World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(REVIEW ARTICLE)

Check for updates

Immunohistochemical study of TGF-β, PDL-1, and IL-10 in patients with *Toxoplasma gondii*

HA Alsailawi [1], SS Ayat [2], Mustafa Mudhafar [1,*], Ruaa K Mohammed [1], Sahi J Dhahi [1], MM Abdulrasool [1], Shahad Shawkat [1] and Hussein A. Raheem [1]

[1] *Department of Anesthesia and Intensive Care techniques, Faculty of AL tuff College, Karbala, 56001, Iraq.*

[2] *Faculty of Pharmacy, National University of Science and Technology, Nasiriyah, 64001, Iraq.*

World Journal of Advanced Research and Reviews, 2022, 14(01), 436–448

Publication history: Received on 17 March 2022; revised on 20 April 2022; accepted on 22 April 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.14.1.0346

Abstract

The aim of this study to evaluate the expression of Programmed cell death-1 (PD-1), TGF- ß, and IL-10 in placental tissue of aborted women and the correlation with *Toxoplasma.gondii (T. gondii*) and the correlation of these cytokines with each other and with toxoplasmosis, during an abortion. The placental tissue samples have been processed and used for immunohistochemistry to study the expression of PDL-1, IL-10, and TGF-β. There was an increase in expression of TGF-β, PDL-1, and IL-10 in the placental tissue of aborted women infected with *T.gondii*, The results of the study showed that the residence of pregnant women in an urban area or rural has no relation with Toxoplasmosis infection (P>0.05), Age shows a significant correlation with Toxoplasmosis, the risk of infection increased with age (P<0.05). The number of recurrent abortions has no significant correlation effect in toxoplasmosis infection and abortion between the positive and control groups (P>0.05), Placental expression of PDL-1, IL-10, and TGF-β show significant correlation during infection with toxoplasmosis (P<0.05).

Keywords: TGF-β; PDL-1; IL-10; *Toxoplasma gondii*

1. Introduction

Toxoplasma.gondii is a protozoan eukaryotic parasite that can enter the body host as an obligate intracellular parasite and causes toxoplasmosis infection, it has the ability to cause latent infection in the host tissue such as cardiac muscle, skeletal muscles, or the central nervous system [1].

The definitive host is wild and domestic feline. It has a variety of intermediate hosts mostly warm-blooded animals. The infections have been notified on all continents and in earthen and aquatic environments. The resistance of the oocyst wall of the parasite permits the distribution of *T. gondii* within watersheds and ecosystems, and long-term stability in several foods [2].

Toxoplasma has adverse outcomes during pregnancy especially in the first trimesters and leads to congenital toxoplasmosis; it can be asymptomatic or can cause a flu-like sickness with low-grade fever, lymphadenopathy, and fatigue. Without regular prenatal screening procedures, it remains undiagnosed and untreated [3].

The immunological strategies during infection consist of the maternal-fetal interface through the first trimester of pregnancy and balance between Th1 and Th2 and other inflammatory cells as NK cells and macrophages, whereas DCs and B cells are rare. Tregs from mother increase during the pregnancy and prevent maternal rejection of the embryo in mice and human. *Toxoplasmosis* cause degrees in Tregs and lead to pregnancy miscarriage [4]. Interferon-γ (IFN-γ) is a

* Corresponding author: Mustafa Mudhafar
Department of Anesthesia and Intensive Care techniques, Faculty of AL tuff College, Karbala, 56001, Iraq.

specific parasitic cytokine that prevents replication and improves its conversion from tachyzoite to bradyzoite stage but its inhibit trophoblast growth result in fetal death and abortion [5].

2. Toxoplasmosis

2.1. Historical review

Early in 20th century *Toxoplasma* was discovered by scientists in the working in the ideological context of the studies of Robert Koch (1843–1910) and Louis Pasteur (1822–1895). At that time Splendore (1908), in Brazil, declared on the identification of this organism in the tissues of a rabbit [6]. There were a numbers of reports between 1908 and 1937, to recognize the toxoplasma-like organism in some animal species including humans; however, the first detailed scientific study was undertaken using techniques beforehand in studies of viruses [7]. In 1937-1939 Wolf, Cowen, and Paige popularized details about the first cases of congenital toxoplasmosis, and gave a clinical description of this disease [8]. Nearly , the first report of a fatal disseminated *T. gondii* infection was reported in a young adult in a study by Wilder (1952), where *T.gondii* parasites were found to be associated with severe intraocular inflammation [9]. Only asexual stages of *Toxoplasma.gondii* were known until 1970, in 1970 the sexual lifecycle ,oocyst, were discovered (Dubey, 2009) At first studies from North America and Europe identified only three genetic diversity, which were classified into genetic types I, II, and III [10].

2.2. Epidemiology

Toxoplasma.gondii is of a high prevalence world wiled because of contaminated food and water with oocyst from feline feces, in Southern Brazil, about (70%) of sheep contaminated with *T.gondii* [11,12]. Cats play a vital role in the transmission of the parasite, infection is high prevalence in area with highest number of cats. Studies showed that about 59.6% of blood donor in Egypt are asymptomatic infected with toxoplasmosis [13,14]. In British Columbia, About 22% of cats found infected with toxoplasmosi [14]. The epidemiological studies in china show there were 11.3% of men and 11.0% of women of different ages with toxoplasmosis. From 1999–2004, studies in U.S show a predominance of 10.8% among persons age from 6–49 years. About 14.1% to 9.0% between 12- to 49-year-old [15,16]. Studies of 4538 samples from Iran show distribution of about 34.4% of *Toxoplasma.gondii* distribution in blood donors were 2.74% (CI95%: 0.55-4.92) and 31.84% (CI95%:45 20.61-43.08), respectively [17]. From 2011-2015, 4365 patients diagnosed with toxoplasmosis in Iraq and a high rate found in Diala during 2011 and 2012; this rate decreased in 2013, during the 2015 Najaf recorded (180) cases while Karbala, Dahok, Muthana, Sulaymaniyah, and Anbar were nil [18]. Recent studies in Qadisiyah show seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies were positive in 55/125 cases (44%) for IgG and 5/125 cases (4%) for IgM, in aborted women [19]. The rate of congenital toxoplasmosis reported in Asia is 0.0008:0.0013 in Central America, in South America it's about 0.0034.

2.3. Causative agent

Toxoplasmosis, caused by the obligate intracellular protozoan *Toxoplasma.gondii*, that infects almost warm-blooded animals, including humans, It is thought the one of the most successful eukaryotic pathogens (Liu *et al.*, 2015).

