Prevalence of Toxoplasma Gondii in Samarra City and Its Effect on Ghrelin and Growth Hormone

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Abstract
The current study included the examination of 200 blood samples, where samples were collected randomly from both sexes of age between 19 and 47 years of age from August 12th, 2021, to May 2nd, 2022. The total number of positive samples, consisting of individuals carrying Toxoplasma gondii parasites, was 95 (47.5%) and the rest were considered to be negative for the disease. The results showed a morally high IgM ratio in infected samples compared to uninfected samples (0.347) picograms/ml and the high IgM in the parasite's precipitin serum may be due to the tendency of the opposite to stay for a long time in the case of newly infected brains and may therefore be against IgM in the case of acute acuity indicating a low level for several years. The results of the current study indicate that there is a significant increase in growth hormone level in the serum in patients with toxoplasmosis moral difference was found at the probability level (P<0.01), with the concentration of infected persons (7.188 ± 2.7997 micro-unit/ml), while the concentration of the hormone in the control group (5.626 ± 1.839 microunit/ml). The current study showed a significant increase in the level of ghrelin in the serum of people carrying the parasite. The concentration of ghrelin in patients was (868.713 ± 188.325 ng/ml) compared to control (682.511 ± 101.907 ng/ml) and at a probability level (P<0.01). The aim of this study is to know the effect of toxoplasmosis on ghrelin and growth hormones.
Keywords: Toxoplasma gondii, toxoplasmosis, ghrelin, growth hormone.

1. Introduction

Toxoplasmosis arises as a result of the infection of the Toxoplasma gondii parasite found in the faeces of cats mixed with Oocysts or eating uncooked meat that has the gastrointestinal phase in the form of Tissues cyst, which has a complex and multiple life cycl. Toxoplasma gondii is spreading globally, as some statistics indicate that the proportion of Toxoplasma gondii is estimated to be between 15% and 85% in the population groups (Emilie et al., 2017). This depends mainly on the sex, age and immune status of individuals. In addition to the presence of an important factor, which is the extent to which an individual mixes with cats in the way he cooks meat. It should be noted here that the infection may not cause obvious pathological symptoms. The individual's attention to medical consultation. The immune system works with both fungal I nate immunity and acquired immunity in controlling the active infections of the parasite during the first weeks or months (Sasai et al., 2018) Toxoplasma gondii is a mandatory parasitic parasite with the ability to infect organisms. The infection may be without clinical symptoms. This parasite also causes a variety of clinical diseases for humans and animals. Therefore, this disease is an opportunistic disease for weakened immunity (Mahfouz et al., 2019).

The epidemiological study in Iraq, which was carried out by (Al-Nasiri, 2013) proved an increase in the frequency of infection in the toxoplasmosis parasite since 1981 and so far compared to the 1970s, that the diagnosis of the Toxoplasma parasite is made by investigating antibodies, whether for acute mingling IgM or chronic IgM. Human body hormones change during pregnancy and thus affect women's immune response. This increases the chance of infection due to the dwindling initial inflammatory response of the body, especially antibodies to parasites. This results in unwanted developments in toxoplasmosis. In the second and third week of pregnancy, there is an increase in the hormones of bergestrogen and strabdol when toxoplasma spreads. Low cellular immunity is associated with a higher level of steroid hormones, which increase the parasite's susceptibility to survival in the body and cause a lot of congenital diseases that occur during pregnancy (Hussain et al., 2017).

