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Detection of Toxoplasma Gondii DNA in Uterine and Malignant Prostate Tissues of Cancer Patients[1]

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ABSTRACT

The current study aimed to find the Toxoplasma gondii parasite in uterine and prostate tissue models infected with cancer, which were collected from the cities of Kut and Nasiriyah, and to identify infected organs using Real-time PCR based on (B1) gene and histological examination, and the other is to identify histological changes in tissues. Cancer as a result of infection with the parasite. By examining the histological sections of samples positive for the parasite, (120) hundred and twenty tumor tissue samples fixed with formalin as well as embedded with paraffin wax were collected from incoming patients and subjected to surgical intervention for samples located in the tissue laboratories of Al-Zahra and Al-Nasiriyah Teaching Hospital and previously diagnosed with prostate and uterine cancer a month ago October 2022 to January 2023 in my province of Wasit and Dhi Qar / Iraq, whose ages ranged from (25-93 years), as it included (40) samples of the prostate for males and (80) samples of uterine tissue from females.

Positive samples were taken using real-time PCR technology, and tissue sections were prepared to diagnose parasite presence and to identify histological changes in infected tissue models. The total number of samples positive for T. gondii parasite, based on the tissue sections, was 53 (44.16%) distributed according to organs, the percentage of infection was recorded in the prostate 5 (35.71%) and the uterus 19 (48.71%). These results indicated the possible role of infection with the Toxoplasma gondii parasite in causing different types of infection cancer.

It is concluded that infection with the Toxoplasma gondii parasite and its presence inside the cells has a role in the development of different types of histopathological changes, which in the future turn into possible cancers in the affected organs.

Keywords: *Toxoplasma cancer (prostate, uterine); real time PCR*

INTRODUCTION

The parasite Toxoplasma gondii, which is an obligate intra-cellular parasite, is the source of the infection known as toxoplasmosis. According to Seyed et al. (2019), toxoplasmosis affects people as well as other warm-blooded animals worldwide and has a considerable negative impact on both public health and the economy. Since they might result in a variety of clinical consequences, including chorioretinitis, miscarriage, mental retardation, hydrocephalus, even lethal mortality, and life-threatening encephalitis in persons with cancer and transplantation, T. gondii infections continue to pose serious public health issues (Davood et al.; 2019, Tooran et al.; 2019).

Infection with *Toxoplasma gondii* begins when the infective stage (sporulated eggs) is ingested in contaminated food or drink, often eliminated with the feces of infected cats. Furthermore, the Toxoplasma gondii parasite can easily spread to almost any other animal that shares the feline environment. As in humans, *Toxoplasma gondii* parasites usually enter the body by ingestion. This can happen when people touch their mouths with dirty hands, or eat raw, undercooked infected animal meat or poultry that contains tissue cysts (Cong *et al*., 2015).

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The development and clinical problems of malignant conditions that involve the majority of organs, like uterine and prostate cancer, may be affected by parasite infection. This parasite infection should be further investigated as a potential human carcinogen because cancer is a disease brought on by the abnormal cell division that affects developed as well as developing nations (Donghua et al., 2011). According to reports, long-term host defensive reactions brought on by ongoing infection could encourage cancer by causing inflammation that raises the rate of mutation in organs (Florian and Michael, 2005). It is possible for intracellular pathogens to compromise cellular defenses against cancer, which permits the accumulation of oncogenic mutations (Paul, 2009). Additionally, there is strong evidence linking toxoplasmosis to human malignancies (Hossein *et al*.; 2012).

According to Rebecca et al. (2015), the risk of cancer rises with smoking, age, air pollution, being overweight, physical inactivity, and other factors. Additionally, there has been a noticeable rise in the disease's frequency among young individuals in the Middle East (Nada, 2014). Through numerous works, the attention on toxplasmosis has significantly increased lately in Iraq (Assim and Saheb, 2018, Nafal *etal*.; 2019).

MATERIALS AND METHODS:

Sample collection

one hundred and twenty samples of tumor tissues fixed with formalin and embedded with paraffin wax were collected from the incoming patients who underwent surgical intervention for the samples present in the tissue laboratories of Al-Zahraa and Al-Nasiriyah Teaching Hospitals, which were previously diagnosed with prostate and uterine cancer, as they included 40 prostate tissue samples for males and (80) tissue samples.

