certification

We, certify that this thesis was prepared under our supervision at the department of microbiology–college of Medicine/ University of Babylon, as a partial fulfillment of requirements for degree of Ph.D. of science in Medical Microbiology.

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We are the examiner committee, certify that we have read this thesis entitled (**Immunogenetic study of Interleukin - 17 Family from Hydatid Disease Patients in Babylon Province)** and have examined the student (**Inass Abbas Khiarulla**) in its content, and that in our opinion; it is accepted as a thesis for degree of **Doctor of Philosophy** in Microbiology with **Excellent** estimation.

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1- Introduction

Echinococcosis is the name given to a prominent silent chronic helminthes zoonotic infection caused by infection with the adult or larval stage of the dog tapeworm *Echicoccous granulosus*, which belongs to the Taeniidae family and genus *Echinococcus* (Larrieu *et al* ., 2019).

Echinococcosis an important pathogenic, zoonotic and parasitic infection (acquired from animals) of humans, following ingestion of tapeworm eggs excreted in the faeces of infected dogs. Hydatid disease is a major endemic health problem in certain areas of the world(Nunnari *et al* ., 2012; Rojas *etal*., 2018).

The two-major species of *Echinococcus* of medical and public health importance are *E. granulosus* and *E. multilocularis*, which cause cystic echinococcosis and alveolar echinococcosis, respectively in humans (McManus *et al.*, 2003; Agudelo *et al*, 2016). The disease can be life-threatening in humans and result in a significant economic impact to livestock producers (Azlaf & Dakkak, 2006). Although Cystic Echinococcosis is typically asymptomatic in affected livestock, the organs affected with cysts, which are detected on inspection at slaughter, are usually totally condemned resulting in financial loss for the producers. Severe cases in livestock may result in reduced productivity through interference of organ function (Torgerson &Heath, 2003; Scala *et al.*, 2006).

High incidences of infection by *E. granulosus* often coincides with rural grazing areas where dogs are able to ingest organs from infected wild and domestic animals, approximately 2-3 million human cases occur worldwide annually. In Africa, the prevalence is higher in the northern part such as in Sudan, Egypt and Ethiopia (Federer *etal*.,2016).

The World Health Organization (WHO) identifies human Cystic echinococcosis as one of the most important neglected zoonoses, as the disease continues to pose a serious socio-economic problem in many parts of the world (Budke *etal*., 2006). Cystic echinococcosis is highly endemic in most of the countries of the Mediterranean basin, including North Africa and the Middle East. The high endemicity of echinococcosis in the Mediterranean region has been attributed to many risk factors, such as a lack of adequate public health education, insufficient application of control programmes, and the common practice of home slaughter of small ruminants (Dakkak, 2010).

The host immune system against cystic echinococcosis is classically divided into the adaptive and innate response (Inclan & Siracusa ,2018). Innate immunity is the first line of defense against various parasites (Akira, *etal*.,2006) that can recognize pathogen-associated molecules patterns (PAMPs), via pattern-recognition receptors (PRRs), such as toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)- like receptors . These receptors are expressed by the host innate immune cells, including macrophages, neutrophils, endothelial cells, dendritic cells (DCs) and lymphocytes, which modulate immune responses through different mechanisms for host defense (Janeway& Medzhitov,2002).

The mechanisms involved in innate susceptibility/resistance to Cystic echinocosis /Alveolar echinocosis are mostly unknown (Gottstein& Hemphill,2008). Neutrophils and macrophages are the first responders to detect and eliminate parasites, but their natural activities can be prevented by parasite metabolites. Antigen B secreted by *E. granulosus* can interfere with neutrophil activity via the elastase secreted by

neutrophilic, granulocytes and enable the parasite to escape from thehost immune response (Virginio *etal*., 2007) .

Humans are accidental intermediate hosts and are infected by ingestion of tapeworm eggs shed in the feces of a definitive host (Casulli *etal*., 2019).

Echinococcus granulosus, genotype G1 has the most cosmopolitan distribution and is responsible for the great majority (almost 90%) of human CE, Its principal intermediate host appears to be sheep (Deplazes *etal*., 2017). Although considerably fewer (7%) infections have been attributed to *E. canadensis* genotype G6 than to *E. granulosus* , genotype G1, the former genotype was found to be the second most common cause of human CE worldwide ,Human infections with genotypes G5, G8 and G10 are rare and no cases of human Cystic echinococcosis caused by G4 have been described (Deplazes *etal*.,2017).

In Iraq, human CE is endemic, and the condition has been identified based on the number of individuals hospitalized to hospitals and surgically treated (Maktoof & AbuTabeekh, 2015). In Iraq's southern provinces, a higher number of cases of human CE have been reported (Abdul Ameer *etal*., 2013; Thweni & Yassen, 2015),and in particular Basrah province (Thamir *et al*., 2015). Despite the substantial burden of the disease, national surveillance programmes for Cyst Echinocosis do not exist in Iraq (Barnett-Vanes *et al*., 2016).

1-1- Aim of study :

To determine the some immunogenetic parameters in patients which infected with hydatid cyst and The role of IL-17 cytokines immune responses in CE disease is yet unexplored, This aim achieved by the following objectives.

1- Study the incidence of hydatid cyst infection in Babylon province

2- Study the role of hydatid cyst location (liver, lung, other) in intensity of cytokine induction.

3- study the role of interleukin-17 (IL-17) family (A, B) in patients with hydatid disease and control .

4- investigate the role of IL-17 receptor A and B (1L-17RA and IL-17RB) with Echinococcosis susceptibility.

5- investigate the correlation between IL-17 A,IL-17RA , IL-17B ,IL-17RB in hydatid disease patients and control .

6- Study the association of IL-17 family gene polymorphism with hydatid disease.

1-2- Literatures Review

1.2.1. Historical discoveries of Cystic Echinococcosis (CE):

Hydatid cyst have been known to humans for thousands of years with documentation of epidemic fever in the Ebers papyrus dating back to 1500 BC (Djuricic *etal*.,2010). Hippocrates (∽460-377 BC) was the first person to document information about hydatid cysts and indicated "In those whose water stuffed liver opens into the omentum, the belly is filled with water, and they die" (Eckert & Thompson, 2017). Al-Rhazes, a Persian physician, described hydatid cysts in the liver as watery balloons (Manouras *etal*.,2007). At that time it was believed that the cysts were eggs or embryos of an insect or a cystic tumour , More than 200 hundred years ago, researchers also reported the presence of cysts in the abdominal cavity of slaughtered goats and pigs, but probably were referring to *Cysticercus tenuicollis* rather than hydatid cysts (Ebrahimipour *etal* .,2019).

Philip Jacob Hartmann (1648-1707) had also described a Cysticercus tenuicollis with a scolex, the metacestode of *Taenia hydatigena* (Eckert &Thompson, 2017). For many years knowledge about hydatid cysts was lacking. Aelius Galenus 1821-1833 (cited in Larrieu, 2017) ,reported that "the liver is very much inclined to produce hydatid cysts in the surrounding fascia".

Hydatid cysts were diagnosed in several patients at that time, but the disease or its cause were not understood, and it was believed the cysts were due to dysfunction of the lymphatic glands (Fornaciari,*etal*.,2020).

In the 17th century many researchers described the features of hydatid cysts. For example, Peter Pallas divided hydatid cysts into adherent and non-adherent forms and named the cyst *Taenia hydatigena* (Malik&

Shams 2019). The protoscoleces in the hepatic cysts were described in detail by Goeze (Eckert & Thompson, 2017), and subsequently described the hydatid cysts in sheep and named them *Hydatigena granulosa*.

During the 18th century advancements were made on the understanding of the life cycle of the parasite in both the definitive and intermediate hosts(Brooks *etal*.,2019). Several experimental studies were performed by Carl Theodor von Siebold, Breslau, and Friedrich Küchenmeister to further the understanding of the life cycle of echinococcosis (Tappe *et al.*, 2010). They fed a group of dogs metacestodes of *E. veterinorum* isolated from slaughtered sheep, which led to the discovery of the strobilar stage. Subsequently Von Siebold named the adult worms *Taenia* (T.) echinococcus (Von Siebold, 1853 cited in Eckert & Thompson, 2017), However, a series of further experiments were undertaken to confirm the life cycle of the parasite.

The faeces of infected dogs were fed to a group of sheep resulting in the production of fertile liver cysts (Wen *etal*.,2019). Finally Rudolphi in 1801 suggested the genus name of *Echinococcus*, being derived from the Greek word meaning "spine and berry" (Al-Shabbani, 2014).

1.2.2. Classification of *Echinococcus granulosus*:

According to classified by Eckert *et al* (2002), the tapeworms whose larval stage causes hydatid cysts belong to the following classification :

Phylum: Platyhelminthes.

Superclass: Eucestoda.

Class: Cestoidea.

Subclass: Cestoda.

Order: Cyclophyllidea

Family: Taeniidae.

Sub family : Echinococcinae

Genus: *Echinococcus*

Species: *Echinococcus granulosus*

1.2.3. -Strains of *Echinococcus granulosus*

Echinococcus granulosus has been divided into several strains according to the host (Mario *et al*.,2011). *Echinococcus granulosus* has been researched extensively and discussed in detail as it is the species that is most widely distributed throughout the world, it has seven strains which have been identified as G1 genotype (common sheep strain), G2 genotype (Tasmanian sheep strain), G4 genotype (horse strain), G3, G5 genotypes (cattle strain), G6 genotype (camel strain), G7 genotype (pig strain)(Rahman *et al*.,2015).