2.4. Classification of *Toxoplasma.gondii*

Table 1 Classification of *Toxoplasma.gondii*

Kingdom	**Protista**
Subkingdom	Protozoa
Phylum	Apicomplexa
Class	Conoidasida
Order	Eucoccidiorida
Sub order	Eimeriorina
Family	Sarcocystidae
Subfamily	Toxoplasmatinae
Genus	Toxoplasma
Species	Toxoplasma gondii

This parasite that cause toxoplasmosis is classified in table 1 [20]. Various species were named for *Toxoplsama* based on the species from which they were isolated. However, no biological and serological differences exist among the different isolates. For that *T.gondii* is the unique species of *Toxoplasma* organisms known to date [21]. Analysis studies of Chinese *T.gondii* isolates of microsatellite typing data show limited genetic diversity and the selected virulence strains and phylogenetic analyses exhibit less divergence, although the strain virulence differs in the Chinese 1 type of *T.gondii* predominantly prevalent in China [15].

2.5. General description of *T. gondii*

The life cycle begins when *Toxoplasma* enters the definitive host, mostly cats, after ingested tissue cysts, bradyzoites release into the cat's intestinal, and then divided within the schizogony process to form male and female gametes and form a zygote. The oocyte shed from the cat's intestine and ingested by a human through contaminated water or food. Inside the human intestine cells, Toxoplasma undergoes asexual reproduction which called endodyogeny and results in the release of rapidly dividing motile tachyzoites and slowly dividing bradyzoites, the latter of which encyst in several tissues such as the brain and skeletal muscle, potentially insisting for the lifetime of the host [22,23].

2.6. Tachyzoites

Tachyzoites is asexual (fast-growing form), tachy means fast in greek was first named by Frenkel describing the rapidly growing stage of *T.gondii* which formed after ingestion of oocyst by mammalian, also known trophozoite "tachyzoites" (fast-growing form) [24].

Tachyzoite is about 2 by 6 μm, crescent-shaped structure, have a pointed anterior (conoidal) end and a rounded posterior end. Atypical plasma membrane is surround this parasite stage, inner and outer without space between them. At the anterior tip of the parasite, The inner membrane is discontinuous above the polar rings, and at the posterior pore at the extreme posterior tip of the zoite. The nucleus located in the center , and the cell contain few positive granules and the microtubules arranged in a gentle spiral. It's have prominent transverse striations [25,26]. During penetration, the toxoplasmas sometimes show longitudinal spiral ridges consistently oriented in a counterclockwise direction. It invades the tissue cell through phagocytosis, and become ovoid and surrounded its self by vacuoles [27].

2.7. Bradyzoites and tissue cyst

Bradyzoite or slow-multiplying form (brady = slow in Greek), multiplying within a tissue cyst. Bradyzoites are also called cystozoites [28]. Its multiply by endodyogeny, which lies inside tissue cyst [29,30]. Bradyzoites can be differentiated from tachyzoites that has nucleus lied toward the posterior end where it's centrally located in tachyzoites, tachyzoites have rhoptries contain labyrinthine wheres bradyzoites are usually electron-dense [31]. Cyst tissue are tolerance to proteolytic enzymes more than tachyzoites, they are capable of tolerance long periods of storage and changes in osmotic pressure also stand as evidence in favor. Tissue cyst organism is not destroyed by gastric juices so it's essential in life cycle of parasite [29].

2.8. Cyst stage

The cyst is the sexual stage of *T.gondii* that exerted by a cat after ingestion of bradyzoites or tissue cyst. And become sporulated oocyst which is infectious for human. Sporulated oocyst can survive the most environmental condition [32]. Unsporulated oocysts have smooth with colorless wall, sporulation beginning in the first few days. Sporulated oocyst contains four sporozoites with nucleus lying towards the middle of each sporozoite, it has two smooth layers, oocyst sporulation and development is better when examined under 1 and 2 % sulfuric acid, and 2.5 % potassium dichromate and it low in 0.1% formalin, 20% ethanol [33].

2.9. Etiology and life cycle of *T.gondii*

T.gondii has both definite (feline) and intermediate hosts (human and mouse). When cat ingested bradyzoite, it will excrete an oocyst after 3 to 10 days in feces which become infective after few dyes [34]. Sporozoite from tissue cyst or sporulated oocyst penetrating the intestinal tissue and releasing tachyzoite by action of protolytic enzyme, five types of *T.gondii* wich are morphologically separated develop in intestinal epithelial cells before the sexual cycle begins. These asexual stages are structurally separated from tachyzoites, The entroepithelial stages (types A–E,) are formed in the intestinal epithelium. Type B and C schizonts develop within enterocytes and Types C, D, and E schizonts multiply by schizogony [35].

Human become infected by ingestion sporulated oocyst from food or water contaminated with cat feces or ingestion of undercooked meat, or from tissue cyst containing bradyzoite ingestion [36]. The sporulated oocyst release tachyzoite

inside digestive trace of intermediate host, which circulated into the blood and lymph during this stages lymphadenopathy may occur. Fatigue ,fever, muscle pain and also sore throat. This called the acute phase of toxoplasmosis, the host can shed the parasite through urine, saliva and other biological fluids but the tachyzoites are easily destroyed in the environment [37]. The conversion from tachyzoites into bradyzoites and tissue cyst lead to chronic phase of disease when the parasite encysted inside the various tissue organs such as skeletal muscles and brain which can persist several years without causing harm [38].

Toxoplasma symptoms are mostly non-invasive and may be more pronounced in immunocompromised or AIDS patients, although there are beliefs that other factors may lead to serious disease in immunocompromised patients, not the parasite itself [39].

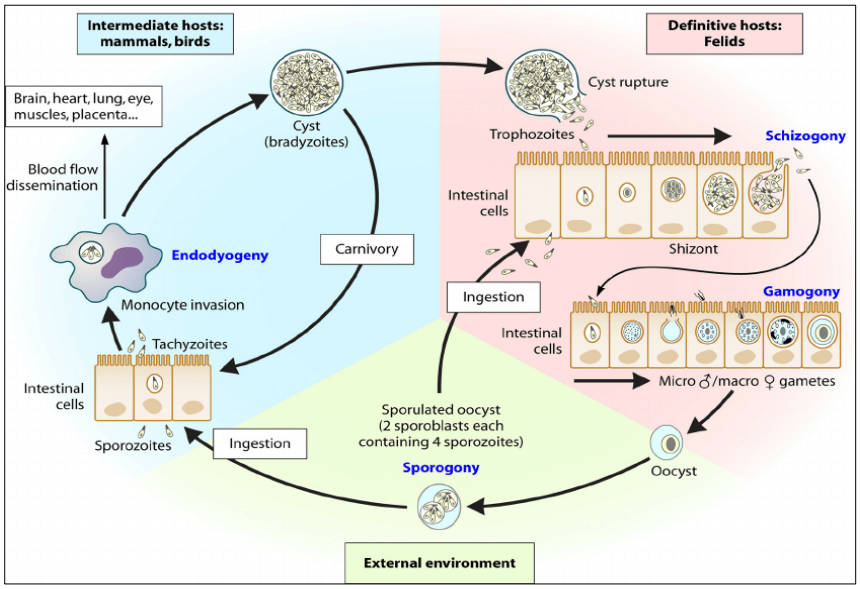

Figure 1 The life cycle of *T. gondii*, explained the biology, contagion, and replication of infective stages [39].