Growth hormone (GH) or somatotropin, also known as human growth hormone (growth hormone or growth hormone) in its human form, is a peptide hormone that stimulates growth, cell proliferation, and cell regeneration in humans and other animals. It is therefore important in human development. Growth hormone also stimulates the production of IGF-1 and increases the concentration of glucose and free fatty acids (Ranabir & Reetu, 2011) is a type of mitrogen that is limited only to receptors to certain cell types. GH is an amino acid 191, a single-chain peptide that is synthesised, stored, and excreted by somatic cells within the lateral wings of the anterior pituitary gland. The effects of growth hormone deficiency (GH) vary depending on the age at which

doi.org/10.31838/hiv22.02.461 Received: 11.04.22, Revised: 25.05.22, Accepted: 04.08.22

they occur. Changes in somatomydin can lead to growth hormone deficiency with two known mechanisms; tissue failure to respond to somatomedin, or liver failure to produce somatomedin (Ignatavicius & Workman, 2015).
Ghrelin hormone is characterised after isolation from the stomach of mice and is oligopeptide consisting of 28 amino acid residues. Ghrelin is an internal bond of the growth hormone secretion receptor (GHSR) and strong growth hormone release activity has been observed after pharmacological administration of ghrelin, although elevated levels of prolactin, Adrenocotyicotropic hormone (ACTH) and cortisol have also been observed after infusion of ghrelin (Nagoya et al., 2001). Growth hormone release activity is likely to have an effect via HGH secretion hormone. (GHRH) The main source of ghrelin is assumed to be the stomach (Moller et al., 2003), at least in non-pregnant individuals, but ghrelin or mRNA has been proven at several other sites, including the ovary, testicle, and placenta in both mice and humans (Gaytan et al., 2013). The research aims to find out the phical relationship between the incidence of toxoplasmosis and the vital levels of these two hormones and their impact and effect on the infection.

2. Materials and Methods

Location and duration of study

This study was conducted in the city of Samarra, where samples were collected from random people of both sexes between the ages of 19 and 48 years, for the period from August 12th, 2021, to June 5th 2022, where samples were taken from women and men who frequent doctors specialised in outpatient clinics and health centres in the city of Samarra. Where 200 samples were collected randomly to detect the presence of the toxoplasma parasite.

Sample Collection

Blood samples are collected from people who are due to medical laboratories by drawing 5 cm3 of venous blood and then placed in plastic pipes and left to clot at room temperature and then discarded at a speed of 3000 cycles / minute for 5 minutes to separate the serum. The serum is transferred to plastic pipes and kept frozen at a temperature of 20 degrees Celsius until the tests are performed. In this research, the questionnaire was adopted as a data collection tool as suitable for such cases. A questionnaire was designed to achieve the research objectives. The questionnaire was distributed to the people from whom samples were collected. The questionnaire included the patient's personal information and the patient's medical history (such as age, weight, height, date of onset of injury, housing, animals in the home, academic achievement, and blood type).

Toxoplasma IgM ELISA

Antibodies to cat disease (Toxo.) were investigated. By following the ready-made steps referred to in several analyses customised for the ELISA device according to its manufacturer. Diluted serum (serum diluent contains sorbent to remove rheumatoid factor and human IgG interference) is added to wells coated with purified antigen. The specific antibody IgM, if any, binds to the antigen. All unrelated substances are washed, and the conjugate enzyme is added to bind a complex (antibodies – antigen), if present. The excess comparative enzyme is washed, and the base material is added. The plate is incubated to allow hydrolysis of the base material by the enzyme. The intensity of the colour generated is proportional to the amount of the specific antibody to IgM in the sample.

Blood group test

Blood group test was performed to determine the relationship between blood group type and infection and their association with hormonal changes during the infection.

Hormonal tests

Determination of serum hGH level

Determine the level of growth hormone in serum using the special Kit test kit prepared by the American company bio-Techne (Catalogue Number: MBS580020). The HGH-ELISA test is based on the solid phase sandwich method. Samples and conjugate reagent (anti-HGH biotin and HRP) are added to wells coated with Streptavidin. Growth hormone in the serum of patients binds to the identical pair of Abs, which leads to the formation of sandwich-like layers and at the same time the compound is stabilised on the plate through streptavidine and biotin reactions. Unbound protein and conjugate HRP are washed off. Through the washing step. When adding the substrate, the intensity of the colour is proportional to the concentration of growth hormone in the sample.