Genomic DNA extraction

DNA was extracted from tissue samples preserved in paraffin block using the (G-spin) TM Total DNA Extraction Kit (Fixed Tissue Protocol) according to the specifications of the manufacture (iNtRON, Korea).

Probe and primers

Specific probe and primers targeted (94 bp) fragment amplification from (B1) gene of Toxoplasma gondii in various samples. Those primers and probe were developed by [18], and supplied through (Macrogen, Korea), the sequences of primer TOXO-R (5'_ AGCGTTCGTGGTCAACTATCGATTG _ 3'), and TOXO-F (5'_ TCCCCTCTGCTGGCGAAAAGT _ 3').; and the TaqMan probe sequences that are specific for (B1 gene) in the Toxoplasma gondii, has been (5_6FAM-TCTGTGCAACTTTGGTGTATTCGCAG –TAMRA_3).

Real-Time PCR

This method was made based on the approach that has been presented in (Mei *et al*.; 2000). A qPCR master mixture was produced using (RealMOD™ Probe HP 5X qPCR Master Mix), made according to the manufacturer's instructions, with all (20 µl) of reaction solution (Template DNA (5.0 µl); (B-1) forward primer (10pmol).) (1.0µL), B-1 Reverse Primer (10 pmol) (1µL), B-1 probe (20 pmol) (1µL), q-PCR master mixture (10 µL), PCR water (2 µL) is placed in Real-time PCR Thermo-cycler (Bio-Rad. USA).

Sectioning

The wax molds containing the tissues were placed in the microtome manual tissue cutting device, and then the required tissue thickness was determined by approximately (3 - 5) micrometers to obtain the form of strip sections of the tissue sections. The strips were placed on a black plate to take the appropriate ones, and Eppendorf tubes with a capacity of (1.5 ml) (Mcmillan & James, 2018).

Tissue staining

The prepared tissue sections were stained using hematoxylin-eosin acid stain according to the steps described by (Woods & Ellis, 1994). The formalin-fixed and paraffin-embedded tissues of prostate and uterine cancer were cut to 4-5 microns, loaded on glass slides and dried at room temperature for (24 hours).

Statistical analysis

The Chi-square test and the percentage have been used in order to analyze the results that were reached in the current study. It was noted that there is a relationship between the presence of DNA of the toxoplasmosis parasite and samples taken from prostate and uterine cancer that were included in the study (Al-Rawi, 1986).

RESULTS AND DISCUSSION:

Percentage of infection with toxoplasmosis parasites in the uterus and prostate) using the Real-Time polymerase chain reaction technique

The statistical results in the current work, which are confirmed in Table (1) and Figure (1), showed that the recorded infection rate with toxoplasmosis parasite in the uterine organs and prostate, using the real-time polymerase chain reaction technique in diagnosis, reached the highest rate of infection of the uterine organs (48.72%), as it gave A positive result was found in (19) samples out of (39) samples, while the lowest rate of infection with the Toxoplasma gondii parasite was recorded around (35.71%) in the prostate organs. There were (5) cases that gave a positive result out of the number of samples that were examined, amounting to14 sample.

Table: 2 Percentage of Toxoplasma parasite using tissue sections divided by organs (uterus, prostate)

Type of samples	The total number	Results	No.of sample	percentage
Prostate cancer	14	**Positive**	5	**35.71%**
uterine cancer	39	**Positive**	19	**48.72%**