These species can infect a specific type of animal or a range of animals: for example *E. felidis* is restricted to the lion (*Panthera leo*) (Hüttner *et al*., 2009); and *E. shiquicus*, discovered in 2005, is found in Tibetan foxes (*Vulpes ferrilata*) with the pika (*Ochotona curzoniae*) and voles (*Microtus limnophilus* and *Lasiopodomys fuscus)* from the Tibetan plateau being the predominant intermediate hosts (Boufana *et al.*, 2013; Wang *et al.*, 2019). This latter species is a "sister species" to *E. multilocularis* (Xiao *et al.*, 2006) and *E. felidis* has been shown to be phylogenetically closely related to *E. granulosus* (Hüttner *et al.*, 2009). In contrast to *E. felidis*, a wide range of hosts are susceptible to *E. granulosus*, including yaks, buffalo, camelids, pigs and equids (Eckert and Deplazes, 2004). The genotype G6/G7 of the pig strain has a wide

allopatric distribution, and has been assigned to the species *E. intermedius* and has been reported in pigs and camels as well as in other animals (goats, horses and humans) (Lymbery *et al.*, 2015).

1.2.4.Life cycle of *Echinococcus granulosus*

1- Final host

According to Lewall (Jenkins *et al* .,2015) the final hosts of *E. granulosus* are dogs, wolves, jackals, dingoes, coyotes and foxes, Feline species are seldom infected naturally, but the parasite has been reported in cats, wild cats and leopards, which can also serve as hosts, but with low efficiency.

2- Intermediate host

The common intermediate host of *E. granulosus* are many domesticated mammals such as human , sheep, cattle, pigs, goats and camels. Sheep, which harbor the most fertile hydatid cysts, are the most important intermediate host and represent the most important source of infection to dogs through the feeding of infected offal, (Rahman *et al.*, 2015).

3- **Development**

The intermediate host ingests eggs of *E. granulosus*, which are passed in the faeces of the final host and are immediately infective. They are resistant to external conditions and are capable of development even after months outside the body (Thompson & McManus, 2001). Following ingestion by an intermediate host, they hatch in the small intestine and the resulting oncospheres invade the blood vessels of the wall. The hatching process uses the disaggregation of the keratin-like blocks of the

embryophore by pepsin, pancreatic enzymes, etc., followed by the activation of the contained oncospheres (Khanfar, 2004).

The released muscular oncosphere attaches to the microvilli of the jejunal region of the small intestine with its six hooks, then enters the lymphatic or mesenteric venules and lodges in numerous organs, affecting the differential distribution of metacestodes between the liver and lungs, Within twelve hours after ingestion, it arrives at the liver, where if not destroyed by phagocytic cells, it develops into a hydatid cyst (Thompson & McManus, 2001).

Then settle in the liver, forming a respective cyst, which is the origin of an *Echinococcus*. However, the liver is not always the primary filtration site (Zarbaliyev *etal*., 2019). Inhaled eggs can also cause pulmonary hydatid disease as it has been shown that eggs administered to sheep by a tracheostomy develop into lung cysts (Torgerson *et al*., 2003). The larvae of *E. granulosus* may also develop in the heart, spleen, kidneys, brain, eyes and long bones (Ramos,*et al*., 2020). The speed of development of the embryo to the fully formed metacestoda is slow, and during the first 10-14 days, it involves cellular proliferation, degeneration of the oncosphere hooks, and formation of a central cavity and development of the laminated and germinal layers. For example, the time taken to produce the first brood capsules from eggs is reported to be not less than 7 months in mice (Ahmed *etal*., 2019) ,10-12 months in pigs and 10-48 months in sheep .

When a final host ingests these hydatid cysts, the protoscoleces project their heads and secure a firm hold among the villi of the small intestine and mature into adults in 40-50 days. Since each worm stays in a host for 5-29 months, the eggs of *E. granulosus* are liberated after

detachment of a gravid segment, which occurs every two weeks. Thus, the number of eggs produced by an adult is less than those for other cestodes (Hijjawi *et al*., 2018) In addition, the small intestine may finally become full of adults as a result of repeated infections or intake of a large number of cysts.

Each scolex that is ingested is capable of developing into an adult worm in the intestinal tract in about seven weeks (Thompson , 2017) figure (1-1). In heavily endemic areas, 50% of the dogs are infected with adult worms, and up to 90% of sheep and cattle and 100% of camels may be infected with hydatid cysts (Saheb *et al*., 2017), However, experimental evidence has been reported that echinococcosis can become established in the lungs of sheep from eggs without their prior ingestion through intra tracheal route.

The pathology caused by the presence of a single unilocular cyst depends very much on its site in the body. In the liver, the cyst may cause compression of the liver cells, leading to biliary stasis and cholangitis. Lung cysts are always intracapsular and their rupture may result in protoscoleces being coughed up in the sputum. Hydatid cysts in the brain or spinal cord are usually small and may cause symptoms earlier than in other sites. Renal cysts sometimes occur and, if they burst into the kidney pelvis, may become secondarily infected (AL-tameemi &Kabakli, 2019) .

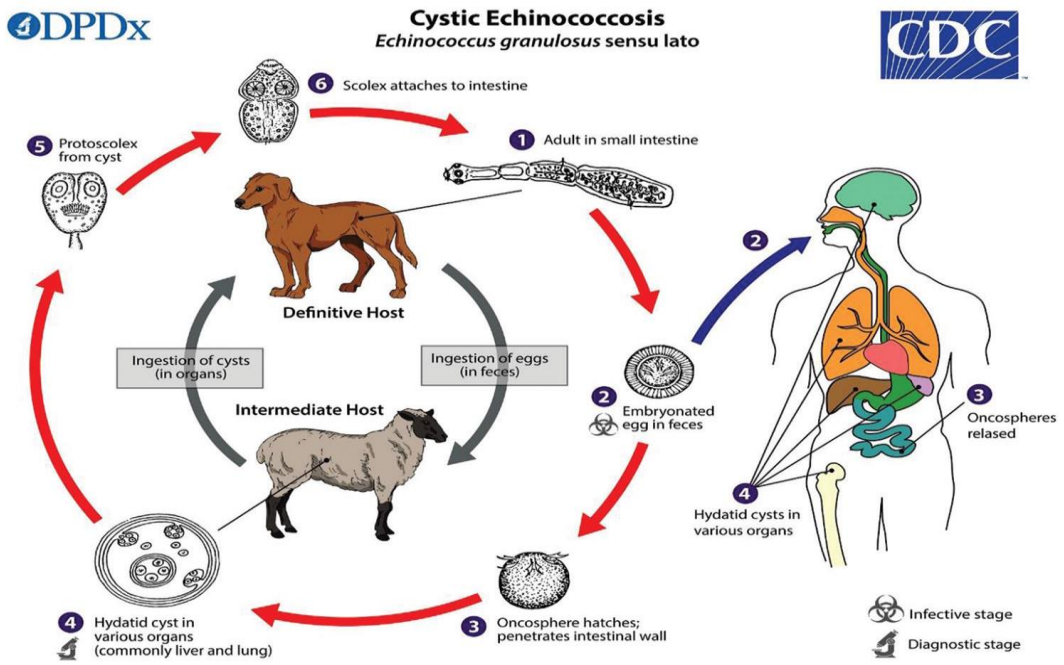
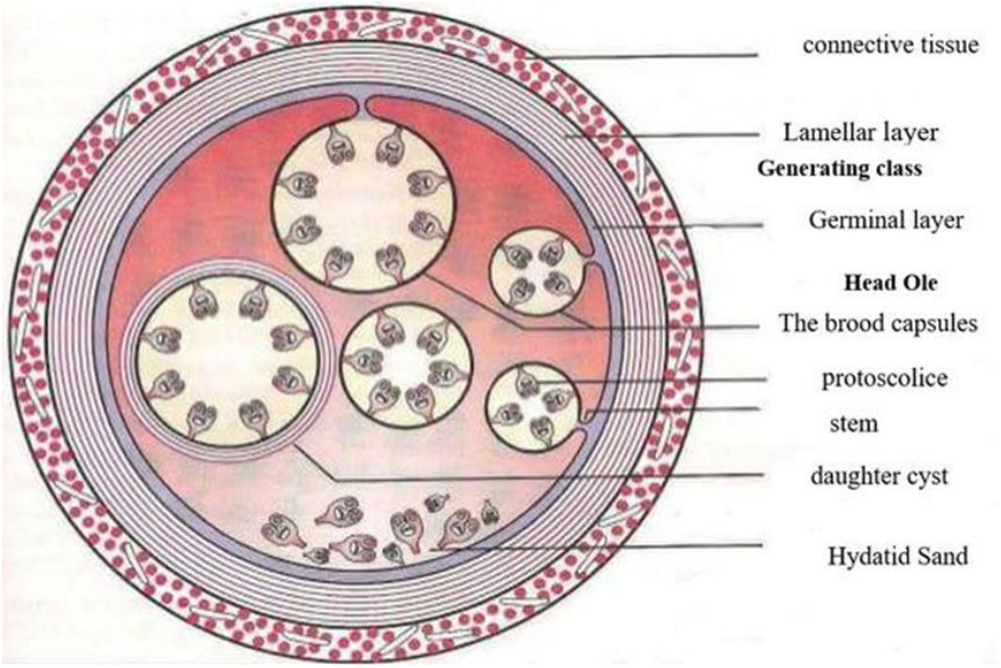


Figure (1.1): A typical domestic lifecycle of *Echinococcus granulosus*. Source:Centers for Disease Control and Prevention (Ndlovu *et al.*,2018).

