Role of Glutathione in male infected with giardiasis

Lecturer Maysoon K. A. Al-Hadraawy/  medical laboratory/ Department Technical institute
Correspondence should be sent to: Mhza814@yahoo.com

Abstract

The study was conducted 69 out patients and 30 healthy males to determine the influences of infected with Giardia lamblia on levels of Glutathione in males infected with G. lamblia in compared with healthy group. Who have visited Al-Sadder medical city and Al-Hakeem Hospital in Al- Najaf Province during the period from January till August 2012.Diagnosis infection with this parasite by using the wet amount microscope for stool from patients. The results showed significant decrease (P<0.05) in Glutathione in G. lamblia infection patients in compared to control group.

Key word : influences , Giardia lamblia , Glutathione

Introduction

Giardia intestinalis is a world wide cause of intestinal infection. It is a flagellated enteric protozoan that infects humans. It exists in trophozoite and cyst forms and the infective form is the cyst. Trophozoites of G. intestinalis are found in the upper part of the small intestine, where they live closely attached to the mucosa. They are found at times in the gall bladder and in biliary drainage. G.intestinalis infection causes severe intestinal disorder, most commonly, diarrhea and related symptoms due to malabsorption [1].

G. lamblia is best known as the organism responsible for “Beaver Fever” or “Backpacker’s Diarrhea” because of its proliferation in streams and rivers, G. lamblia can completely destroy the surface of the mucosal barrier. The destruction of the small intestine’s barrier causes inflammation, reduction of surface area for nutrient absorption, lactose and sucrose intolerance, and inability to digest fats and oils. It can also result in the formation of deep pockets, in which mucus plugs form, creating an environment that harbors and protects infectious organisms. Another effect of giardiasis infection is hypermotility (or colonic dumping), in which food moves through the small intestine too quickly and therefore isn’t completely digested. The undigested food dumps directly into the large bowel, which creates protein putrefaction and a fertile environment in which yeast, fungus, and other unwanted microorganisms proliferate. [2].

Glutathione is synthesized by the successive action of Y-glutamylcysteiny1 synthetase (GGCSS) and glutathione synthetizes. Both require ATP. Reduced glutathione inhibits GGCS by non-allosteric feedback. Glutathione is degraded by Y-glut amyl Trans peptidase, Y-glut amyl cyclotransferase and 5-oxoprolinase and by a peptidase, thus conforming the Y-glut amyl cycle [3]. Glutathione (YL-glutamly-L-cysteinyl-glycine) is low molecular weight thiol that is found in highest concentration in mammalian cells. It protects against toxicity from highly electrophilic compounds or from their metabolites and against free radicals, [4]. The defense mechanism used by mammalian cell to eliminated free radicals are multiple and diverse [5].It is also participates in detoxification processes of xenobiotic of electrophilic character, via glutathione -s- transferases [6]. In addition it is important in protecting DNA and lipid membrane [7].
Glutathione reductase is a flavoprotein catalyzing the NAD PH-dependent reduction of an active form of glutathione which is disulfide (GSSG) to an active form of glutathione which is reductase glutathione (GSH). The reaction is essential for the maintenance of glutathione level: \( \text{NADPH} + H^+ + \text{GSSG} \rightarrow \text{NADP}^+ + 2\text{GSH} \). Selenium dependent glutathione peroxidase and catalase activity have not been detected [8], and superoxide dismutase activity is very low [9].

The glutathione (GSH) and the thioredoxin (Trx) system have overlapping and differential targets, and function in great variety of biological processes. These pathway operate through redox cascades that involve transfer reducing equivalents from NADPH to targets through a series of dithiol-disulfide reaction or variations of them [10,11]. The differences in the thioredoxin and glutathione pathway between parasitic flatworm and their mammalian hosts, and the lack of redundancy of these redox pathways have prompted studies which have recently resulted in validation of TGR as a novel drug target for Platyhelminthes [12].

In most organisms, including the mammalian hosts of Platyhelminthes parasite, cellular redox homeostasis, ant-oxidant defenses and supply of electrons for deoxyribo-nucleotide synthesis rely on two major and independent pathways the glutathione (GSH) and the thioredoxin (Trx) systems [11,13].

**Subjects and Methods**

**Specimens**

From January till August, 2012, 69 samples were collected from patients and 30 healthy male who attended the clinics in AL-Sadder Teaching Hospital and AL-Hakeem Hospital in AL-Najaf province. Stool samples were collected into clean, wide-mouth specimen bottles, from male patients and blood samples were also drawn from the same patients by vein-puncture into specimen tubes and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Backman/counter, Germany) to separate the serum and collected in other sterile tubes, each sample of serum was kept in deep freeze at -20Cº till used for the determination Glutathione.

**Specimen processing**

Freshly voided stool specimens were processed and examined microscopically using X40 objectivens for intestinal parasites as described by Paniker (1989). Ten X40 objective fields of the stool smears were examined before a slide was considered negative.