3. Clinical manifestation

3.1. Ocular Toxoplasmosis

Toxoplasmosis of the eye in immunocompetent person is initially subclinical and may result in mild inflammatory response that form scars in the choroid of the eye, if left untreated it will severe and cause necrotized and granulomatous inflammation, tissue destroyed and scares appear in the choroid (Pathogenesis of ocular toxoplasmosis, 2020). If left untreated, decreased vision occurs from sever humeral inflammatory reaction [40]. Studies of active ocular toxoplasmosis show that blindness may occur with eyes in scares, short-term treatment has no effect in visual recurrence after time [41].

3.2. Cerebral Toxoplasmosis

Relapsing of latent toxoplasmosis in AIDS patients lead to invasion of tachyzoites which released from tissue cyst to the cerebral tissue because of depletion of CD4 cells, decreased of cytokines [42,43]. Multiplication of parasite inside brain tissue leads to the formation of tissue necrosis and then cerebral lesion, Granulomatous lesion. MR imaging. The parasite result in cranial nerve palsies, change in level of awareness, or seizures and had full body pain distribution, the hemorrhagic lesion leads to headache. Differential diagnosis of cerebral toxoplasmosis is require brain biopsy to investigate [44,45,46].

3.3. Toxoplasmosis and pregnancy

Congenital toxoplasmosis is a serious infectious cause of miscarriage and pregnancy complications, transmission of *T.gondii* to the embryo occurs when women acquired the infection for the first time through pregnancy [47].

Syncytiotrophoblast (SYN), a multinucleated cell layer that contains the external layer of the placenta and has direct contact with maternal blood which helps in nutrient and gas exchange. The cytotrophoblasts (CYTs) is the

mononucleated and proliferative cells that blend to and supply the SYN layer during pregnancy. these layers form a primary barrier to the entrance of pathogens by the hematogenous route [48,49]. Treg cells are necessary to maintain tissue homeostasis and establish immune tolerance. HLA-G positive extravillous trophoblast (EVTs) regulate the activation of T cells through the initiation of tolerogenic dendritic cells (DCs) and cause an increase in Treg cells, Toxoplasmosis infection reduced the Treg cells and TGF-β level, which may result in adverse pregnancy outcomes [50].

Studies show multiplication of *T.gondii* in maternal tissues may induce a systemic immune response that harms cells in the fetal brain and course inflammatory necrosis. Similarly, elevated levels of interferon (IFN)-γ, transforming growth factor (TGF)-β and tumor necrosis factor (TNF)-α have been measured in the serum of pregnant women in the acute infection which has a harmful effect and cause abortion. Maternal infections induce clinical signs such as Ocular lesions, a different type of hydrocephalus and sometimes children are born with acute toxoplasmosis that characteristic by petechial rash, fever, jaundice, microphthalmia, hepatosplenomegaly, myocarditis and cataract [51].

4. Immunity

4.1. Immune changes during pregnancy

The immune system represents the host's protection against the different pathological and physiological conditions. Pregnancy is a particular condition when the immune system contributes to the tolerant and normal growth of the fetus without stimulating a harmful response [52].

Recognition and response of the maternal immune system to the fetus require Tcell activation via both classes I and class II MHC. The syncytiotrophoblast, which serves as the main maternal/conceptus interface lacks these MHC classes, maternal Tcell activated human placental tissue is novel in that extravillous trophoblast (EVT) exposes three of the non-classical MHC class I antigens, HLA-E, HLA-G, and HLA-F [53,54]. Human chorionic gonadotropin (HCG) plays an important role in blastocyst implantation; It produced by the trophoblast [55].

The anti-fetal pro-inflammatory immune response is downregulated in successful pregnancies by the explanation of regulatory T cells (Treg cells) in the circulation following expression to paternal antigens. These cells, identified as CD4+, Foxp3+ T cells, And CD25+ actively overcome allogeneic cell-mediated immune responses to fetal antigens, The absence of these cells leads to the collapse of gestation after implantation [56]. CD8 T cells play a major effector role through *T. gondii* infection by lysis the infected cells, While the CD4 T cell subset gives the vital help needed for their duration, depletion of both CD4 and CD8 T cell populations affects the reactivation of latent toxoplasmosis and, As an outcome [57].

4.2. Innate immune response for *T.gondii*

Infection in the intermediate hosts (such as human) begins through oral ingestion of cysts that are made in tissues of intermediate hosts or oocysts that are formed in cat. Oocysts or bradyzoites included in the ingested cysts invade the intestinal of the host epithelium via the wall of digestive tract and transform into tachyzoites. [58,59].

First recognition of innate immunity is by the recognition of pathogen by pattern recognition receptors (PRRs), Such as C-type lectins, Toll-like receptors (TLRs) and Nod-like receptors. And thes induced neutrophils production of interleukin (IL)-12. Monocytes sense Toxoplasmosis-infection through Alarmin S100A11 release from infected cells, that results in generation of the chemokine (C-C motif) ligand 2 (CCL2) [60]. Macrophages and neutrophils do not sense the parasite in the same way because they do not display pyroptosis or IL-1β secretion [61].

4.3. Adaptive immunity for *T.gondii*

Acquired immune defense to *T. gondii* infection is mediated by the mucosal and systemic Th1 cellular resistance which in turn produces IFN-γ, Dendritic cells have a critical role in cellular immunity by secreting interleukin (IL-12) , The main cytokine triggering an adaptive immune resistance by developing IFN-γ production [62,63].

T. gondii also involves a different secretory pathway by the rhoptries, Rhoptries are organelles that direct their discharge contents within host cells during the invasion manner. Chronic infection leads to functional weakness of T cells, Loss of IL-21R that signaling for T cells which lead to T cell exhaustion, dysfunction, and exhibition of inhibitory receptors like PD-1, Blockade of PD-1-PDL-1 pathway retains the CD8 response activity [64].

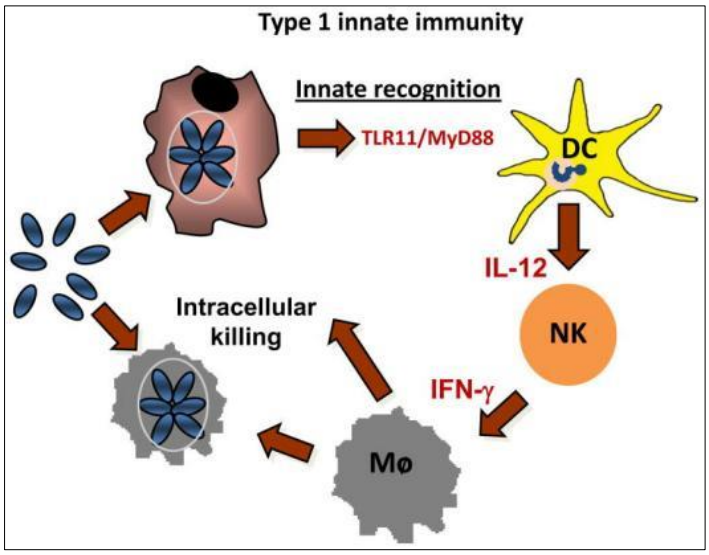

Figure 2 Innate immunity to *T.gondii* [65].