The hydatid cyst has three layers: (*a*) the outerpericyst, composed of modified host cells that form a dense and fibrous protective zone; (*b*) the middle laminated membrane, which is acellularand allows the passage of nutrients; and (*c*) the inner germinal layer, where the scolices (the larval stage of the parasite) and the laminated membraneare produced. The middle laminated membraneand the germinal layer form the true wall of the cyst, usually referred to as the endocyst, although the acellular laminated membrane is occasionally referred to as the ectocyst (Malik, 2016;Hassan, 2017). Daughter vesicles (brood capsules) are small spheres that contain the protoscolices and are formed from rests of the germinal layer. Before becoming daughter cysts, these daughter vesicles are attached by a pedicle to the germinal layer of the mother cyst. At gross examination, the vesicles resemble a bunch of grapes (Fig 1-2). Daughter

cysts may grow through the wall of the mother cyst, particularly in bone disease (Osman, 2017).

Cyst fluid is clear or pale yellow, has a neutral pH, and contains sodium chloride, proteins, glucose, ions, lipids, and polysaccharides. The fluid is antigenic and may also contain scolices and hooklets. When vesicles rupture within the cyst, scolices pass into the cyst fluid and form a white sediment known as hydatid sand (Subramanyam *et al*., 2015).



Figure(1.2) :structure of hydatid cyst (Al-Khalidi *et al.*,2020)

1.2.5. Distribution of Cystic Echinococcosis

1.2.5.1. Global distribution of Cystic Echinococcosis

Anthropogenic influences, including increased globalization of animals and animal products, and altered human/animal interfaces are thought to have played a vital role in the global emergence of this pathogenic cestode (Davidson *et al.*, 2012; Deplazes *etal*.,2017). Expansion of the human population, resulting in a shrinkage of natural habitats and the associated increased urbanization of some wild carnivore

species, have resulted in the spread of *Echinococcus* spp. from the traditional rural environs (Carmena & Cardona, 2014). In some parts of the world, such as Central Europe, the Baltic States and the Scandinavian countries, hydatid cysts are less prevalent in humans and other animals (Dakkak, 2010).

In contrast a high prevalence has been reported in Russia (Torgerson *et al.*, 2010), and CE is considered to be re-emerging in Wales (Buishi *et al.*, 2005) . Re-emergence in some countries may be as a result of failure of existing control campaigns or socio-economic changes resulting from the collapse of previous political systems ,Transmission of CE in Europe relies primarily on dogs serving as the definitive hosts and domestic ungulates, including sheep, goats, cattle, buffaloes, horses and pigs, acting as the intermediate hosts(Cardona & Carmena, 2013).

Human CE remains endemic in Europe with regular recording of sporadic cases (Torgerson, 2017). In Germany CE cases are considered autochthonous with an annual incidence of approximately 0.05 per 100,000 population, being reported from 2001 to 2013. In Austria, the annual incidence of CE has been estimated to be 0.4 per 100,000 inhabitants (Schneider *et al.*, 2010).

Hydatid cysts are endemic in livestock and humans in Africa (Dakkak , 2010). Although some countries of Africa have undertaken studies to determine the prevalence of hydatid cysts (Mohammed , 2016), others have not and it is likely that the real burden of infection has been underestimated (Cardona & Carmena , 2013).

Molecular studies of CE have been carried out on cysts from production animals in African countries (Algeria, Mauritania, Ethiopia, and Sudan), revealing the presence of *E. granulosus*, *E. ortleppi*, *E.*

canadensis and *E. equinus* (Omer *et al.*, 2010). In Uganda and Kenya, *E. felidis* has been detected in lions (*Panthera leo*) and warthogs (*Phacochoerus* spp.), although no evidence of infection in humans has been reported (Hüttner *et al*., 2009).

In contrast, a genetic survey of cysts isolated from human patients in Mauritania and Kenya have detected the sheep strain G1, as well as the camel strain G6 (Salem *et al.*, 2011). In Egypt, *E. granulosus* have been isolated from sheep and *E. canadensis* from camels and sheep (Amer *et al.*, 2015).

In South America, CE is a common disease with around 5,000 new human cases reported annually in the five countries of Argentina, Brazil, Chile, Peru and Uruguay (Pavletic *et al.*, 2017). The life cycle of CE is maintained by domestic cycles of transmission involving dogs and herbivores (sheep, swine, cattle, goats, horses and camelids) and multiple species/genotypes including *E. granulosus* (G-G3), *E. ortleppi* (G5) and *E. intermedius* (G6/7) (Deplazes *et al*., 2017). In Argentina the annual incidence was reported as 0.95 per 100,000 inhabitants between 2005 and 2010 (Bingham *et al.*, 2014).

1.2.5.2. Distribution of Cystic Echinococcosis in the Middle East

Echinococcosis has been reported to be a public health concern in Iran, Iraq, Jordan, Egypt and Turkey as evidenced by the large number of hospitalised cases (Sadjjadi, 2006). A number of factors have been identified contributing to the increase in the disease's prevalence in humans in the Middle East, including poor slaughter-house hygiene, low general public awareness of the disease, inefficient veterinary services and large numbers of free-roaming stray dogs (Dakkak, 2010).

The home slaughter of animals (small ruminants particularly) is still common in a number of Middle Eastern countries, and large numbers of animals are often slaughtered for specific festivals such as the Muslim Eid (Othieno *et al*., 2016). Free roaming dogs have been shown to have high infection rates of *Echinococcus* with a prevalence of 7.9 to 14.3% and 14.2% observed in Palestine (Abdel-Hafez and Kamhawi, 1997). *Echinococcus granulosus* has been reported to be the most dominant species in dogs in Egypt, Jordan and Iran (Shariatzadeh *et al*., 2015).

In Iraq, cystic echinococcosis is one of the main health concerns (Al-Rishawi & Al-Mayali, 2019), which is endemic and a major health problem in the country, It is even more complicated because of that CE didn't have a real systematic national surveillance and control program in Iraq (Athmar & Ban-Abbas, 2014). The largest number of human cases were reported in the central and southern governorates of Iraq including Basrah, Dhi Qar and Al-Muthana (Abdulhameed *et al*., 2019).

1.2.6. Pathology of cystic echinococcosis

Despite carrying a massive parasite burden, definitive hosts do not normally show any symptom while being infected by adult worms on contrary, the larval stage of *Echinococcus granulosus* induces significant pathology in the,intermediate host (Torgerson& Budke, 2011). Indeed, the pathogenicity of hydatid cyst differs from host to host and depends on many factors such as age, sex, genetic traits, physiological condition and, species of the host (Cappello *etal*., 2013). Besides, severity of clinical symptoms is closely correlated to the size, number and localization of evolved cysts (Deplazes *etal*., 2017).

Almost in all intermediate hosts, hydatid cyst is principally located in the liver with a frequency of about 70%, although can be found in

other organs such as the lungs (20%), kidneys, spleen, brain, heart and bones with less frequency (Zhang *et al* ., 2012). About 20–40% of human patients have multiple cysts or multiple organ involvement. After an undefined incubation period which may last months or years, the exerted pressure on adjacent tissue by a grown cyst may cause symptoms and can be followed by other pathologic events(Craig *etal*., 2017). As hydatid cysts grow slowly, the host often tolerates it remarkably well and therefore hydatid patients may come to clinical attention only when the normal function of the infected organs is interfered by the mechanical pressure of the cyst (Chai *et al*., 2021). Other clinical signs such as allergic reactions, eosinophilia or accidental cyst rupture which triggers acute hypersensitivity responses can also indicate the existence of the infection, cysts or a cystic mass may also be discovered by chance during body scanning or surgery for other clinical complications (Nunnari *et al* . , 2012).

High temperature and desiccation are limiting factors for human infection with hydatid disease (Kwa, 2018), Other factors are type of the soil, wind dispersal, and vegetation cover also persons of both sexes appear equally susceptible but sex ratios of patients vary in different regions and host factors are human behavior such as widespread use of dogs and the habit of feeding on viscera of home butchered sheep or other livestock,Personal hygiene and cleanliness ,socioeconomic and cultural characteristics are among the risk factors for human infection (Caldas *et al* ., 2008).

1.2.6.1. Cystic echinococcosis of the liver

Once the onchosphere passes through the intestinal wall, it is carried by the portal venous or lymphatic system to the the liver as the first line of defense. That is why the liver is the most frequently involved organ. Most cysts tend to be harbored in the right lobe (Kanan & chain ,2006). Natural history of the hydatid cyst can be divided into two phases (Stojkovic & Junghanss , 2013).

1- During the first phase, continuous growth and the enlargement of the cyst can cause increased compression on the surrounding parenchyma and may result in upper abdominal pain and other non-specific signs. While hydatid cyst is growing, the cyst wall may lose its resistance against the pressure of the hydatid fluid, thus cyst rupture occurs. As well, this condition can happen due to a trauma or even surgical intervention. In general, symptoms such as acute allergic reactions, obstructive jaundice and emesis can be detectable as consequences of the cyst rupture.