Determination of serum ghrelin hormone level

The level of growth hormone in serum was determined using a special kit and prepared by the American company bio-Techne. GHRE ELISA has applied competitive enzyme immunoassay technique using GHRE antibodies and GHRE-HRP consortium. The assay sample and insulating solution are incubated with conjugate GHRE-HRP in a pre-painted plate for one hour. After the incubation period, the wells are poured and washed five times. The wells are then incubated using the base material of the HRP enzyme. The result of the reaction of the main substance with the enzyme is a blue-coloured compound. Finally, the stop solution is added to stop the reaction, which in turn will turn turn turn turn the solution to yellow. Colour intensity is measured by a spectrophotometer measurement method at 450 nm in the microplate reader.

3. Results

Table (1) Shows a significantly high IgM level in

patients (3.6 Pg/ml) compared to the control group. As for IgG, its level was (0.107) pg/ml in patients, while it was (0.017) pg/ml in control group.

Table 1: Concentrations Of IgG, IgM Antibodies of Toxoplasmosis.		
Study groups	Toxoplasmosis	
	Positive IgG	Positive IgM
Patients	1.037 ± 0.107	3.600 ± 0.3
Control	0.381 ± 0.017	0.348 ± 0.065
P- value	0.0004**	0.005*

Table (2) shows that there is a significant difference between IgG & IgM levels with respect to blood groups A+, B+ and AB. Group O+ was noted that there is no significant difference between the level of ecunoglobulin. Where the concentration of IgG was in type A+ (0.5032±0.1789) compared to the concentration of IgM (03971±0.0949) for the same family. Type B+ was the concentration of IgG (0.5457±0.1588) compared to the concentration of IgM (0.3915±0.1397). The concentration of IgG was in the AB+ family (0.4382±0.0439) compared to the concentration of IgM (0.4798±0.173) for the same family. The O+ family was the concentration of IgG (0.4953±0.1247) compared to the concentration of IgM (0.4172±0.1569).

Table 2: Concentrations of IgG, IgM antibodies Toxoplasmosis in study totals compared to blood aggregates.		
Blood group	IgM (Toxoplasma Ab)	IgG (Toxoplasma Ab)
A+	03971±0.0949 b	0.5032±0.1789 ab
B+	0.3915±0.1397 b	0.5457±0.1588 a
AB+	0.4798±0.173 a	0.4382±0.0439 b
O+	0.4172±0.1569 ab	0.4953±0.1247 ab
Control	0.2405±0.0574 c	0.2405±0.0574 c
P-value	0.0008 **	0.00002 **

The results in the current study indicate that there is an increase in the level of growth hormone GH in serum in patients with the parasite *T. gondii*, where a significant difference was found at a probability level (P<0.01), with the concentration of those infected (7.188 ± 2.7997 microunit/ml), while the concentration of the hormone in the control group, table (3).

Table 3: Concentrations of growth hormone HGh, Toxoplasmosis in study groups.		
Study groups	Growth hormone (hGH) Mean ± SD	P-value
Patients	7.188 ± 2.7997	0.0002**
Control	5.626 ± 1.839	
**significant		

The current study showed a significant increase in the level of ghrelin in the serum of people carrying the chondular arch parasite, as shown in table (4), where the concentration of ghrelin in patients with parasite (868.713±188.325 ng/ml) compared to control totals (682.511 ± 101.907 ng/ml).