4.4. Immune evasion of *T.gondii* from the immune system

Toxoplasma is an obligate intracellular microorganism, infecting the nucleated cells. It remains within a parasitophorous vacuole (PV) in the host cell, Physically isolated from the cell cytoplasm. The parasitophorous vacuole membrane (PVM) forms the barrier between host and parasite and forms a rest for survival and replication which is the target for recognition by the host defense mechanisms [66].

The parasite has several evasion strategies focused at or around the PV. The PV is formed by invagination of the host cell plasma membrane and contain tubules and filamentous structures. The PVM is host-derived by its lipid structure. A fluorescent lipid probe entered into host membranes internalized with the PVM on the attack. The vacuole is furnished nonfusogenic, So avoiding lysosomal acidification, by selectively removing away transmembrane proteins [67].

4.5. Toxoplasmosis And Abortion

Acute toxoplasmosis during pregnancy has a strong suitability for women to suffering Spontaneous abortion. A study found that there is a strong relationship between IgG antibodies of *T.gondii* and women with repeated miscarriage. It should be educated the risk factors of toxoplasmosis especially is childbearing women [68]. Th2 cell plays a major role during immune pathology against *T.gondii* infection, by produced cytokines such as IL-6 which promoting generation of acute-phase protein to produce antibody and pro-inflammatory effect. IL-5 increases the eosinophil number in the site of infection. IL-10 has a regulatory effect that inhibits synthesis of cytokines. Increasing in IL-8 and appealing of neutrophil and lymphocyte to endometrium cause abortions. Switching to Th1 responses causes an increase in IL-12 levels that stimulate NK cells to release INF-γ and releasing of TNF-α by T-lymphocytes and macrophages leads to loss of the fetus [69].

5. Cytokines

5.1. Interleukin-10 (IL-10)

Interleukin 10 (IL-10) is an essential pleiotropic immunoregulatory cytokine principally secreted by macrophages, and also by T helper 1 (Th1) and Th2 immune cells, dendritic cells, CD8T cells, B lymphocytes, mast cells, and monocytes. The IL-10 family of cytokines part of the Class II cytokine group and consists of nine members: IL-10, IL-19, IL-20, IL-24, IL-22, IL-26, and the also distantly related IL-29, IL-28B, and IL-28 based on their relations with regard to the building and location of their encoding genes and their protein constructions and receptor complexes [70,71].

IL-10 activity is mediated by the IL-10 receptor (IL-10R) which is a member of the class II cytokine receptor family. IL-10 inhibits the ability of monocytes and macrophages to exhibit antigen to T cells by an inhibitory impact on the expression of major histocompatibility complex (MHC) class II, molecules as CD80 (B7.1) and CD86 (B7.2) and hence downregulates the expression of IL-1, IL-8, IL-6, IL-12, and tumor necrosis factor-alpha (TNF-α) [72].

5.2. Transforming growth factor-beta (TGF-β)

Transforming growth factor-beta (TGF-β) is anti-inflammatory immune cytokines that belong to the family groups of three isoforms. TGF-β family involves control of numerous features and cell functions, such as differentiation, proliferation, and migration, in all tissues of the individual body. The TGF-β family is regarded as a group playing one of the various roles in the control of physiological aspects regarding the continuance of metabolic homeostasis in the bone tissue [73]. The transforming growth factor-beta (TGF-β) superfamily is an extended and continuously enlarged group of regulatory polypeptides, including a pattern transforming growth factor-beta family and other families [74].

TGF-β is synthesized as a pre-pro-TGF-β presage, a latent form, And it's proteolytically cleaved toward a non-covalently linked mature TGF-β and latency-associated protein, This (LAP) complex is further bound to a different protein called latent TGF-b-binding protein (LTBP) during the secretion process The bioavailability of TGF-β is limited when bound to LAP and LTBP and several mechanisms are required to release the active TGF-β from the latent complex like proteases, Relative Oxygen Species acidic environment [75,76].

5.3. Programmed death ligand 1 (PD-L1)

(PD-L1) well-known as B7-H1 or CD274 is the first functionally defined ligand of the co-inhibitory programmed death receptor 1 (PD-1). Collectively with its related ligand PD-L2, PD-L1 acts a key role in managing peripheral and central protected cell tolerance by adhering to the PD-1 receptor [77].

TLR-mediated control of PD-L1 relies on the stimulation of the MEK/ERK kinases, which improve PD-L1 messenger RNA transcription through nuclear factor-kappaB. Interferon-γ receptors 1 and 2 are further involved in directing PD-L1 expression. PD-1/PD-L1 also involved in Immune exhaustion, The impairment of effector T cell function after persistent antigen presentation is a mechanism that limits tissue destruction in chronic infection [78].

5.4. The role of TGF-β, IL-10, and PDL-1 in abortion in pregnant women with Toxoplasmosis

Trophoblasts, cytotrophoblasts, and syncytiotrophoblasts, in addition to fetal mesenchymal and endothelial cells, form the placental barrier, where nutrients, hormones, and gas exchanges occur to supply the embryo. However, the placental barrier can also permit the passage of pathogen microorganisms as *T. gondii*, as leucocyte-bearing parasites could facilitate the infection of trophoblasts due to close contact with these cells [79].

Tregs secrete interleukin 10 (IL-10) and transforming growth factor β (TGF-β), Increased local levels of IL-10 and TGF-β are critical for protecting tolerance induced by Tregs during pregnancy. Interaction of IL-10 with its receptor leads to activation of intracellular signaling cascades with the participation of kinase proteins such as Janus protein tyrosine kinases that promote phosphorylation of the signal transducer and activator of transcription 3 (STAT3) factor [73].

5.5. *T. gondii* isolation and culturing

Isolation of parasite can be done by mouse inoculation or tissue culture to detect organism during acute infection more widely obtainable is cell culture. In *vitro* cell, culture approach are alternative ways to bioassays the *T. gondii* oocysts obtain following sporozoite excystation, Which relied on a mechanical approach to obtain free sporocysts, and incubation with bile salts to release the sporozoites. Enteroid culture methods are one of culturing methods that based on the constant of intestinal epithelial stem cells division in *vitro* 3-dimensional (3D) culture [80, 81].