2- If protoscolices and daughter cysts are overproduced during the first phase, the hydatid fluid will be replaced by these components which results in stiffness of the cyst cavity and is followed by the calcification of the cyst wall. In this phase, cyst growth usually halts and the ectocyst is detached from the fibrous capsule, Partial calcification of the cyst does not always indicate the death of the parasite; nevertheless, densely calcified cysts may be assumed to be inactive (Kanan & chain ,2006) .

Secondary infection (i.e. bacterial, fungal) of the hydatid lesion is the most common complication and can be somewhat symptomatic. Infection occurs only after communicating and direct rupture when both the

pericyst and endocyst fracture, which allows pathogens to pass easily into the cyst, in 5%–8% of cases (Lin *et al*., 2014). The evolvement of an infected hydatid cyst is usually dormant, sub-acute and is clinically identified by pain in the right hypochondrium, hepatic abscess, and fever (Mnati,*etal* ., 2020)

Biliary rupture may occur through a small fissure or bile duct fistula (Yilmaz *et al*., 2012). A wide perforation allows the access of hydatid membranes to the main biliary ducts, which can cause symptoms simulating choledocholithiasis (Bricault, 2012). Intrabiliary rupture of a hepatic cyst can be indicated as an occult drainage of hydatid fluid into the biliary tree and is observed in 10-37% of patients mainly in centrally localized cysts (Ramia *et al*., 2012; Touma,*et al*., 2013).

The increased pressure of the hydatid fluid can be also a prompting factor of the rupture usually in the right hepatic ducts, although the left hepatic ducts are sometimes involved, More severe complication can be detected due to an overt passage of intra-cystic material to the biliary tract in 3-17% patients (Abdelraouf *etal*.,2015), Perforation into the gallbladder can be detected in 5-6% of cases.

Hepatic cyst rupture to the gastrointestinal tract is very rare (Vidoura *etal*., 2017), On the other hand, released protoscolices settle other visceral organs and every one of them can potentially evolve to a hydatid cyst. This condition is termed as secondary cystic echinococcosis and may occur either spontaneously or after trauma. Formation of secondary cysts has been also observed as a complication after inattentive surgery(Sokouti *etal*.,2017). Involvement of the pulmonary parenchyma or peritoneum is usually the most frequent trait of secondary cystic

echinococcosis. Nonetheless, primary infection of the peritoneum has been also reported (Panteleyev ,2018) .

1.2.6.2. Cystic echinococcosis of other organs

In human hosts, the lungs are the second most frequent sites of infection in adults, while the involvement of the lungs is the most common feature of cystic echinococcosis in children (Vatankhah ,2016). In organs such as the lungs and the brain, hydatid cysts may grow faster and achieve larger size more likely due to the softness of the tissues which is easy to compress, Calcification in pulmonary cysts is very rare (0.7% of cases)(Kern *etal*.,2017), although it may be seen in pericardial, pleural, and mediastinal cysts (Al-Yasari *et al*.,2013). Expectoration of the fluid or other materials of the cyst and its rupture into the pleural cavity may also occur. Bacterial infection of the cyst is the most serious complication commonly seen after rupture (Pezeshki *et al*.,2012).

The prevalence of renal infection is 3% and the involvement of the kidneys usually remains asymptomatic for many years, although symptoms such as flank mass, pain and dysuria can be commonly seen (Othieno,*et al*.,2016). Several round masses may be seen in the excretory system due to daughter cysts (Murtaza *et al*.,2017).

The splenic involvement in human hydatid disease has been reported in 8% to 9% of cases (Della *et al*.,2015). The metacestode can be harbored in the spleen majorly after systemic or inter-peritoneal dissemination of protoscolices due to the rupture of hepatic cysts. Consequently, the spleen is usually considered as the third most frequent site of infection in humans. Clinical symptoms such as abdominal pain, splenomegaly and fever are often observed in patients with splenic hydatid infection (Akbulut *etal*.,2013).

The osseous involvement in hydatid disease is most commonly seen in the spine and pelvis, followed by the femur, tibia, humerus, skull, and ribs with a frequency ranged between 0.5-4% (Pakala,2016). The absence of the cellular infiltrate and fibrosis (pericyst) around the cysts in the skelet al system allows them to grow vastly in an irregular manner and they may produce subsidiary branches that penetrate through less resistant compartments of the tissue, especially in the bone canals (Craig *etal*.,2019).

Hydatid disease affects the central nervous system in 1% of cases and is usually diagnosed during childhood, The hemispheres are the most common locations of the cerebral cysts, particularly around the middle cerebral artery, although the meta cestode may be harbored anywhere in the brain(Maraby-Salgado *et al* .,2017).

1.2.7. Clinical manifestations

The *Echinococcus* species are of medical and veterinary importance, since infection with the metacestode can cause severe illness (even death of the intermediate host) and livestock associated production losses (Miran *etal*.,2017). Clinical manifestations of the disease in intermediate hosts primarily depend on the size, location and number of cysts (Abo-Aziza *etal*.,2019). Patients can remain asymptomatic for months, years, and even longer, with clinical symptoms showing from below one year to over 75 years of age in all sexes (Seid& Melese ,2018). The clinical complications are therefore a result of mechanical pressure exerted on surrounding vital organs by the growing cyst, Accidental rupture of cysts can also happen, releasing fluid filled with protoscolices and possibly leading to anaphylactic reactions and secondary multiple cyst infections

(as protoscolices can develop into secondary cysts within the intermediate host) (Teshome *et al* ., 2017)

Lung infection may present as chronic cough, pneumothorax, pleuritis, lung abscess and parasitic embolism (Díaz , 2017). Heart involvement can present as a tumour, complete heart block and sudden death (Baumann *etal*.,2019),The effects in the brain are usually headache and mass effects with neurological symptoms, and patients with eye infection can present with pain, ptosis and visual disturbances (Baumann *et al*.,2019).

The tapeworm cysts in humans can be found mostly in the liver (62 to 70%), lungs (20%), and other organs (brain, wall of the heart, kidneys, spleen, orbit of the eye, marrow cavity of bones) can be involved in 10% of the cases (Yuan *et al*.,2017), Multiple cysts or multiple organ involvement can also be seen in 20% to 40% of patients .

The clinical manifestations of cystic echinococcosis can also be complicated by other factors such as co-infection with human immunodeficiency virus (HIV) (Lozneanu *etal*.,2019). It was found that the profound immunosuppression of the patient can result in extensive CE disease and can interfere with immunodiagnostic tests, leading to false-negative results (Lozneanu *etal*.,2019). The adult tapeworms are considered to be rather harmless to the definitive host, except when they occur in large numbers, when they might cause severe enteritis (Al-Khalidi *et al*.,2020).

1.2.8. Diagnosis of cystic echinococcosis

Due to the bad prognosis of developed infection, early diagnosis is an essential part of the treatment and control procedure in cystic echinococcosis, Considerably long incubation period of the infection

during which clinical manifestations are usually absent, is an important challenge to plan an efficient strategy for early diagnosis of the infection (Minaev *et al*.,2018). Parasite larvae show the highest susceptibility to chemotherapeutic compounds during the pre-encystment phase or before maturation of the established cyst (Hijjawi *et al*.,2018).

Because of the distinctive natural history of the infection, patients are usually found accidentally or when physical damage to the harboring organ and cyst rupture has occurred. A reliable diagnosis requires combination of physical examination, imaging techniques and serology methods. Upper abdominal discomfort, loss of appetite and pain are of the major complaint that along with results of physical examinations such as hepatomegaly, presence of abdominal palpable mass and abdominal distention may lead clinicians to consider potential occurrence of cystic echinococcosis (Khatonaki *et al*.,2020).

1.2.8.1. Imaging techniques

Generally used imaging techniques include ultrasonography, CT-scanning and magnetic resonance imaging (MRI). Ultrasonography is the most common imaging method to identify hydatid lesion and is useful to determine the number and size of hydatid cysts in almost all anatomical sites (Orsten *et al*.,2018), This technique can be used in field surveys by applying portable machines . Ultrasonography can only visualize cysts with at least partial calcification where echogenic regions (either hyperechogenic or hypoechogenic) are scattered throughout the lesion. Regarding this trait, it has been tried to introduce an internationally accepted system to standardize the hydatid cyst diagnosis based on classification of its ultrasonographic features (Turgut,*et al*.,2007). In 2003, the World Health Organization (Abdulhameed *et al*.,2019)

published a new standard system which was actually an amended version of Gharbi‟s classification method introducing six categories: cystic lesion (a unilocular cyst with unknown origin which is to undergo more investigation), CE1 (unilocular fertile cysts with visible wall), CE2 (multivesicular septated fertile cysts), CE3 (laminated layer is detached from the cyst wall which makes „water lily‟), CE4 (cysts with scattered hypo- and hyperechoic degenerative contents and no visible daughter cysts), and CE5 (cysts with thick wall showing partial or complete calcification) (Brunetti *et al.*,2018).

1.2.8.2. Immunological and molecular diagnosis

Although imaging techniques provide valuable information about physical appearance, dimension and anatomical site of hydatid cysts, diagnosis can be sometimes presumptive (Stojkovic *et al*.,2009).