Table 4: Concentrations of ghrelin hormone GHRE for parasite arched chondosis Toxoplasmosis in study groups.		
Study groups	Ghrelin hormone (GFRE) Mean ± SD	P-value
Patients	768.713 ± 188.325	0.0002**
Control	682. 511 ± 101.907	
**significant		

4. Discussion

The reason for the increase in the IgM in the serum of the parasitic to the tendency against to stay for a long time in the case of recent infections. Thus, IgM antibodies in case of acute infections may have a low-level indicator for several years. The presence IgM antibodies is usually detected to know when the infection occurs, if it is old or new. The IgM increases when the parasite divides and multiplies inside the body in response to the occurrence of reproduction. IgM begins to appear during the first two weeks of infection. It is an important indicator of acute infection and when the mother acquires infection during pregnancy or shortly before pregnancy, which puts the foetus to the risk of congenital infection (Belaz et al., 2015). The reason for the rise in IgG may be due to the continued presence of IgG antibodies, which is one of the most important components involved in the humoral immune response in controlling the spread of the parasite, which begins to appear one to two weeks after the injury and peaks at 8-6 weeks and then begins to gradually decrease for (2-1 years). The low calibre may last for life, while others have high titibre for many years and because of the IgG stays for a long time in the body, it controls the infection of the parasite and prevents its spread (Hussien et al., 2017).

In previous studies investigated on the relationships between the ABO blood type system and antibody resistance to T. gondii, but their conclusions were conflicting. Four studies reported an association between infection by this parasite and blood and

groups B and AB (Kolbekova et al., 2007). These studies suggested that antigen B could serve as a potential future for T. gondii. However, there are two other similar investigations in which there was no evidence of this association (Lecolier, 1990). The expression of glycoconjugates for ABO blood groups occurs in the epithelial cells of the gastrointestinal mucosa in approximately 80% of the population (Schenkel-Brunner, 2000). T. gondii uses the human digestive system (GIT) as one of the methods of infection (Sibley, 2004). Therefore, the occurrence of these two processes in the same tracts and the contribution of glycococonogates to infection by T. gondii justifies current research (Sibley, 2004).

The result is similar to those conducted in the Hawiji and Beiji areas of Iraq by Jubouri et al. (2013) and those conducted in Al-Qadisiyah governorate in Iraq by Hadi et al. (2016), they found a high rate of serum prevalence of toxoplasmosis among abortive women (Al-Jeboury et al., 2013; Hadi et al., 2016). But it differs from those done by Ahmad and others in Iran in 2014 who found a large linear trend to increase the general serovalence of toxoplasmosis by age (Ahmed et al., 2014).

The current results do not show any association between infection with *T. gondii* gondii and the ABO blood type system except for the moral differences between the concentrations of antibodies, and therefore, do not support those studies that suggest that antigen B, expressed in B and AB blood groups, serves as a potential receptor for this parasite in the gut GIT (Kolbekova et al., 2007). On the other hand, the results are consistent with investigations conducted with blood donors in Tanzania and pregnant French women, which did not report any association between this system and infection by *T. gondii*, (Lecolier, 1999). The suggestion that antigen B represents *T. gondii* receptors does not seem to be valid for the Brazilian population. The disagreement between these results may result in other studies from several factors. Low antigen B can exert an effect on the adhesion of *T. gondii* to the mucous membrane of the gastrointestinal tract and its contribution is obscured by the high prevalence of infection by these parasites in the world population, as well as through the molecular variation of their strains identified in some regions of South America (Colombo et Furthermore, the use of only female patients in this study may not be sufficient to detect the possible effects of sex in the ABO blood type system with infection with *T. gondii*. (Mustafa & AL-Samarraie, 2020).

T. gondii infection is also associated with the effect on hormones in the blood, including testosterone and glucocorticoids, although positive and negative associations have been reported in previous studies. These hormones are known to target the amygdala and other interconnected areas of the brain, including the hippocampus and hypothalamus, which in turn regulate circulating steroid hormone levels that influence behaviour (Heany et al., 2015; McEwen et al., 2016). And the immune response (Bereshchenko et al., 2018). Two close explanations for the relationship between *T. gondii* infection and host hormone levels are: 1) a direct mechanism in which topical cysts work directly on specific areas of the brain that affect physiology and behaviour) an indirect mechanism in which the immune response to infection and elevated cytokine levels affect brain function, physiology, and behaviour (McConkey et al., 2013; Webster & McConkey et al., 2010).