5.6. Diagnosis Of *T. godii* Infection

The diagnosis of *T. gondii* infection or toxoplasmosis may be established by serologic tests, amplification of specific nucleic acid sequences (PCR), histologic demonstration of the parasite and/or its antigens, or by isolation of the organism. Other rarely used methods include demonstration of antigenemia and antigen in serum and body fluids, a toxoplasmin skin test, and antigen-specific lymphocyte transformation [82].

5.7. Erological Detection

Serological diagnosis of toxoplasmsis can be done by screening for IgG and IgM antibodies in patients' serum such as using the ELISA technique. The present of IgM antibody refers to the acute infection while IgG refers to chronic or past infection. IgG antibody may present for years after infection so that the acute infection identified by the avidity test of IgG. Acute infection associated with low avidity IgG antibodies [83]. Recent studies show that IgA antibody is positive in many pregnant women with toxoplasmosis,The PAMF-TSL serology panel test which is (dye test IgM, IgA, IgG, IgE, AC/HS, IgG avidity) can be used to know the time of infection in pregnant women [84].

The plasmonic gold chips technique (GOLD) test is found to have 100% sensitivity and specificity for *T.gondii* IgG and IgM. Bead-based multiplex assays (BBMAs) is a quantification method used for the detection of anti-toxoplasmosis antibodies in serum. Studies show that used Synthetic glycan antigen GPI1 from *T.gondii* in this technique gives good results in the detection of IgM antibodies during acute infection. Chemiluminescent immunoassay (CLIA) technique which based on used recombinant antibody and recombinant antigen with novel acridinium labels show to have promising future in diagnosis of toxoplasmosis IgG [85].

5.8. Polymerase Chain Reaction (PCR)

The amplification technique of *Toxoplasma* DNA is a highly specific technique to diagnose the parasite in large congenital numbers of samples. Real-time PCR technique shows good promising for routine screening of Toxoplasmosis infection synchronism with other diagnostic techniques. It is useful to diagnose a patient who can't formation specific IgM or IgG such as. Multiplex PCR assay is a new amplification approach which is simple,easy and rapid can be used in the detection of *T.gondii* such as in congenital toxoplasmosis [86, 87].

5.9. Immunohistochemistry (IHC)

Immunohistochemistry (IHC) is a technique for localizing particular antigens in formalin-fixed, Paraffin-embedded (FFPE) tissues based on antigen-antibody interaction, its used to visualize organisms in tissue section. The technique is based on the manifestation of antigens in tissue sections by the use of specific antibodies. when antigen-antibody binding done, the colored histochemical reactions obvious by light microscopy with ultraviolet light. When used PCR and IHC can be useful detection techniques to enhance histologic evaluation by visualizing the parasite within tissue sections [88].

5.10. Radiological Examinations

MR imaging is a noninvasive technique, rapid and can help in the differentiation between toxoplasmosis and lymphoma of the brain in AIDS patients which characterized by no metabolic lesion as saw in cerebral lymphoma. CT scan technique found to have a vital role in the demonstration and localization of toxoplasmosis and follow up of patients during medical therapy [89].

5.11. Fetal Maternal Toxoplasmosis Diagnosis

In order to recognize and limit the severity of fetus infection, prenatal investigation must be done. Transmission occurs after the placenta has infected and then lead to congenital toxoplasmosis of the fetus. Detection can be done by fetus blood sample screening for IgM or IgG, amniotic fluid is can be obtained during pregnancy. Ultrasound examination can help in the detection of any enlargement of the cerebral ventricles. Fetal ultrasounds may detect brain or hepatic calcifications hydrocephalus, splenomegaly ,and ascites. PCR assay for *T.gondii* DNA is more sensitive and reliable for prenatal diagnosis during pregnancy, The infection can be detected by amplification of parasitic nucleic acid sequences in amniotic fluid. Infants with potential congenital infection, anti-*Toxoplasma* IgA and IgM antibodies, and cerebrospinal fluid PCR are recommended to detect Toxoplasma DNA to give a highly sensitive for congenital toxoplasmosis diagnosis [90, 91].

6. Conclusion

The results of the study showed that the residence of pregnant women in an urban area or rural has no relation with Toxoplasmosis infection ($P>0.05$), Age shows a significant correlation with Toxoplasmosis, the risk of infection increased with age ($P<0.05$). The number of recurrent abortions has no significant correlation effect in toxoplasmosis infection and abortion between the positive and control groups ($P>0.05$), Placental expression of PDL-1, IL-10, and TGF-β show significant correlation during infection with toxoplasmosis ($P<0.05$).

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this document.

References

[1] Mendez OA, Koshy AA. Toxoplasma gondii: Entry, association, and physiological influence on the central nervous system. PLoS pathogens. 2017 Jul 20;13(7):e1006351.

[2] Shapiro K, Bahia-Oliveira L, Dixon B, Dumètre A, de Wit LA, VanWormer E, Villena I. Environmental transmission of Toxoplasma gondii: Oocysts in water, soil and food. Food and Waterborne Parasitology. 2019 Jun 1;15:e00049.

[3] Rostami A, Riahi SM, Contopoulos-Ioannidis DG, Gamble HR, Fakhri Y, Shiadeh MN, Foroutan M, Behniafar H, Taghipour A, Maldonado YA, Mokdad AH. Acute Toxoplasma infection in pregnant women worldwide: a systematic review and meta-analysis. PLoS neglected tropical diseases. 2019 Oct 14;13(10):e0007807.

[4] Mudhafar MU, Zainol IS. Medical values, antimicrobial, and anti fungal activities of Polyalthia genus. International Journal of Pharmaceutical Research. 2019 Jan;11(1):90-6.

[5] El-Sherbini MS, El-Aal A, Amany A, El-Sherbiny WS, Attia SS, Abdel Aziz IZ, Nasr GM, Salama MS, Badr MS. Toxoplasmosis and abortion: pro-and anti-inflammatory cytokines gene expression of the host immune cells. Egyptian Journal of Medical Human Genetics. 2019 Dec;20(1):1-0..

[6] Weiss LM, Dubey JP. Toxoplasmosis: a history of clinical observations. International journal for parasitology. 2009 Jul 1;39(8):895-901.

[7] Dubie T, Terefe G, Asaye M, Sisay T. Toxoplasmosis: Epidemiology with the emphasis of its public health importance. MRJMMS. 2014;2(4):097-108.

[8] Majhooll AA, Zainol I, Jaafar CN, Ha A, Hassan MZ, Mudhafar M, Majhool AA, Asaad A. A brief review on biomedical applications of hydroxyapatite use as fillers in polymer. J. Chem. 2019;13:112-9.

[9] Innes EA. A brief history and overview of Toxoplasma gondii. Zoonoses and public health. 2010 Feb;57(1):1-7.

[10] Mudhafar M, Zainol I, Desa S, Jaafar CN. Mini-review of phytochemistry for polyalithia Longiflia. Eurasian Journal of Analytical Chemistry. 2019 May 23;14(2):119-47.