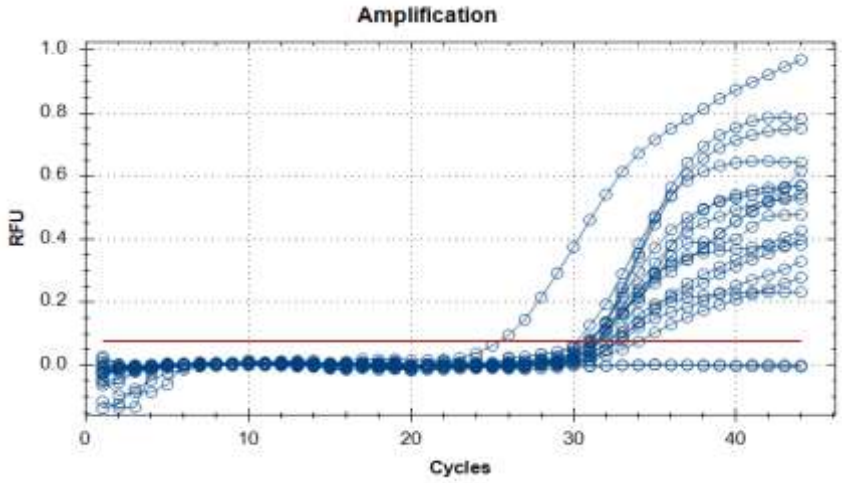

Figure. 1: Real Time amplification plots of B1 gene in the positive *Toxoplasma gondii* from Human samples.

The present work's findings were in agreement with a survey study that was carried out to identify the parasite's presence in 900 patients in China by (Cong et al; 2015), in which the parasite's DNA has been extracted from cancer tissues of (510) patients and T. gondii B1 gene has been amplified with the use of an approach called PCR nested. The survey study found a total infection rate of (35.56%) in cancer patients divided by organs. Lung cancer patients had the highest prevalence rate of the T. gondii parasite (60.94%), followed by patients with cervical cancer (50%) and patients with brain cancer (42.31%) and endometrial cancer (41.67%). T. gondii infection in cancer patients has been substantially correlated with exposure to the soil and eating undercooked meat.

Because the parasite might not have spread uniformly across the tissues from which samples or biopsies were collected, the infection rate in the prostate organs decreased in the current investigation (Cenci-Gogat et al., 2011). A few works suggested that the hormonal system in tissues infected with the parasite Toxoplasma gondii plays a significant role in the stability of the parasite through influencing the tissue activities of the parasite and the ability of the parasite, by living in the tissues of the host, to use these cells to its advantage. Through their effect on the immune system of people infected with *Toxoplasmosis*, the parasite also has the ability to exploit these changes to escape from the immune system, through the attachment of these hormones to special receptors on the parasite, which leads to preventing the effect of antibodies secreted against the parasite by the host (Aabasian *et al*.; 2016).

Histological changes in cancerous tissues in the organs (prostate, uterus) and their causes

The existence of *Toxoplasma gondii* parasite in the present work with its rapidly multiplying phase (tachyzoite) and tissue cyst containing the slow multiplying phase (Bradyzoite) was recorded in the histological sections of the organs examined from the tissues of the prostate and uterus. For the prostate, the follicles of the prostate gland appeared proliferating and full of follicle proliferation, where it was observed that the alveolar cells lining the prostate follicles proliferation with the disappearance of the inner lining (Acinus of the follicle), in addition to the proliferation of collagen in the interstitial tissue with infiltration of inflammatory cells, and the presence of hemorrhage within the tissue of the gland was observed in addition This indicates the infiltration of inflammatory cells of large macrophage cells, and the proliferation of transformed cancer cells in the dysplastic cells that line the follicle, which appear in a manner resembling papillary projections. The instar is slow to reproduce, as shown in Figure (2).