Serology of hydatid cyst is normally based on tracing specific antibodies, parasite circulating antigens and circulating immune complexes in serum samples (Siles-Lucas *et al*.,2017). So far, various serological methods have been tested for diagnosis of the infection. Due to the lack of sensitivity and presence of non- specific results, early methods such as Cassoni intradermal test and complement- fixation test were replaced by more sensitive examinations. Routine laboratory tests include indirect hemagglutination (Nunnari *et al*.,2012) , enzyme- linked immunosorbent assay (ELISA), latex agglutination (LA), indirect immunofluorescence antibody test (IFA), immune- electrophoresis (IEP), and immunoblotting (IB) (Carmena *et al*.,2006), Immunoassays have appeared to be expedient not only for detection of the infection and follow- up analyses but also for screening studies in endemic areas.

Recent advances of molecular genetics have suggested application of more accurate methods such as polymerase chain reaction (PCR) for detection of hydatid antigens or expression of specific antibodies, although performing these methods requires high- tech equipment and well- trained laboratory staff and other conditions which are often difficult to provide particularly in endemic areas of the Third- world or developing countries(Zheng *et al*.,2013).

Molecular techniques have provided much more valid methods for identification and characterization of parasite (Craig *et al*.,2007). Recently analysis of DNA has been used to categorise variants of *E. granulosus* into distinct genotypic strains, only 10 genotypes (G1-10) have been identified (Sharbatkhori *etal*.,2016) .

Genetic studies have principally confirmed the concept of strain diversity within the species of *E.granulosus*, previously based on morophological, biochemical and epidemiological studies, This variation may reflect variable in characters which affect the life cycle pattern, host specificity, development rate, pathogenicity, sensitivity to chemotherapeutic agents, transmission dynamic, epidemiology and control of echinococcosis (Barazesh *etal* .,2019).

1.2.9. Treatment

Cystic echinococcosis treatment is based on surgery, chemotherapy or both, Cyst removal followed by anthelminthic medication is often proposed as the best choice for the disease management Schantz (Lupia *et al*.,2021).

Surgical operation is normally associated with many risk factors and may not be very cost- effective, although seems to be the best option for precise depletion of the parasite (Schantz, 2006). Furthermore, clinical

status of the patient and presence of other health complications as well as cyst indications (such as size, number and location which may make it surgically inaccessible) and presence of well- experienced medical team can restrict surgery (Monteiro *et al*.,2010). The most common techniques are used for hydatid cyst surgery include open procedure, laparoscopy and percutaneous treatment. Cyst removal by surgery is divided into radical (when pericyst and parasitic content of the lesion are entirely excised or cystectomy) and conservative (when only parasite cyst is removed or hydatidectomy) methods (Bayrak & Altıntas, 2019).

Chemotherapy has recently received more attention for treatment of cystic echinococcosis, Benzimidazole and albendazole are current chemotherapeutic choices for the disease, although albendazole is more recommended because of its better absorption (Muhammedoglu *et al*.,2021) . Although anti-hydatid drugs have had some success, adverse medication responses, non-responsiveness to treatment, resurgence of infection, parasite resistance to the administered drug, and in some cases severe illnesses have been reported in a significant number of patients (Khalkhali, *et al*.,2018)

1.2.10. Immunity response to the *E.granulosus* :

The host immunity plays an important role in determining the relationship between the host and the parasite. Parasite produces excretory compounds, which influence the immune-competent cells in the human host and stimulate pro-inflammatory immune responses, releasing antibodies, and activate T-cells in the body (Slimane *et al*.,2018).

Continuing presence of parasites in the body indicates that they have developed some of the evasion mechanisms from host immune mechanisms to preserve their development. *E. granulosus* can use two

mechanisms to reduce the host immune response: (Al-Tameemi & Kabakli,2019) Passive escape by developing into a hydatid cyst so avoiding the ruining effects of an immune response and, immunomodulation through which *E. granulosus* interacts with the host immune system to reduce the efficiency of the host response (Mohamed .,*et al*.,2017) Recent studies showed that *E. granulosus* secretes molecules that can modulate the immune responses so changing the cytokine balance toward Th2.

Antibodies play a major role in parasite killing as a protective immune response against *E. granulosus* involving antibody-dependent cell-mediated cytotoxicity reactions (Profumo *et al*.,2014), Cystic echinococcosis (CE) induces two (Th1 and Th2) cytokine secretion patterns in the active and inactive stages of hydatid disease. Early Th1 cytokine production (which kills the metacestode at the initial stages) changes to a predominant Th2 cytokine as a response in the chronic stage of *E. granulosus* and *E. multilocularis* infections (Zhang *et al*.,2012). Th2 cells produce interleukin (IL)-4, IL-5, IL-6, and IL-10 which are associated with susceptibility to the disease, whereas Th1 cells express IL-2 and interferon-gamma and they are well related to protective immunity. Some of the studies showed an increase in the production of some cytokines such as gamma interferon, IL-4, and IL-5(Craig *et al*.,2017).

The six family members identified (IL17A-F) exert mostly pro-inflammatory activities (Noda *etal*.,2011). IL17A and IL17F, mediators of the recently described proinflammatory Th17-type immune responses, have been associated with inflammatory disorders like rheumatoid arthritis and inflammatory bowel disease (Robert& Miossec, 2019).

1.2.10.1. Host- parasite immune interaction in cystic echinococcosis

Existing data about host immune reactions to cystic echinococcosis is still insufficient,Many studies have been carried out to unveil the association of immune responses to the host- parasite relationship in different stages of the infection, although a definitive picture to show the clear-cut traits of hydatid- induced immune mechanisms cannot be concluded by the achieved results (Yang *et al*.,2012). It probably reflects the impact of the intermediate host phenotype on immune reactions, as a wide range of mammalian species is involved in the parasite life cycle. Furthermore, genotypic variability within a certain species is likely determinative to the immunity against cystic echinococcosis (Shen *et al*.,2014). The main problem is raised by taking into account that these results are primarily derived from studies on laboratory animals, so cannot be a precise indication of immunity against the parasite in naturally infected intermediate hosts(Gottstein *et al*.,2017). Immunoserological assays have also provided indirect evidence to characterize parasite- induced immune reactions in human patients and other mammalian intermediate hosts(Mourglia-Ettlin *et al*.,2016). Nevertheless, such findings seem inadequate to thoroughly illustrate the real feature of host- parasite immune interaction, particularly in human(Mourglia-Ettlin *et al*.,2016).

Immunopathology of the infection can be studied during two distinctive phases: the establishment (pre-encystment) phase and established (encystment) phase (Farid& El Amir,2015), however this concept has appeared to be controversial as the *Echinococcus* species are multicellular organisms shown to bear a large number of antigenic compounds that may frequently change during their life span (Mnati *et al*.,2020).

1.2.10.2. Immune responses during the pre-encystment phase

Little is known about innate immune mechanisms are primed against early stages of hydatid infection, study has suggested the innate immune responses have a crucial role in host susceptibility/resistance to the infection (Vuitton& Gottstein2010). Evidently, various effector mechanisms corresponding to innate and adaptive immune functions are induced against experimentally produced hydatid infection either with eggs (primary infection) or with active onchospheres (secondary infection) in laboratory animals. Perhaps activation of complement system, particularly through its alternative pathway, associates with the host resistance against the infection during the pre-encystment phase. It has been implied that C5- mediated complement reaction followed by activation of inflammatory cells may cause pathologic changes when the embryo is trapped in the harboring organ (Kendall,2014). Infiltration of leukocytes such as eosinophils, neutrophils and macrophages is a hallmark of cellular reaction against invasive larvae of tissue-dwelling helminthes and is detectable after 3-5 days post-infection in mice (Rinaldi *et al*.,2014). Eosinophils seem not to have participation in the host defense against adult *Echinococcus granulosus* (Mezioug & Touil-Boukoffa, 2012).

Both humoral and cellular schemes of adaptive immune system are shown to contribute to the host resistance against developing hydatid cyst, Detectable levels of immunoglobulin (Ig) G are produced against primary infection after 2 and 11 days in mice and sheep, respectively (Wang *et al*.,2019). Activation of neutrophils and macrophages upon presence of IgM and IgG suggests that the antibody-dependent cell-mediated cytotoxicity (ADCC) has likely a pivotal role in depletion of the parasite, as well conducts the pathologic changes during early stages of

the infection (Sutton *et al*.,2019). There is a lack of information about early cytokine production in primary infection of hydatid cyst is insufficient, although it is believed that T lymphocyte profile activated against developing cyst is initially polarized toward Th1 cytokines which is capable to deplete the parasite and provoke inflammatory changes in the tissue(He *etal*.,2021).

1.2.10.3. Immune response during the encystment phase

Host immune reaction to the established cyst has received more attention for investigation about immunopathology of cystic echinococcosis. As soon as the embryo is lodged in a suitable organ, it starts to develop into hydatid cyst with complete endocyst and ectocyst (Zhang *et al*.,2012). In both animal and human host, humoral immune reaction against the established cyst is characterized by presence of circulating IgM, IgG1 and IgG4, and IgE (Díaz *et al*.,2011). Animal models challenged with either parasite eggs or active protoscolices initially show lower levels of expressed IgG subclasses, however gradual increase in antibody response is observed along with the cyst growth (Feng *et al*.,2013). Expression of IgM, IgG1, and IgG3 may induce complement activation and suggests the role of innate immune mechanisms during the early stage of encystment phase,Although complement factors are not clearly shown to have contribution to the host defense against established cysts, their activation has been indirectly confirmed (Sharma *et al*.,2020). Complement effector molecules such as C3 and C4 were also measured in sera obtained from hydatid patient and remained detectable after surgery (Barrios *et al*.,2019).