In pregnant women with toxoplasmosis, low levels of progesterone and oestrogen can trigger severe infection. However, the mechanism is unknown (Al-warid and Al-qadhi, 2012). Current studies indicate that there was no statistical effect in progesterone levels among infected and uninfected women with *T. gondii*, although higher progesterone levels were observed in uninfected women compared to a lower level in infected women. Furthermore, oestrogen levels in both chronic and non-chronic injuries did not appear in infected women and have shown a significant difference, although infected women had a higher level, compared to uninfected women. The results of the current study are in line with a previous study conducted in Mexico on the Halation of growth hormone in *Amoeba histolytica*, the results of which showed an increase in GH levels in people with liver infection resulting from the same parasite (Aldaba-Muruato et al., 2017). High levels of GH in the blood may be due to the exposure of the body to stress, as in the case of infections caused by intestinal parasites and others, because this hormone is one of the stress hormones (Ranabir & Reetu, 2011).

There are not many studies to support the effect of growth hormone on people with toxoplasms or vice versa due to poor physiological explanations, but in a study conducted by Natsheh et al., (2018) on growth hormone levels in people with congenital toxoplasmosis, where the hormonal study revealed growth hormone deficiency, after which the correction stabilised blood glucose levels without further episodes of hypoglycaemia. Growth hormone has been shown to modify bile acid synthesis and secretion, because it increases the secretion of hepatic bile acids, so a lack of pituitary hormones may be linked to diseases that cause cholestasis by promoting the accumulation of bile acid precursors and producing cholestasis effect. Therefore, growth hormone therapy will improve liver function. Although eye examination and computed tomography of the brain were normal, we believe that congenital toxoplasmosis diagnosed in the patient is the cause of pituitary weakness manifested in growth hormone deficiency. Pituitary disfunction here was partly where other hormones were within normal ranges (Natsheh et al., 2018).

Ghrelin is a hormone that is produced and released mainly by the stomach, with small amounts also released by the small intestine, pancreas, brain, kidneys, myocardium, hypothalamus, and pituitary gland. Ghrelin is transported in the blood circulation associated with high-density lipoprotein (VHDL) and

high-density lipoprotein (HDL). Changes in ghrelin levels were reported in parasitic infections (Erensoy et al., 2010; Parvin et al., 2014). Acile ghrelin affects glucose metabolism by modulating insulin secretion, amino acid absorption and bone formation, appetite, increased food intake, energy balance, digestive mobility, heart performance and anxiety (Leite-Moreira & Soares, 2007).

It is believed that ghrelin concentrations decreased in patients with intestinal parasitic infections compensate for an increase in glucose concentration. Thus, the relationship between insulin and ghrelin. The low concentration of ghrelin in patients observed in this study is consistent with the suggestion that this may be the main cause of anorexia in patients with parasitic infections (De Vriese et al., 2007). Another possible cause-effect relationship that may explain, at least in part, low endogenous ghrelin levels in infected patients may be the reduction of lipid peroxide that has increased as a result of parasitic infection (Kilic et al., 2004).

The low level of ghrelin in the blood in people with intestinal parasites may be due to exposure of the gastrointestinal tract to inflammation, which affects the hormone secretion process, which takes place mainly in the stomach and small intestine (Poher et al., 2018). The results of the current study were in line with a study conducted by AL-Hadraawy and others (2016) when studying the levels of ghrelin in patients with the Giardiasis parasite, where the level of ghrelin in those infected people was much higher than that of the hormone in the control group. This study contradicted the results of a study conducted by Erensoy and his group (2010) in Turkey, where a decrease in the level of ghrelin was observed in the serum of people with intestinal parasites, including Blastocystis compared to control groups.

5. Conclusion

From this study, we conclude that there is a correlation between the hormonal variables studied in patients with condescending toxoplasmosis, while it was noted that there is a relative relationship between the chemical variables of patients with the parasite.

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