[11] Ding H, Gao YM, Deng Y, Lamberton PH, Lu DB. A systematic review and meta-analysis of the seroprevalence of Toxoplasma gondii in cats in mainland China. Parasites & vectors. 2017 Dec;10(1):1-2.

[12] ALsailawi HA, Mudhafar M, Majhool AA, Asaad A. Study of Cystatin C as Early Biomarker of Nephropathy in Patients with Type 2 DM and Risk Stratification in Tarnaka Hospital of Hyderabad City in India. Journal of US-China Medical Science. 2019;16:232-41.

[13] HA AS, MISNAN R, MUDHAFAR M, DESA S, ABDULRASOOL MM. Effects of Storage period on protein profile and allergenicity of purple mud crab (Scylla. tranquebarica) under various storage conditions. International Journal of Pharmaceutical Research. 2020 Jul;12(2).

[14] Aramini JJ, Stephen C, Dubey JP, Engelstoft C, Schwantje H, Ribble CS. Potential contamination of drinking water with Toxoplasma gondii oocysts. Epidemiology & Infection. 1999 Apr;122(2):305-15.

[15] Li, K. et al. (2017) 'Epidemiology of Toxoplasma gondii infection in native Tibetans in Tibet , *China'*, 62(3), pp. 529–532.

[16] Mudhafar M, Zainol I, Alsailawi HA, Aiza Jaafar CN. Green Synthesis of Silver Nanoparticles using Neem and Collagen of Fish Scales as a Reducing and Stabilizer Agents. Jordan Journal of Biological Sciences. 2021 Dec 15;14(5).

[17] Mansouri A, Mojarad MR, Badfar G, Abasian L, Rahmati S, Kooti W, YektaKooshali MH, Soleymani A, Azami M. Epidemiology of Toxoplasma gondii among blood donors in Iran: A systematic review and meta-analysis. Transfusion and Apheresis Science. 2017 Jun 1;56(3):404-9.

[18] Mudhafar M. Review of Photochemistry for Polyalithialongiflia. Discovery Phytomedicine. 2019;6(2):33-55.

[19] Mosa IS, Saheb EJ, Mahdi SG, Abdul-Karim MI, Khistawi AN. An epidemiology study of some protozoan parasitic diseases in Iraq from 2011 Till 2015. Al-Nahrain Journal of Science. 2017 Sep 1;20(3):115-20.

[20] Mudhafar M, Zainol I, Alsailawi Ha, Jaafar Cn, Mohammed Rk, Dhahi Sj. Preparation And Characterization Of Beads Of Fish Scales Hydroxyapatite/Collagen/Silver Nanoparticles By Using Infiltration Method. Malaysian Journal Of Microscopy. 2021 Dec 20;17(2).

[21] Wójcik-Fatla A, Sroka J, Zajac V, Sawczyn A, Cisak E, Dutkiewicz J. Toxoplasma gondii (Nicolle et Manceaux, 1908) detected in Dermacentor reticulatus (Fabricius)(Ixodidae). Folia parasitologica. 2015;62:1.

[22] Dubie T, Terefe G, Asaye M, Sisay T. Toxoplasmosis: Epidemiology with the emphasis of its public health importance. MRJMMS. 2014;2(4):097-108.

[23] Mudhafar. M, Alsailawi H. A, Hussein A. Raheem and Ruaa K. M. (2022). A Review Study on the Biomaterials and Bone Grafts for Bon Defects Applications. European Journal of Biomedical and Pharmaceutical Sciences. 9(4), 63-73.

[24] Milne G, Fujimoto C, Bean T, Peters HJ, Hemmington M, Taylor C, Fowkes RC, Martineau HM, Hamilton CM, Walker M, Mitchell JA. Infectious causation of abnormal host behavior: Toxoplasma gondii and its potential association with Dopey Fox syndrome. Frontiers in Psychiatry. 2020:1007.

[25] Mudhafar M, Alsailawi H, Abdulrasool M, Jawad Rk, Mays A. Mini-Review Of Phytochemicals Of Ten Ficus Species. International Journal Of Chemistry Research. 2021 Oct 1:7-18.

[26] Alsailawi HA, Mudhafar M, Mohammed RK, Bashi AM, Mays AD, Abdulrasool MM, Khalaf A. Clinical Implications of Cross-Reactive Shellfish Allergens. J. Chem. 2021;15:16-31.

[27] CHIAPPINO ML, NICHOLS BA, O'CONNOR GR. Scanning Electron Microscopy of Toxoplasma gondii: Parasite Torsion and Host-Cell Responses during Invasion 1. The Journal of protozoology. 1984 May;31(2):288-92.

[28] Sakban FM, A'aiz NN. Investigate the Toxoplasma gondii infection in the consumed beef in Al-Diwaniyah province. Iraqi Journal of Veterinary Sciences. 2020 Jan 1;34(1):95-9.

[29] Mudhafar M, Zainol I, Alsailawi HA, Aiza Jaafar CN. Green Synthesis of Silver Nanoparticles using Neem and Collagen of Fish Scales as a Reducing and Stabilizer Agents. Jordan Journal of Biological Sciences. 2021 Dec 15;14(5).

[30] Dubey JP. The history and life cycle of Toxoplasma gondii. InToxoplasma gondii 2020 Jan 1 (pp. 1-19). Academic Press.

[31] Ha A, Rosmilah M, Keong BP, Ateshan HM. The effects of thermal and non-thermal treatments on protein profiles of Scylla tranquebarica (purple mud crab). Plant Archives. 2019;19(2):813-6.

[32] Mudhafar M, Zainol I, Aiza Jaafar CN, Alsailawi HA, Majhool AA. Two green synthesis methods to prepared nanoparticles of Ag: two sizes and shapes via using extract of M. dubia leaves. Journal of Computational and Theoretical Nanoscience. 2020 Jul 1;17(7):2882-9.

[33] Ali AH, Rosmilah M. Effects of Food Processing on the stability and quality of shellfish allergens. Journal of US-China Medical Science. 2019;16:149-63.

[34] Howe DK, Sibley LD. Toxoplasma gondii comprises three clonal lineages: correlation of parasite genotype with human disease. Journal of infectious diseases. 1995 Dec 1;172(6):1561-6.

[35] Mudhafar M, Zainol I, Jaafar CN, Alsailawi HA, Majhool AA, Alsaady M. Phytochemical Screening and Characterization of Meliadubia Leaves Extract for Antimicrobial Activity against Escherichia coli and Staphylococcus aureus. Indian Journal of Ecology. 2020;47(2):493-6.

[36] Abdulrasool MM, Ruaa KM, Mays AD, ALsailawi HA, Mudhafar M, Bashi AM. Regeneration of Chitosan-Based Adsorbents Used in Heavy Metal Adsorption. Journal of Life Sciences. 2021;15:11-9.

[37] Jones JL, Kruszon-Moran D, Elder S, Rivera HN, Press C, Montoya JG, McQuillan GM. Toxoplasma gondii infection in the United States, 2011–2014. The American Journal of Tropical Medicine and Hygiene. 2018 Feb;98(2):551.