The established cyst is thought to induce activation of different immune effector cells in the intermediate host (Weatherhead et al.,2020).

Cytokine profiling assays have demonstrated the presence of IL-2, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-4, IL-5, ,IL12 and IL-17 in the sera obtained from animals with the primary or secondary infection and from human patients (Magatti et al.,2018; (Fereig& Nishikawa,2020). This cytokine may associate with the parasite survival and its production reclines after successful treatment but remains high in patients who does not respond to chemotherapy (Naik *et al*.,2015).

1.2.11. *E. granulosus* and Cytokine Induction :

Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells (Cassatella *et al*.,2019). Cytokine is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes) (Cameron& Kelvin, 2013). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action)(Scully *et al*.,2017).

There are both pro inflammatory cytokines and anti-inflammatory cytokines, There is significant evidence showing that certain cytokines/chemokines are involved in not only the initiation but also the persistence of pathologic pain by directly activating nociceptive sensory neurons(Ramesh *et al*.,2013). Certain inflammatory cytokines are also involved in nerve-injury/inflammation-induced central sensitization, and are related to the development of contralateral hyperalgesia/allodynia. (Eguchi *et al*.,2018).

E. granulosus that encounter the immune system can influence the differentiation decision. Th1 and Th2 cells are not precommitted phenotypes but rather, represent endpoints of a multistep differentiate process, whereby a common precursor population acquires a distinct cytokine secretion profile (Jankovic *et al*.,2001). During CE, the evidence concerning antibody levels of IgG4 and IgE isotypes and frequent eosinophilia, suggested that the immune response to established *E. granulosus* infection is Th2 dominated and that *Echinococcus* antigens modulate polarized T cells (Siracusano *et al*.,2008).

The IL-17A is a key pro-inflammatory cytokine in the T helper 17 pathway and it plays a critical role in host defense and inflammation. Evidences highlighting crucial role of cytokines in the host-parasite relationship come from studies on parasite-driven cytokine production in a large number of albendazole-treated patients with CE (Mévélec *etal*.,2020)

The T cell lines from a patient with an inactive cyst had a Th1 profile whereas T cell lines derived from patients with active and transitional cyst had mixed Th1/Th2 and Th0 clones (Farid& Amir ,2015) . Since PBMC from seronegative patients produced no parasite antigen driven- IL-5 and scarce IL-4 and IL-10(Della Bella *et al.*,2017).

Study of Li *et al*(2020) indicated that in CE a strong Th2 response correlates with susceptibility to disease (active cyst) whereas a Th1 response correlates with protective immunity (inactive cyst) and that Th1 and Th2 responses coexist .

1.2.11.1. Discovery and expression of IL-17 cytokines.

1.2.11.1.1. IL-17 (IL-17A)

Interleukin 17 (IL–17) also referred to as IL-17A, was discovered in a search for T-cell-derived molecules with immune functions. It was cloned from a T cell hybridoma produced by fusion of a mouse cytotoxic

The human IL-17 gene was originally mapped on human chromosome 2q31 (Basu *et al*.,2013). however, it has also been located in a sequence from a chromosome 6p12 clone (Tsai *et al*.,2013). The gene encodes a 20–30 kDa protein of 155 amino acids (Matsuzaki & Umemura, 2018).

The IL-17 polypeptide comprises at least one N-glycosylation site and six cysteine residues that form intermolecular interactions during dimerization. It has a 19-amino-acid signal sequence followed by a 136-amino-acid mature portion (McGeachy *et al*., 2019).

Sources of IL-17 appear to be rather restricted; expression of IL-17 has been detected mainly in activated CD4+ and CD8+ T lymphocytes (predominantly of the memory CD45RO+ subset) , Later studies detected the presence of IL-17 messenger RNA (mRNA) transcripts also in neutrophils and eosinophils (McGeachy *et al.*, 2019).

The IL-17 family and its receptors, which share minimal homology with other cytokines or known proteins, have been recognized as a distinct cytokine-receptor family and is crucial for normal host immune responses; this family is associated with many human pathogeneses, including those of inflammation and cancer (Lin & Leonard, 2018).

IL-17A, the prototypic member of this family, was first identified in 1993 (Rouvier *et al*., 1993) and named CTLA8. It was subsequently

renamed IL-17, and more recently, IL-17A. it is a 35-kDa, disulfide-linked, homodimeric protein with variable glycosylation (Morita *et al* .,2015; Ghanemi *et al*.,2020).

1.2.11.1.2.Interleukin 17 B (IL-17 B)

Interleukin17B was originally identified as a proinflammatory mediator that accelerates neutrophil recruitment and migration (Ge *et al*., 2020) ,IL-17B inhibits IL-25 signaling and attenuates mucosal inflammation .Although low IL-17B mRNA is detected in several organs, its expression is high in chondrocytes and neurons (Alves *et al.*,2018). Like IL-17E, IL-17B binds to IL-17RB with lower affinity than IL-17E. (Chan , 2019); however, the signal transduction mechanisms of IL-17B-IL-17RB are unknown. Both IL-17B and IL-17C induce TNF and IL-1b expression from a monocytic cell line and cause neutrophil infiltration (Cao *et al*.,2019).IL-17B, IL-17C, and IL-17D may have similar activity to induce inflammatory mediators, and contribute to inflammatory responses like IL-17A and IL-17F. Future experiments using cytokine-blocking Abs or cytokine gene-targeted mice may help to understand the functions of these cytokines in immune responses(Hadian *et al*.,2019).

Th1 and Th17 cells may have roles in clearing the parasites; however, with the extension of infection time, particularly after 3 months, Th2-type cytokines may begin to inhibit Th1-cell proliferation and immune response (Zheng , 2013). Thus, Th1 cells were downregulated, and the high levels of IL-17 secreted by Th17 cells resulted in a strong immune pathological injury of the host liver(Monin& Gaffen,2018).

Lechner *et al* .(2012), described how proper immune regulation, in response to an CE infection, depends on IL-17 regulation in pro-inflammatory immune responses , This can promote parasite tissue

infiltration and long-term growth *in vivo*, and can result in immune escape in a host of echinococcosis.

1.2.11.3. IL-17 Receptor and Its Signal Transduction:

The IL-17 cytokines bind to five cytokine receptors (IL-17RA to IL17 E-) on target cells to drive their biological actions , IL-17R is expressed in a variety of cell populations, including keratinocytes, fibroblasts, mesothelial cells, epithelial cells, and leukocytes (Xiang *et al*.,2020). IL-17RA is a shared receptor for different IL-17 isoforms. IL-17 cytokines can trigger signals via an IL-17RA/IL-17RC receptor complex (Fabre *et al*., 2018).. IL-17RB and IL-17RE serve as the specific receptors for IL-17B and IL-17RA/IL-17RB heterodimeric complex, respectively (Stevens *et al*.,2018). IL-17A and IL-17F act through the same IL-17RA/IL-17RC receptor complex, Studies suggest that IL-17RD also drives IL-17-mediated signaling, but the ligand of IL-17RD remains unknown(Bosmann *etal*.,2013).

1.2.12. Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are mostly biallelic point mutations, present within a population in a frequency higher than 1%. SNPs are also believed to be the main source of variability among humans, especially when they influence gene expression or function depending on their location in the DNA sequence. Moreover, since SNPs are relatively easy to be detected, they are considered as one of the best biological markers in association or case-control studies. Therefore, a large number of SNPs in cytokine loci have been described and studied in complex illnesses like infectious and autoimmune diseases and cancer (Pacheco & Moraes, 2009).

Single-nucleotide polymorphisms (SNPs) and single-nucleotide mutations result from the substitution of only a single base. The SNP or mutation can be relevant to disease susceptibility, pathogenesis of disease, and efficacy of specific drugs. It is important to detect SNPs or mutations clinically (Matsuda, 2017).

The promoter region SNPs affect gene expression by altering promoter activity, transcription-factor binding, DNA methylation and histone modifications (Schirmer e*t al*, 2016). The exonal SNPs affect cancer susceptibility by suppressing gene transcription and translation (Griseri *et al*., 2014). SNPs in intron regions generate splice variants of transcripts and promote or disrupt binding and function of long non-coding RNAs (lncRNAs),(Xiong *et al*., 2015).

SNPs in genes that regulate DNA mismatch repair, cell cycle regulation, metabolism and immunity are associated with genetic susceptibility to cancer (Ulaganathan *et al*., 2015).

The PCR and DNA sequencing have subsequently been used to construct a phylogenetic tree of the genus *Echinococcus*, with intraspecific variation in *E. granulosus* being identified Based on molecular characteristics (Ahmed,2016) .

1.2.13. **Single Nucleotide Polymorphisms (SNPs) and gene expression of IL 17Family**

The gene for human IL-17 is located in the human chromosome and have 1874 base pairs long (Wang *et al*., 2014).

Single nucleotide polymorphisms (SNPs) serve as important mutations that can affect transcription and translation. Numerous studies have reported the associations of IL-17A and IL-17F polymorphisms and

susceptibility to digestive system neoplasms, however, the results were not consistent. The IL-17A rs2275913 and IL-17F rs763780 polymorphisms were associated with susceptibility to digestive system (Gao *et al*., 2019).