**Glutathione detection**

The test intended for quantitative of human glutathione (GSH) concentration in serum through the Immunosorbant assay (ELISA) using bio Elisa reader EL x800 (bio kit, U.S.A.) in virology laboratory of AL-Sadder Medical City in Al-Najaf province. The assay Max Human Glutathione (GSH) ELISA kit was achieved according to the manufacturing company (CUSABIO, U.S.A.) as a follows:

**Procedure**

1- Prepared all reagents, working standard, and samples as directed in the previous sections.
2- Incubate for 2 hours at 37Cº. A plate layout is provided to record standards and samples assayed.
3- Remove the liquid of each well, do not wash.

4- 100 µl was add of Biotin-antibody (1 X) to each well. Cover with anew adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.

5- Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with wash buffer (200 µ l) using a squirt bottle. Multi-channel pipette. Manifold dispenser, or auto washer, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the lost wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6- 100 µl of HRP-avid in (1 X) were added to each well. Cover the microtiter plate with a new adhesive strip incubates for one hour at 37°C.

7- Repeat the aspiration /wash process for five times as in step5.

8- 90 µl of TMB substrate were added to each well. Incubate for 15-30 minutes at 37°C. Protect from light.

9- 50 µl of stop solution were added to each well, gently tap the plate to ensure thorough mixing.

10- The absorbance on bioelisa reader EL x800 was read at a wave length of 450 nm immediately. Results were provided within one minute on the LCD display and printed out on the printer.

**Results**

GSH concentrations of *Giardia lamblia* infection patients in result of this study were highly significant increase (P< 0.01) in compared to healthy group, as seen in Figure (1).

![Figure(1): Comparison of GSH Concentration between Healthy Group and Patients Suffering from giardia lamblia Infection.](http://www.uokufa.edu.iq/journals/index.php/ajb/index / http://iasj.net/iasj?func=issues&jId=129&uiLanguage=en)

E.mail: biomgzn.sci@uokufa.edu.iq
*Significant difference (P<0.01) between control group and patients.

**Discussion**

The current result showed that the serum concentration of GSH was significantly decreased in the giardiasis infection patients compared to healthy groups. The decrease of GSH level in the serum of patients can give an idea on oxidative damage. Decrease level of this parameter maybe due to increasing in it's the catabolism, due to decrease in the GSH synthesis and due to increase transformation it to disulfide form GSSG [14,15]. It may be due to the resistance of parasite to phagocytosis by increasing the free radical and this leads to decrease in level of GSH in serum of patients. [16].

Further more, the low level of GSH may be attributed to the low level of the substrate (which necessary for building it) during oxidative stress such as NAD pH which promise important stimulation material for action of Glutathione reductase which act to restatement the active form of glutathione from inactive form[17]. It also may react with superoxide anion to form oxidative factor called peroxynitrite which lead to peroxidation cell membrane [18] and product increases in the MDA and decrease in GSH. The decrease of level of GSH in serum maybe attributed to the increase in the nitric oxide which considers the more toxic than another free radical that increase in the giardiasis infection disease and consider a mechanism defense against the harm influence of parasite [19]. This result is study similar to the findings of [20] who study the decrease of GSH level in three tissue organ (liver, spleen and heart)of rats which are infected with Myocardia asteroids compared to the healthy control groups. Also similarly [21] who recorded that the level of glutathione decrease in the tissue of infected mice by *T. vaginalis* compared to healthy groups.

It is also similar to the current study was by [22] who recorded significant decrease in the levels of glutathione that provided that the mean values for GSH were 42.38 ± 0.10 in serum from human patients infected with *Entamoeba coli* compared to healthy groups. It is also similar to the study by [23] who recorded a significant decrease in the level of glutathione in serum from women infected with *Toxoplasma gondii* parasite compared to healthy control groups.

**Reference**


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دور الجلوتاثيون في الذكور المصابين بالجبارديات

ميسون خضير الحدراوي

الخلاصة

صممت هذه الدراسة لتحديد تأثير الإصابة بطفيلي الجبارديا على مستوى الكلوتاثيون للأشخاص المصابين بهذا الطفيلي في مدينة النجف الأشرف وذلك باستخدام طريقة الفحص المجهرية المباشر لبراز المصابين حيث اشتملت الدراسة الحالية على 69 حالة إصابة بطفيلي الجبارديا و30 حالة من أشخاص غير مصابين ارتداه مدينة الصدر الطبية ومستشفى الحكم في محافظة النجف الأشرف لمدة من كاثن الثاني حتى اب 2012 . اظهرت الدراسة الحالية حصول انخفاض معنوي (P<0.05) في مستوى الكلوتاثيون في الأشخاص المصابين بطفيلي الجبارديا مقارنة بمجموعة السيطرة.