Level of Dopamine, LipoxinA٤ and Testosterone in mice infected with Toxoplasmosis

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Abstract

Toxoplasma gondii **is a parasitic infection induced by a protozoan. results in alterations in the concentrations of brain neurotransmitters, including dopamine, lipoxin A٤, and testosterone hormone. Behavioural modifications may transpire in affected hosts. This study was conducted to evaluate the serum levels of dopamine, lipoxin A٤, and testosterone in mice infected with chronic toxoplasmosis. The research was conducted from August ٢٠٢٤ to February ٢٠٢٥.A total of ٤٢ serum samples were collected from mice BALB\c infected.** ***Toxoplasma gondii*** **was injected intraperitoneally into mice to induce chronic toxoplasmosis. The infected mouse serum were separated after ٤٠ days. The ELISA technique was utilized to evaluate Dopamine, Lipoxin A٤ and Testosterone levels in Toxoplasmosis infected mice. Results: The ELISA test revealed on ٣٢ positive samples (٧٦,١٩٪) and ١٠ negative samples (٢٣,٨٠٪). Dopamine levels were significantly higher in patients, with an average concentration of ٧٤٤,٤ ± ٣٣,٢٤ pg\ml, compared to the control group, whose average concentration was ٥٤٤,٤ ± ٢٤,١٠ pg\ml. LipoxinA٤ levels in patients were signified higher compared to control group, with an mean of ٣٦٨,١ ± ١٧,٣١pg\ml , ٣٠٠,١ ± ١٤,١٣ pg\ml. In contrast testosterone hormon level were significantly lower in patients with an average concentration of ١٣٠,٢ ± ٤,٧١٤ pg\ml, compared to the control group, whose average concentration was ١٧٢,٦ ± ١٨,١٥ pg\ml. Conclusions: The present study demonstrates elevated of Dopamine and lipoxinA٤ and lower testosterone in patientsز**

Keywords: ***Toxoplasm gondii*** **, Dopamine , LipoxinA٤ ,mice BALB\c Testosterone hormone , ELISA**

مستوى الدوبامين والليبوكسين A٤ والتستوستيرون في الفئران المصابة بداء المقوسات

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المستخلص:

داء المقوسات عدوى يسببها طفيلي احادي الخلية يؤدي الى تغيرات في الاعصاب والنواقل العصبية في الدماغ بما فيها الدوبامين واللايبوكسين والتستوستيرون والتي تؤدي الى حدوث تغيرات سلوكية في المضيف وبناء على ذلك اجريت هذه الدراسة لقياس مستويات الدوبامين واللايبوكسين وهورمون التستوستيرون في مصل الفئران المصابة بداء المقوسات المزمن . تم اجراء هذه الدراسة من شهر اب ٢٠٢٤ الى شباط ٢٠٢٥ جمعت ٤٢ عينة من مصل الفئران البيض السويسرسة BALB\c المصابة بداء المقوسات . حقنت الفئران بطفيلي *T.gondii* داخل الصفاق وبعد مرور ٤٠ يوم سحب الدم و تم فصل المصل بعد التأكد من تحول الإصابة الى الطور المزمن . استخدمت تقنية ELISA لتقييم مستويات الدوبامين واللايبوكسين والتستوستيرون . اظهرت نتائج اختبار ELISA التي اجريت على ٣٢ عينة مصابة (٧٦,١٩٪) و ١٠ عينة سيطرة (٢٣,٨٠٪) لوحظ ارتفاع مستوى الدوبامين في المصابات بمتوسط تركيز ٣٣,٢٤ ± ٧٤٤,٤ pg\ml مقارنة بمجموعة السيطرة التي كان متوسط تركيزها ٥٤٤,٤ ± ٢٤,١٠ pg\ml بالمقابل كانت مستويات اللايبوكسين A٤ للمصابات اعلى مقارنة مع مجموعة السيطرة بمتوسطات ٣٦٨,١ ± ١٤,١٣ pg\ml , ٣٠٠,١ ± ١٧,٣١ pg\ml . اما بالنسبة لهورمون التستوستيرون انخفض مستواه بشكل ملحوظ في المصابات بمتوسط تركيز ٤,٧١٤ ± ١٣٠,٢ pg\ml مقارنة بمجموعة السيطرة التي كان متوسط تركيزها ١٨,١٥ ± ١٧٢,٦ pg\ml . اضهرت الدراسة ارتفاع ملحوظ في مستويات الدوبامين واللايبوكسين وانخفاض في هورمون التستوستيرون في مجموعة المصابات.