Several studies have reported that IL-17 levels triggered in autoimmune diseases and elevated significantly in patients with rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, and autoimmune hepatitis (Liu *et al*., 2018).

Since then, some studies have reported the association of variation in species/genotypes of *E*. *granulosus* are reflected in morphological and biological characteristics of the parasite and it can influence the life cycle pattern, host specificity, development rates, pathogenicity, treatment, transmission dynamics, epidemiology and finally control of CE (Li *et al.*, 2015).

1.2.13.1. The types of SNPs

1-SNPs in non-coding regions :

Can manifest in a higher risk of cancer (Li *et al*., 2014), and may affect mRNA structure and disease susceptibility (lu *et al*., 2015). Non-coding SNPs can also alter the level of expression of a gene, as an eQTL (expression quantitative trait locus).

2-SNPs in coding regions:

A-synonymous substitutions by definition do not result in a change of amino acid in the protein, but still can affect its function in other ways. An example would be a seemingly silent mutation in the multidrug resistance gene 1 (MDR1), which codes for a cellular membrane pump that expels drugs from the cell, can slow down translation and allow the peptide chain to fold into an unusual conformation, causing the mutant

pump to be less functional (in MDR1 protein (Kimchi-Sarfaty *et al*., 2007).

B-nonsynonymous substitutions:

1-missense – single change in the base results in change in amino acid of protein and its malfunction which leads to disease (e.g. c.1580G>T SNP in LMNA gene – position 1580 (nt) in the DNA sequence (CGT codon) causing the guanine to be replaced with the thymine, yielding CTT codon in the DNA sequence, results at the protein level in the replacement of the arginine by the leucine in the position 527(Al-haggar *et al*., 2012) at the phenotype level this manifests in overlapping mandibuloacral dysplasia and progeria syndrome).

2-nonsense – point mutation in a sequence of DNA that results in a premature stop codon, or a nonsense codon in the transcribed mRNA, and in a truncated, incomplete, and usually nonfunctional protein product (e.g. Cystic fibrosis caused by the G542X mutation in the cystic fibrosis transmembrane conductance regulator gene) (Cordovado *et al*., 2012).

3- Results and Discussion:

3.1. Percentage infection of Hydatid disease.

Hydatidosis is remain a main public health problem in our country as in some other parts of the world (Sarhan,2008) . A total of clinical samples in this study include 93(53 patients with Hydatid cyst attended to hospitals in AL- Hilla city(AL-Imam Al_Sadiq Hospital, AL- Hilla Teaching Hospital, and private hospitals) and 40 case of control . The results of this study showed that the total percentage of infection with *E. granulosus* was (56.9%) as show in figure (3-1).

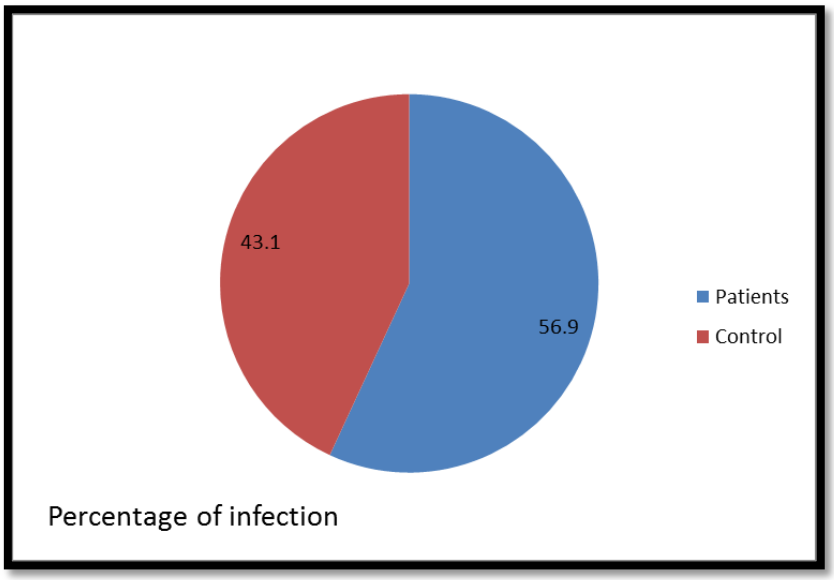


Figure (3-1) : The total percentage of infection with hydatid cyst.

A person becomes infected with this disease through accidental consumption of water, soil or food contaminated with the faeces of infected dogs, which is the most common type of method of infection(Al-Marsomy,2021).

A study of Saida & Nouraddin (2011), was disagreement with present study which confirm infection with hydatid cystic disease (HCD) in human and slaughtered animals in Erbil province. For humans a statistical analysis of documents, 149 cases have been recorded due to

cystic echinococcosis, and treated surgically in private and governorate hospitals in Erbil province. Among total of patients admitted to the surgical department, 0.846 % of which were found to be infected with cystic echinococcosis, and about 6.3 /100,000 persons among Erbil population. This different of our results explents that, low education of individuals on the disease leads to a high prevalence rate of infection while illegal home slaughtering of animals lead the infected organs to be at the access of the definitive hosts (stray dogs) for completing the life cycle of the parasite, These hosts act as a source of infection to the intermediate hosts by discharging huge numbers of eggs with feces.

Also, out of 480 cases, only 24 (5%) were positive with ELISA by Al-Mukhtar & Qasim,2017 in Mosul City, because this disease is usually detected late due to its silent growth and symptoms can appear years after infection when the internal organs are crushed by the cyst (Cappello *et al*. 2013).

This present result differs from many studies carried out in Iraq such as a study Obaid (2017),which Depending on the results of clinical and ultra sonographic, which show that 15.8 % of the patients were infected with cystic echinococcosis (CE), while 84.2% had simple cysts (SC), While the results of serological examination by ELISA revealed 14.1% for CE and 85.9% for simple cysts.

Another study which was carried out in Baghdad teaching hospital and in Al-Kut governorate by Al-Obaidi *et al* (2014), with liver hydatid cyst for elective surgery, The study found that patients with hydatid cyst represents 48 cases (80%) .

In both previous studies, This difference may be due to the variations of the sample sizes of these studies or it may be related to the early diagnosis of this disease in these countries due to more utilization of updated diagnostic tools, well functioned health facilities and high level

of awareness of public and health care providers about the epidemiological and clinical features of this health problem or due to the different inclusion criteria used for diagnosis.

Also study of Fadhil& Baiee (2018), Which included cross sectional was conducted by reviewing the clinical records of 208 hydatid cyst patients who were admitted to eight public hospitals in Babylon province and This study revealed that the average and the standard deviation of patients were (34.13±16.17).

Furthermore, our result differs than those of several countries study , such as, the study of (Khamesipour *etal*.,2021), in Iran, which included seropositive in terms of Hydatid cysts infection (an average of 7.08%, in Iran while the average of sero epidemiology of hydatid cysts in Jordan, 4.2%, and China 5.9 % have been reported of Hydatid cysts infection.

Also , the overall prevalence of echinococcosis in humans, are partially in accordance with findings of some workers who reported the prevalence rate ranged from 2.3 to 8.5 percent, and it's lower than findings of Al-Shaibani, *et al* (2015), Saida and Nouraddin (2011), AlShibani *et al*.(2012), Singh *et al*. (2013),and Abdulhameed,*etal* (2018) that his study included 748 cases of human with CE were diagnosed and operated in Basrah hospitals, equivalent to an annual clinical incidence of approximately 4.5 cases per 100 000 people.

3.2. Distribution of hydatid cyst:

3.2.1. Distribution of hydatid cyst infection according to patients sex :

The results of this study showed that the percentage of infection with *E. granulosus* was higher in female 37 (69.8%) than male it was 16 (31%), as shown in figure (3-2).

The observed percentage of higher in women because may be more frequently exposed to the infection than men due to being involved with

activities such as confined to house working and this will make them more exposed to the source of infection especially in rurual area. A higher occurrence of cystic echinococcosis in women has similarly been reported in other countries including Jordan, Tunisia, and Iran , Abdul hameed *et al*.(2018).

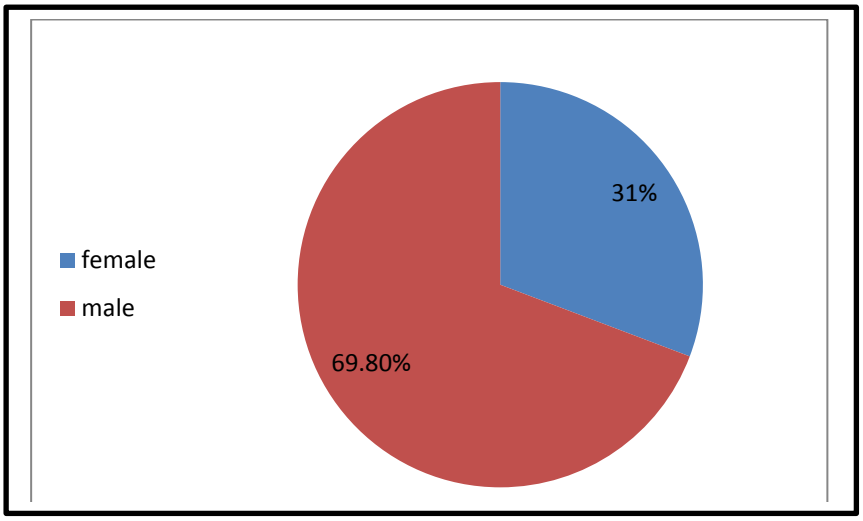


Figure (3-2): the percentage of infection in patients with hydatid cyst according to the sex .