[38] Ruaa KM, ALsailawi HA, Abdulrasool MM, Mudhafar M, Mays AD, Bashi AM. A Review Study on the Effects of Estrogen Level on Vaginal Candida spp. of Women with Estrogen-Related Receptor Breast Cancer. Journal of Life Sciences. 2021;15:10-20.

[39] Saadatnia G, Golkar M. A review on human toxoplasmosis. Scandinavian journal of infectious diseases. 2012 Nov 1;44(11):805-14.

[40] Majhooll AA, Zainol I, Jaafar CN, Mudhafar M, Ha A, Asaad A, Mezaal FW. Preparation of fish scales hydroxyapatite (FsHAp) for potential use as fillers in polymer. J. Chem. 2019;13:97-104.

[41] Robert-Gangneux F, Murat JB, Fricker-Hidalgo H, Brenier-Pinchart MP, Gangneux JP, Pelloux H. The placenta: a main role in congenital toxoplasmosis?. Trends in parasitology. 2011 Dec 1;27(12):530-6.

[42] Majhool AA, Zainol I, Azziz SS, Jaafar CN, Jahil MM. Mechanical properties improvement of epoxy composites by natural hydroxyapatite from fish scales as a fillers. 2019.

[43] Silveira C, Muccioli C, Holland GN, Jones JL, Yu F, de Paulo A, Belfort Jr R. Ocular involvement following an epidemic of Toxoplasma gondii infection in Santa Isabel do Ivai, Brazil. American Journal of Ophthalmology. 2015 Jun 1;159(6):1013-21.

[44] Tavares HD, Da Silva GR. Therapeutic alternatives for the treatment of ocular toxoplasmosis. Journal of Applied Pharmaceutical Science. 2020 Sep;10(09):140-9.

[45] Basavaraju A. Toxoplasmosis in HIV infection: An overview. Tropical parasitology. 2016 Jul;6(2):129.

[46] Mudhafar M, Zainol I, Jaafar CN, Alsailawi HA, Majhool AA. Microwave-Assisted Green Synthesis of Ag Nanoparticles using Leaves of Melia Dubia (Neem) and its Antibacterial Activities. Journal of Advanced Research in Fluid Mechanics and Thermal Sciences. 2020 Dec 31;65(1):121-9.

[47] Al Sailawi HA, Misnan R, Yadzir ZH, Abdullah N, Bakhtiar F, Arip M, Mudhafar M, Ateshan HM. Effects of different salting and drying methods on allergenicity of purple mud crab (scylla tranquebarica). Indian Journal of Ecology. 2020;47(4):1173-9.

[48] Elsheikha HM, Marra CM, Zhu XQ. Epidemiology, pathophysiology, diagnosis, and management of cerebral toxoplasmosis. Clinical microbiology reviews. 2020 Nov 25;34(1):e00115-19.

[49] Hadi HS, Kadhim RA, Al-Mammori RT. Seroepidemiological aspects for Toxoplasma gondii infection in women of Qadisiyah province, Iraq. International Journal of PharmTech Research. 2016;9(11):252-9.

[50] Ander SE, Rudzki EN, Arora N, Sadovsky Y, Coyne CB, Boyle JP. Human placental syncytiotrophoblasts restrict Toxoplasma gondii attachment and replication and respond to infection by producing immunomodulatory chemokines. MBio. 2018 Jan 9;9(1):e01678-17.

[51] Mudhafar M, Zainol I, Alsailawi HA, Aiza Jaafar CN. Synthesis and characterization of fish scales of hydroxyapatite/collagen–silver nanoparticles composites for the applications of bone filler. Journal of the Korean Ceramic Society. 2022 Mar;59(2):229-39.

[52] Tsuda S, Nakashima A, Shima T, Saito S. New paradigm in the role of regulatory T cells during pregnancy. Frontiers in immunology. 2019 Mar 26;10:573.

[53] Gutiérrez-Expósito D, Arteche-Villasol N, Vallejo-García R, Ferreras-Estrada MC, Ferre I, Sánchez-Sánchez R, Ortega-Mora LM, Pérez V, Benavides J. Characterization of fetal brain damage in early abortions of ovine toxoplasmosis. Veterinary Pathology. 2020 Jul;57(4):535-44.

[54] Gilman-Sachs A, Dambaeva S, Garcia MD, Hussein Y, Kwak-Kim J, Beaman K. Inflammation induced preterm labor and birth. Journal of Reproductive Immunology. 2018 Sep 1;129:53-8.

[55] Hackmon R, Pinnaduwage L, Zhang J, Lye SJ, Geraghty DE, Dunk CE. Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. American journal of reproductive immunology. 2017 Jun;77(6):e12643.

[56] Alsailawi HA, Mudhafar M, Abdulrasool MM. Effect of Frozen Storage on the Quality of Frozen Foods—A Review. J. Chem. 2020;14:86-96.

[57] Schumacher A, Zenclussen AC. Human chorionic gonadotropin-mediated immune responses that facilitate embryo implantation and placentation. Frontiers in Immunology. 2019:2896.

[58] Sisti G, Kanninen TT, Witkin SS. Maternal immunity and pregnancy outcome: focus on preconception and autophagy. Genes & Immunity. 2016 Jan;17(1):1-7.

[59] Khan IA, Hwang S, Moretto M. Toxoplasma gondii: CD8 T cells cry for CD4 help. Frontiers in cellular and infection microbiology. 2019 May 1;9:136.

[60] Sasai M, Yamamoto M. Innate, adaptive, and cell-autonomous immunity against Toxoplasma gondii infection. Experimental & molecular medicine. 2019 Dec;51(12):1-0.

[61] Mudhafar M, Alsailawi HA. An expression study profile of proinflammatory cytokines in asthma patient. Journal of Asian Scientific Research. 2019 Dec 9;9(12):227-34.

[62] Safronova A, Araujo A, Camanzo ET, Moon TJ, Elliott MR, Beiting DP, Yarovinsky F. Alarmin S100A11 initiates a chemokine response to the human pathogen Toxoplasma gondii. Nature immunology. 2019 Jan;20(1):64-72.

[63] Lima TS, Gov L, Lodoen MB. Evasion of human neutrophil-mediated host defense during Toxoplasma gondii infection. MBio. 2018 Feb 13;9(1):e02027-17.

[64] Hwang S, Khan IA. CD8+ T cell immunity in an encephalitis model of Toxoplasma gondii infection. InSeminars in immunopathology 2015 May (Vol. 37, No. 3, pp. 271-279). Springer Berlin Heidelberg.

[65] Ruaa KM, ALsailawi HA, Abdulrasool MM, Mudhafar M, Mays AD, Bashi AM. A Review Study on the Effects of Estrogen Level on Vaginal Candida spp. of Women with Estrogen-Related Receptor Breast Cancer. Journal of Life Sciences. 2021;15:10-20.