الكلمات المفتاحية: داء المقوسات , الدوبامين , اللايبوكسين A٤ , الفئران البيض السويسرسة BALB\c , ELISA , هورمون التستوستيرون

Introduction

Both humans and animals are susceptible to contracting the parasite disease known as toxoplasmosis. It is estimated that approximately one-third of the global population is chronically infected with this protozoa infection [١], which is caused by Toxoplasma, a parasite that is distinguished by its capacity to infect a large number of intermediate hosts[٢]. *Toxoplasma gondii* is capable of infecting a broad variety of mammalian and avian species, however felids, which are the definitive hosts, are the ones that carry the sexual form of the

parasite in their intestinal cells and are the ones responsible for the transmission of oocysts across the environment through their faeces [٣]. *Toxoplasma gondii* is a neurotropic parasite that travels through the brain tissue and causes it to become localised in astrocytes, microglia, and neurological cells. There is a substantial relationship between the distribution of cysts throughout the brain and the behavioural changes that occur in affected participants [٤]. Research conducted on animals has demonstrated that chronic T. gondii infection is linked to hyperactivity, as well as decreased fear or increased risky behaviour. Furthermore, a more recent study reveals that T. gondii infection is linked to a more general loss of predator avoidance behaviour[٥]. This infection, when it is chronic, has the potential to bring about a change in the specific behaviour of its host. Furthermore, it is linked to the development of neuropsychological symptoms in mice. changes in neurotransmitters` levels such us Dopamine, LipoxinA٤ and Testosterone hormone[٦]. Dopamine dysregulation is consistently implicated in behavioural changes and neuropsychiatric manifestations[٧]. *T. gondii* infection alters dopamine metabolism via parasite-encoded tyrosine hydroxylase, leading to increased dopamine levels in brain regions and serum, which correlates with behavioural changes such as increased impulsivity and altered fear responses in mice. According to research, infections can cause neurochemical and synaptic changes in dopaminergic systems, which suggests that dopamine may play a role in the neuropsychiatric manifestations that are associated with toxoplasmosis[٩]. A modulator of endogenous anti-inflammatory activity, lipoxinA٤ helps to maintain a healthy balance of immunological responses[١٠]. There are changes in testosterone levels that occur during infection, that either increases or decreases depending on the species, the phase of infection, and the host, which has an effect on reproductive parameters and behaviours. Reduced levels of testosterone are connected with greater sexual behaviour, which may make it easier for parasites to spread, whereas increasing amounts are associated with

decreased levels [١١]. This study aims to isolation and diagnosis of a parasite *Toxoplasma gondii* from the placenta of aborted women in various areas of Salah al-Din and inducing an experimental injury in male and female mice (BALB \c) by injecting a tissue cyst sub peritoneal and evaluation of some standards nervous, immune and hormonal since it includes Dopamine, LipoxinA٤ and testosterone.

Materials and working methods

Time and location: The research was conducted from August ٢٠٢٤ to February ٢٠٢٥ .The experiments were conducted at tow locations, the first being The animal house at Tikrit University ,The second location is the Central laboratories at Tikrit University.

Parasite`s preparation

The tissue cyst from the aborted placenta of the affected women were isolated according to the method [١٣]The placenta was cut in to small pieces and then incubated with ٤ml of pepsin enzyme at ٣٧C° temperature the mixture was filtered ,and centrifugation process was carrid out for ١٠ minutes and then the precipitate was suspended in a saline solution until it was injected.

Animals

Forty two meal and female BALB\c mice aged ٦ weeks and weighing ٣٠_٣٥ g were Animal house at Tikrit University . All animals were kept in standard conditions; temperature of ٢٢±٢ C° , the humidity of ٦٠-٤٠٪, dark –light cycles of ١٢ hour, proper ventilation, and access to adequate water and food.[١٢] the parasites were subcutaneously injected(١٠٠tissue cyst per mice)[١٣] .

Blood Samples

Capillary pipettes were used to draw ١ml from mice's retro-orbital sinus. In gel tubes, blood samples were centrifuged at ٣٠٠٠ rpm for ١٠ minutes to collect serum. The tubes were left at room temperature for ١٥-٢٠ minutes to coagulate blood. Serum samples were maintained at -٢٠°C in ٢٠٠ µl Eppendorf tubes for tests to maintain quality until immunological testing. After sampling, all samples

were evaluated simultaneously to minimise repeated freezing and thawing, which could affect results[١٤].

Mice Dopamine , LipoxinA٤ ,Testosterone ELISA test

The serum concentrations of dopamine , lipoxinA٤ and testosterone were assessed using the enzyme –link immunosorbent assay (ELISA) (China Sunlong). ELISA paltes were coated with mice dopamine, lipoxinA٤, and testosterone antibodies. After adding samples to the plates, dopamine, lipoxinA٤, and testosterone levels linked with substrate solution colour development. A stop solution stopped the process and ٤٥٠nm absorbance was measured.

Statistical Analysis

All statistical analyses and graph generation were performed using Graph Pad Prism (Graphpad Software, La Jolla CA ,USA) .A p-value ≤ ٠,٠٥ was considered statistically significant.