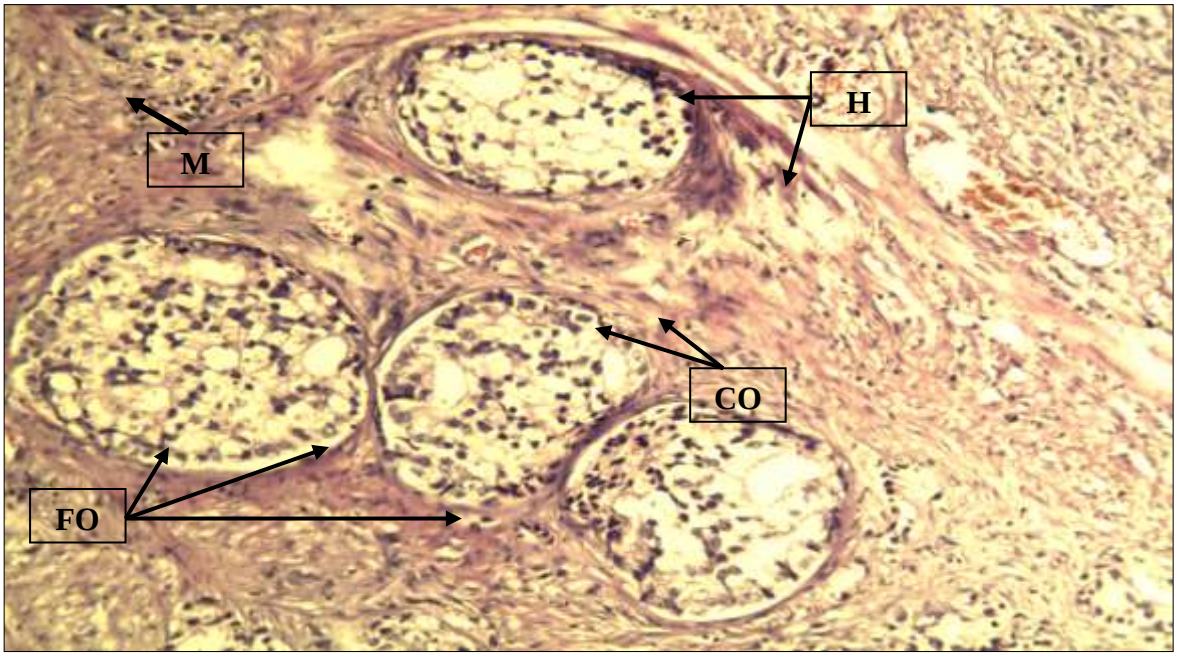

Figure (2) A section of prostate cancer tissue infected with the Toxoplasma gondii parasite, *T. gondii*, in which the proliferation of follicles filled with cells (FO) with collagen proliferation in the interstitial tissue (CO) is noted, in addition to infiltration of inflammatory cells (M) with bleeding inside the gland (H). 10 x H&E

As for the histological changes of uterine cancer, the presence of a parasitic cyst containing the slow proliferation phase and the rapid proliferation phase was recorded within the serous tissue of the uterus, as well as clear bleeding with the existence of volatile inflammatory cells in the serous tissue, in addition to the proliferation of the endometrium, which can be observed as follows: Invade smooth muscle bundles. The uterine wall as a result of the proliferation of columnar cells, which appear as an extension within the uterine tissue, as in Figure (3).