[66] Moretto MM, Hwang S, Khan IA. Downregulated IL-21 response and T follicular helper cell exhaustion correlate with compromised CD8 T cell immunity during chronic toxoplasmosis. Frontiers in immunology. 2017 Oct 31;8:1436.

[67] Pifer R, Yarovinsky F. Innate responses to Toxoplasma gondii in mice and humans. Trends in parasitology. 2011 Sep 1;27(9):388-93.

[68] Clough B, Frickel EM. The Toxoplasma parasitophorous vacuole: an evolving host–parasite frontier. Trends in parasitology. 2017 Jun 1;33(6):473-88.

[69] Coppens I, Romano JD. Hostile intruder: Toxoplasma holds host organelles captive. PLoS pathogens. 2018 Mar 29;14(3):e1006893.

[70] Kheirandish F, Ezatpour B, Fallahi S, Tarahi MJ, Hosseini P, Rouzbahani AK, Tabaei SJ, Akbari S. Toxoplasma serology status and risk of miscarriage, a case-control study among women with a history of spontaneous abortion. International Journal of Fertility & Sterility. 2019 Oct;13(3):184.

[71] Aldabagh MA, Hachim SK, Qassim KW, Al-Mayah QS, Hassan JS, Salloom DF. Immune profile in aborted Iraqi women with toxoplasmosis. Medical Journal of Babylon. 2018 Jan 1;15(1):48.

[72] Ouyang W, O'Garra A. IL-10 family cytokines IL-10 and IL-22: from basic science to clinical translation. Immunity. 2019 Apr 16;50(4):871-91.

[73] Ha A, Misnan R, Mudhafar M. Major and minor allergen Ige reactivity of purple mud crab (Scylla tranquebarica) against a cross-reactive allergen in crustacean and molluscs in patients with a seafood allergy. Research Journal of Pharmacy and Technology. 2021 Jan 29;14(1):239-44.

[74] Brockmann L, Gagliani N, Steglich B, Giannou AD, Kempski J, Pelczar P, Geffken M, Mfarrej B, Huber F, Herkel J, Wan YY. IL-10 receptor signaling is essential for TR1 cell function in vivo. The Journal of Immunology. 2017 Feb 1;198(3):1130-41.

[75] Zhang Y, Alexander PB, Wang XF. TGF-β family signaling in the control of cell proliferation and survival. Cold Spring Harbor perspectives in biology. 2017 Apr 1;9(4):a022145.

[76] Verhamme FM, Bracke KR, Joos GF, Brusselle GG. Transforming growth factor-β superfamily in obstructive lung diseases. more suspects than TGF-β alone. American journal of respiratory cell and molecular biology. 2015 Jun;52(6):653-62.

[77] Sheng J, Chen W, Zhu HJ. The immune suppressive function of transforming growth factor-β (TGF-β) in human diseases. Growth Factors. 2015 Mar 4;33(2):92-101.

[78] Mudhafar M, Abdulrasool MM, Nasser L. Investigation on the Antibacterial Activity and Phytochemical Screening of the Fruit Seed Extracts of Agro Waste. Journal of US-China Medical Science. 2019;16:223-31.

[79] Boussiotis VA. Molecular and biochemical aspects of the PD-1 checkpoint pathway. New England Journal of Medicine. 2016 Nov 3;375(18):1767-78.

[80] Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nature Reviews Immunology. 2015 Aug;15(8):486-99.

[81] Arora N, Sadovsky Y, Dermody TS, Coyne CB. Microbial vertical transmission during human pregnancy. Cell host & microbe. 2017 May 10;21(5):561-7.

[82] Luu L, Johnston LJ, Derricott H, Armstrong SD, Randle N, Hartley CS, Duckworth CA, Campbell BJ, Wastling JM, Coombes JL. An open-format enteroid culture system for interrogation of interactions between Toxoplasma gondii and the intestinal epithelium. Frontiers in Cellular and Infection Microbiology. 2019:300.

[83] Nasrat M, Misnan R, Yadzir ZH, Bakhtiar F, Abdullah N, Ali H. Comparison of protein profiles and allergenicity of different body parts and genders of Scylla paramamosain. Int J Sci Environ Technol. 2018;7(5):1759-65.

[84] Rostami A, Karanis P, Fallahi S. Advances in serological, imaging techniques and molecular diagnosis of Toxoplasma gondii infection. infection. 2018 Jun;46(3):303-15.

[85] Rahimi-Esboei B, Zarei M, Mohebali M, Valian HK, Shojaee S, Mahmoudzadeh R, Salabati M. Serologic tests of IgG and IgM antibodies and IgG avidity for diagnosis of ocular toxoplasmosis. The Korean journal of parasitology. 2018 Apr;56(2):147.

[86] Olariu TR, Blackburn BG, Press C, Talucod J, Remington JS, Montoya JG. Role of toxoplasma IgA as part of a reference panel for the diagnosis of acute toxoplasmosis during pregnancy. Journal of clinical microbiology. 2019 Feb 1;57(2):e01357-18.

[87] Garg M, Stern D, Groß U, Seeberger PH, Seeber F, Varón Silva D. Detection of anti-Toxoplasma gondii antibodies in human sera using synthetic glycosylphosphatidylinositol glycans on a bead-based multiplex assay. Analytical chemistry. 2019 Aug 12;91(17):11215-22.

[88] Mousavi P, Mirhendi H, Mohebali M, Shojaee S, Valian HK, Fallahi S, Mamishi S. Detection of Toxoplasma gondii in acute and chronic phases of infection in immunocompromised patients and pregnant women with real-time PCR assay using TaqMan fluorescent probe. Iranian Journal of Parasitology. 2018 Jul;13(3):373.

[89] Jasim HA, Misnan R, Yadzir ZH, Abdullah N, Bakhtiar F, Arip M, Ateshan HM, Keong PB. Identification of Common and Novel Major Crab Allergens in Scylla tranquebarica and the Allergen Stability in Untreated and Vinegar-treated Crab Identification of major crab allergens in Scylla tranquebarica. Iranian Journal of Allergy, Asthma and Immunology. 2021;20(1):76.

[90] Meixner N, Sommer MF, Scuda N, Matiasek K, Müller M. Comparative aspects of laboratory testing for the detection of Toxoplasma gondii and its differentiation from Neospora caninum as the etiologic agent of ovine abortion. Journal of Veterinary Diagnostic Investigation. 2020 Nov;32(6):898-907.

[91] Khalatbari H, Parisi MT. Management of hydrocephalus in children: anatomic imaging appearances of CSF shunts and their complications. American Journal of Roentgenology. 2021 Jan 11;216(1):187-99.

[92] Vidal JE. HIV-related cerebral toxoplasmosis revisited: current concepts and controversies of an old disease. Journal of the International Association of Providers of AIDS Care (JIAPAC). 2019 Aug 14; 18:2325958219867315.