Results and Discussion

The study found that dopamine infected mice had a significantly higher average concentration (٧٤٤,٤±٣٣,٢٤ pg\ml) than the control group (٥٤٤,٤±٢٤,١٠ pg\ml) (Table ١).

Table ١; Serum Dopamine level in patients with *T.gondii* and control groups

Parameters	Control N =١٠	Patients N = ٣٢	p. value
	Mean±S.E		٠,٠٠٠١*
Dopamine Pg\ml	٧٤٤,٤±٣٣,٢٤	٥٤٤,٤±٢٤,١٠	

In mice with toxoplasmosis, lipoxinA٤ concentrations were significantly higher (٣٦٨,١±١٧,٣١ pg\ml) compared to uninfected animals (٣٠٠,١±١٤,١٣) (Table ٢).

Table ٢; Serum LipoxinA٤ level in patients With *T.gondii* and control groups

Parameters	Control N = ١٠	Patients N = ٣٢	p. value
	Mean±S.E		
LipoxinA٤ Pg\ml	٣٠٠,١±١٤,١٣	٣٦٨,١±١٧,١٣	٠,٠٠٤٤*

In mice with toxoplasmosis, testosterone levels decreased significantly (١٣٠,٢±٤,٧١٤) compared to uninfected animals (١٧٢,٦±١٨,١٥) (Table ٣).

Table ٣; Serum Testosterone level in patients with *T .gondii* and groups

Parameters	Control N = ١٠	Patients N = ٣٢	p.value
Testosterone Pg\ml	١٧٢,٦±١٨,١٥	١٣٠,٢±٤,٧١٤	٠,٠٤٧٠

Discussion

The findings of this study appear to further substantiate the alterations in neurotransmitter signals and hormonal levels in hosts infected with toxoplasmosis. We have demonstrated that persistent infection by the parasite induces significant modifications in dopamine levels in mice, attributable to the parasite's invasion of neuronal and glial cells, potentially affecting the brain's neurotransmitter processing [١٥] .similarly the inflammation caused by the presence of the parasite may lead to an imbalance in the nervous system and affect dopamine metabolism . Dopamine is not only a neurotransmitter it also plays an immune role regulating the activity of T cells and macrophages . it may be elevated in response to stress or inflammation . some studies have shown that chronic infections such as Toxoplasmosis or neuroinflammation raise dopamine levels are an indicator of activation of the brain –immune –endocrine axis. This research aligns with the studies [١٦,١٧,١٨].

In toxoplasmosis a chronic stages the level increases lipoxinA٤ because key endogenous regulator of inflammation during infection ,influencing disease outcome and host pathogen balance. the presence of tissue cysts in brain cells stimulates inflammation in the area increasing expression lipoxinA٤ [١٩] its elevation often reflects the body attempt to suppress inflammation and activate tissue repair mechanisms studies have shown that LXA٤ is associated with regulating cytokines and reducing excessive inflammatory responses. The

increase may represent a compensatory response to protect tissues from inflammatory damage .These findings are in agreement with the studies conducted by [٢٠,٢١,٢٢] This study provides association between *T.gondii* infection and testosterone alteration .This study showed a clear decrease in the level of testosterone and this decline is attributed to decrease reproductive fitness in mice in chronic stages [٢٣]. The results showed an increase in serum testosterone concentrations, consistent with its known role in regulating immunity and reducing inflammation. The literature indicates that testosterone can inhibit the production of certain inflammatory cytokines, such as TNF-α and IL-٦, reinforcing its role in maintaining immune balance. The observed hormonal changes may be related to activation of the hypothalamic-pituitary-adrenal-gonadal (HPA-HPG) axis, particularly in cases of stress or chronic inflammation. The results of this research matched with both studies [٢٤,٢٥], and this research disagreed with the results [٢٦,٢٧,٢٨] Some studies have shown a link between elevated testosterone levels and toxoplasmosis, which may negatively impact fertility and contribute to infertility. Testosterone is one of the primary male hormones responsible for regulating reproductive functions and sperm production, but an imbalance can lead to impaired sperm formation and reduced sperm quality. This supports the hypothesis that the parasite may induce a dual hormonal and immune imbalance that contributes to impaired reproductive performance. Therefore, elevated testosterone in cases of toxoplasmosis may be considered an important biomarker that may be used to explain some cases of infertility [٢٨]. The combination of elevated serum dopamine, lipoxinA٤, and testosterone supports the hypothesis of a functional synergy between these molecules, with dopamine enhancing neural and immune activity, while lipoxinA٤ and testosterone suppress the inflammatory response to maintain homeostasis. Therefore, the current findings may reflect an adaptive defense mechanism of the body in the face of stress or chronic inflammation, and may

also open the way for a deeper study of the relationship between these markers in immune, neurological, and hormonal diseases.

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