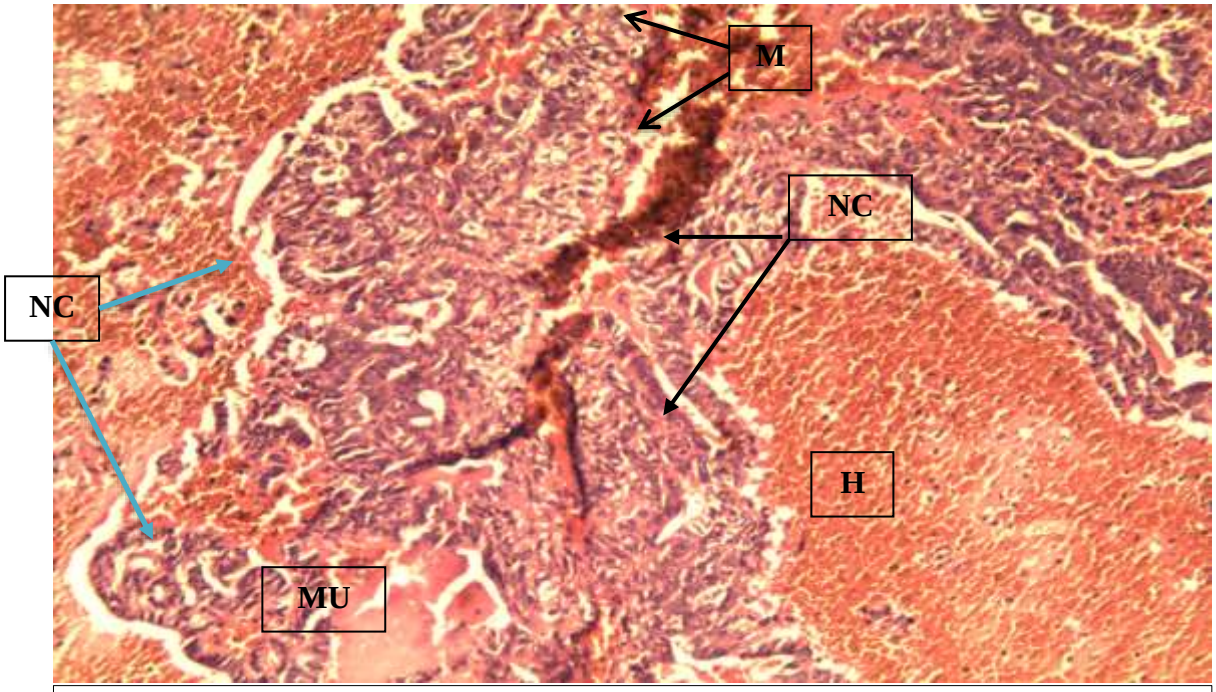

Figure (3) A section of tissue from uterine cancer infected with *Toxoplasma gondii* parasite, in which the proliferation of columnar cells lining the uterine tissue (NC) is observed within the smooth muscle of the uterine wall (MU) with volatile inflammatory cells (M) and severe bleeding in them (H). (10 x H&E)

The immune response to Toxoplasma gondii may be a contributor to histological and neurological changes (Rivest, 2009). The irregular distribution of necrosis within infected tissues has been detected by Toxoplasma gondii (Evans *et al*.; 2014) and there is real confirmation of the presence of the parasite in neurons (Cabral *et al*.; 2016).

It causes infection with the *Toxoplasma gondii* parasite In prostate tissue, tissue damage contributes to the production of several cytokines and inflammatory chemicals that promote the formation of inflammation in the surrounding blood vessels, immune cells and fibrous tissue, which leads to hypertrophy and proliferation in the tissues. Layers of epithelial cells and defective basal or cuboidal cells in prostate tissue, which proliferate in a rapidly spreading phase within the host cell of chronic toxoplasmosis (Colinot *et al*.; 2017). Toxoplasmosis of prostate tissue causes significant chronic inflammation with monocyte overexpression with histologic hyperplasia and basal epithelial cell proliferation (Colinot *et al*.; 2017).

The resulting necrosis in the tissues is the invasion of the rapidly multiplying phase of the parasite to the cells of the host, where they are added inside, leading to the formation of large voids in the tissues as a result of the formation of cysts of the parasite. Parasite tissue. Damage to these tissues leads to cell death through secretions and toxins produced through *Toxoplasma gondii* parasite, leading to deformities. or disturbances in the cells that make up the quadrant prophase stage (Jabbar *et al*.; 2020).

Toxoplasma gondii parasite was found in the rapidly growing phase in the uterine tissues as well, and it is thought that the parasite's capacity to migrate through the endometrium's muscles and invade its tissues is what causes blood vessels to become congested. Along with having the potential to release certain protein substances which stimulate platelets as well as endothelial cells through secreting certain chemical media, it distributes throughout the blood. This increases blood vessels and aids in the aggregation and adhesion of platelets and blood components on the walls. vascular permeability, which allows the quickly growing phase to enter the tissue and settle in the organ (Bayat *et al*.; 2013).

CONCLUSIONS

A thorough understanding of the seroepidemiology of the toxoplasmosis infection in the cancer patients is provided by this study. There is a discrepancy in the rate of toxoplasmosis parasite infection among the different cancer organs

used in the current study, based on the stage of infection, and the importance of the organ in relation to the parasite, while chemotherapy and radiotherapy in cancer patients led to changes that weakened the immune system, which caused the activation of the parasite toxoplasmosis.

Conflicts of interest: The authors declare that there have not been any conflicts of interest.

Ethical Clearance: The Iraqi government's health, environmental, higher education, and scientific research ministries have ethically approved the research conducted there.

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