic oxidative stress and inflammation.[11] Many previous studies have kept focus on the role of ROS in obese diabetic patients. [12]Although both hyperglycemia and obesity go together to cause oxidative stress ,the contribution of obesity to this stress has not been quantified.[7] Glutathione peroxidase an enzyme protects the organism from oxidative damage by free radicals and optimal level of GSH is required in the body to potentiate glutathione peroxidase activity to stay healthy. [13] Many studies have found association between obesity leading to oxidative stress and diabetes mellitus type 2,[14]and many others have shown that the level of ROS increase in obesity. [15,16] Even with all these findings, this study did not reveal any significant differences between healthy weight volunteers and obese subjects or overweight subjects. Many reasons may explain and support this inconsistent results .The glutathione concentration may be affected by many factors related to the life style such as non healthy food

and psychological stress, leading to decrease antioxidants level.[17]A study performed by (Huchul de Campus et al,2006) has shown that people with anxiety and sleep apnea show a depletion in GSH level .[18]Other study performed by (Gulec and et.al,2012) has concluded that primary insomnia causes significantly lower glutathione peroxidase activity compared with controls.[19] Drugs intake, such as non steroidal anti-inflammatory drugs dramatically decreases glutathione level.[20]

In conclusion, in spite of the importance of obesity in contributing to oxidative stress, this study did not find significant differences in GSH concentration between normal weight volunteers and obese subjects or overweight subjects. This may be attributed to many factors cause dysregulation in GSH level rather than obesity and other oxidative stress associated diseases.

References

1. Medicinenet.com/obesity-weight-lose/article.htm
2. Medicalnewstoday.com/info/obesity
3. Isomaa B.et al. ***Diabetes Care***.; 2001,**24**,683-689.
4. Furukawa S,Fujita T and Shimamura L. ***J clinInves.;*** 2004,**15;114(12)**:1752-1761.
5. Pompella A,Visivikis A, Paolicchi A, Tatan U ,Casini AF.***Biochemical pharmacology .;*** 2003, **66(8)**,1499-1503.
6. Pastore A ,Ciampalini P,Tozzi G ,Pecorelli L, et al. All ***Diabetes*** .,2010,**13**. 272-277.
7. Goyal R ,Singhai M, Faizy A. ***.J Midlife health.;*** 2011,**2(2)**,72-76.
8. Riener CK ,Kada G,Gmber H J. ***Anal Bbioanal Chem.;*** 2002,3**73(4-5)**,266-276.
9. Al Aubody N.M. Profile patients.Basra University.2004.
10. SPSS Statistical Package of Social Science .Version 15,USA.2006.
11. Devaraj S ,Goyal R, Jialal I.***.US endocrinology.;***2008,**49**,32-37.
12. Kochar NI ,Umathe S N. ***Pharmacol Res.;*** 2009, **61**, 665-672.
13. Lee Y S,Kim AY,Choi JW,Kim M,et al. ***Mol Endocrine.;*** 2008,**22**,2176-89.
14. Bhatia S,Shukla R,Venkata MS,et al. ***Clin. Biochem.;*** 2003,**36**,557-562.
15. Mokdad AH,Ford ES, Bowman B A, et al. ***JAMA.;*** 2003,**289**,76-79
16. Kawakita S,Kitahata H,Oshita S. ***World J Gastroentirol.;*** 2009,**15**,4137-4142.
17. Patki G,Solanki N ,Atrooz F,Allam F,Salim S. ***Brain Res.;*** 2013, **3** , s0006- 8493 (13)01307-3.
18. Hachul de :Campus H ,Brandao LC, et al. ***Climaleric.;*** 2006,**9(4)**,312-9.
19. Gulec M ,Ozko H,Selvi Y ,et al. ***Bio Psychiatry.;*** 2012,**37(2)**, 247-51
20. Micheli L,Fiaschi Al,Giorgi G,Cerretani D. ***Agent Action.;*** 1992,**c**,106-108.