Other study indicated that the rate of hydatid cyst in female is significantly higher than in male, or nearly equal in two sexes, confirming the findings of others (Khan *etal*.,2020), perhaps this because the females are more commonly involved in dealing with meat, contaminated food and vegetables, and with animals (Kadhim& Al-Mayali , 2021). In spite of this, investigational studies showed that the males were more prone to contract the disease than the females (Al-Fatlawi and Al-Mayali , 2021).

The findings of this study are corroborated by a number of prior studies (Moosazadeh *et al*., 2017), in which the percentage of males was lower (42.2%) than females (63.4%), also study by Rafiei *etal*. (2019) in Iran included 314 (77.7) % patients . females and males were 244(75%) and 70 (22.3%), respectively. And also study in Iraq by Hammad *et al* (2018).

Study of Fadhil& Baiee (2018) revealed that females (61.2) % were significantly ($p \leq 0.05$) more infected with hydatid disease than males(38.8)% .

This difference in sex distribution varies according to socioeconomic, traditional, and cultural factors (Tamarozzi *et al*., 2018). also differences between genders may be associated with occupation because considering that infection is frequently associated with living conditions, work with cattle and other domestic animals, in different countries there are different traditions ,it could also be important, and that supports these gender differences ,also In economically less developed regions the infection risk may be associated with contaminated water, including work with sewerage systems (Lymbery, 2017).

3.2.2. Distribution of hydatid cyst according to the location of organ infection :

The most organ which was infected with hydatid cyst in patients was the liver with the infection percentage is (75.4%). The second highest rate of infection was in the Abdominal cavity with percentage (9.4 %) of followed with infection for kidney (7.5 %) of infection as show in table (3-1).

Table (3-1) : Distribution of hydatid cyst infection among organs of patients .

Organs	Number of CE case	Percentage %	Sex (male case / female case)	P value
Liver	40	75.4	10/30 case	0.05*
Lungs	2	3.7	2/0 case	0.01
Abdominal cavity	5	9.4	1/4 case	0.04*
Kidney	4	7.5	1/3 case	0.01*
Brain	2	3.7	2/0 case	0.01
Total	53	100	53	

***Significant $p \leq 0.05$.**

The liver acts as the primary filter for the parasite, on the contrary, the lungs acts as the secondary filter but in few studies some researchers found that the lungs were the predominant site of hydatid disease this is probably because most frequently ,the oncosphere enter the portal vein in the liver and then to the first capillary filter ,if it passes the liver ,it reaches the lung and other distant foci (Husain *etal*.,2018).

The current study disagree with Ahmed (2013) who confirmed the majority of human infection in the lung 58.82%, while in the liver was the lowest rate 41.18%, while agreement with Saida (2019),who confirm that according the liver was highly infected 31(55.3%) and then followed by lungs 9(16.07)% in Sulaymania Province, also agreement with Al-Saeed & Al-Mufty (2016) study that the liver was the most common site of hydatid disease in 26 (54.2%) of cases .

During the years 1986-1990 in Iran 4.850 cases of hydatid disease were operated, the lung cysts was with a rate of 46.2% while liver cysts were 42%(Wen, *etal*.,2019)

In the present study as in usual in hydatid infection, the liver in both sexes was more frequently site involved than in other organs. However,

the Abdominal cavity is being the next. These findings are in agreement with results of other studies (Saida & Nouraddin ,2011) ,and in many cases, the resistances shown by the liver tissue surrounding the cyst determines a slow growth or even avoids the growth for many years. On the other hand, the lungs show lower resistance to the growth of the hydatid cyst due to their elasticity, This state allows an increase of the cyst size (Ali *etal*.,2020).

The study of Taha & Hassen(2018) confirm the number of hydatid cysts in any of the organs ranged the total number of organs containing a hydatid cyst or more was 58 (78.38%) for the lungs and 14 (18.92%). for the liver, 1 (0.8%) for the spleen, and 1 (0.8%) for the heart.

The reason for the variation in the number of cysts in the organs may be due to the number of embryos that reach the affected organs, and this may be due to the repeated eating of food contaminated with the parasite eggs as a result of the spread of the final hosts in the pastures in which these animals graze. Infested with eggs of the *E. granulosus*, or the cause may be attributed to variation in strains and geographic regions, different environmental conditions, or host immunity(Al-Mayali & Kadhim,2020).

3.2.3. Distribution of hydatid cyst infection according to Age :

In regarding to the age distribution of hydatid disease it was found the highest prevalence of infection was between the age 31-40(28.3%) year old ,and recorded lower infection in (61-70) age was(13.2)% also there significant between age (20-31)year and (61-70)year . As show in table(3-2).

Table(3-2) :-Distribution of hydatid cyst infection according to the age groups:

Age/years	Number of CE cases	Percentage %	Sex(male/ female cases	P value
20-30	12	22.6	4/8 case	0.01*
31-40	15	28.3	3/12case	0.00*(HS)
41-50	8	15.09	3/5case	0. 03
51-60	11	20.75	4/ 7case	0.02
61-70	7	13.2	2/5case	0.01*
Total	53	99.9	53 case	

*** Significant ≤ 0.05 .HS, higher Significant.**

These results are not consistent with Al-Yasari *etal*.(2013) , as his results show that the highest prevalence was between the age of 25-54 years old.

Also, the result was disagreement with the results obtained by Obaid (2019) during his study of infected people in Kirkuk, where he recorded the highest rate of infection in 56-64 year which were infected with the CE in male was with rate of 40%, followed by 20-28 with rate 18.2%, no positive sample were detected in each of (11-19), (47-55), (65-73) age group.

Study of Abdul hameed *et al*. (2018) show the age (41-50) year was higher infection percentage (42.5)% and lower in age (≤10) year it was (4) % infection .

The results of the present study were disagree with Mukhlisun (2007) who showed that there is a relationship between age and sex for the infection ,that the infection with this disease is the age between (21- 40) years in rate (26)% of patients .

This difference between the current results and previous studies is because high number of hydatid cyst acquired infections are in the period of the childhood but needs several years to patent themselves as harmful lesions.

The results of Al-Rishawi and Al-Mayali (2019) confirm that the most affected age groups were 16-30 years of age the highest (50%), followed by 31-45 years (30),this study was identical with the present study.

The finding that maximum prevalence is found among patients in the fourth decade of age is supported by several previous studies (Saida, 2019), though others have found high incidences in younger age groups (Cardona& Carmena,2013.; Hassan *etal*.,2017). Children are considered to be more exposed to infection from playing in the soil and having close contact with dogs, and since the clinical signs of hydatidosis may take 10–15 years to develop, their infections may not be detected until much later in life.

Also result the similar was to many studies carried out in Iraq such as study which was carried out in Baghdad teaching hospital and in Al-Kut governorate by Al-Obaidi *et al.*(2014), in both previous studies, the age of the majority of hydatid cyst patients were between(20-30) years, while results of (Mor, *et al.* 2015)in which the most affected age group was(16-30) and similar with study of Iran (31-40) years, (Vejdani, *et al.* 2013). This difference may be due to the variations of the sample.

This different between results may due to the type of social life, because this group is able to work outside the home and thus be more susceptible for infection, with noncompliance with public health rules and indifference to eat foods, as well as spend a long time outside the home, making them more compatible with the causes of infection. These results were similar to the previous results conducted by (Taher,2012) who

showed that they age range between (21- 40) years had the highest rate of disease incidence.

3.2.3. Distribution of hydatid cyst infection according to the residence .

The results of this study showed that the percentage of infection with *E. granulosus* in rural area was 40(75%) higher than urban area 13(24.5%), as seen in table(3-3):

Table (3-3): Distribution of hydatid cyst infection according to the residence area.

residence	*E. granulosus* infection		*P* value
	No of patients	Percentage(%)	
Urban	13case	24.5	0.02*
rural	40 case	75	0.001*
Total	53	100	

*** Significant ≤ 0.05**

The increasing distribution of hydatid disease in rural area in compare with urban area may be due to the many factors, including poor living conditions and lack of adequate health education in rural areas and economic instability and financial restrictions in control and prevention, This result is supported by many researches, (Mahmoudi *et al.*,2019) where they found that the infection rate in rural are higher than that in urban.

The study of Saeed *et al*. (2000)showed that the incidence rates were estimated to be 2 per 100,000 inhabitants this dis agreement with our study .

Saida & Nooraldeen, (2014) show the percentage of cystic hydatidosis among 149 patients , the result showed that, 82 (55.03%)

were in areas around or outside the city (rural) while the others 67 (44.97%) were urban, This result agree with a current study

The study done by Ahmadi& Badi (2011) show not Similarly, urban dwellers was also over-represented among the cases (87% urban vs. 13% rural; P<0.001) , also the investigation revealed(Rahi *et al* ., 2015) that the highest number of patients of 18 human hydatid cyst samples were collected from AL-Zahra'a and Al Karamah teaching hospital in Wasit province in iraq was in high infection appeared in urban than rural areas, this is not identical to the current study . This was not optimal since both of the studies were conducted in rural/urban , it appears that CE is being urbanized and can no longer be considered solely as a rural disease(Bait Almal., *et al*,2020) .

The human behavior also helps to perpetuate the domestic cycle of *E. granulosus* (Romig, *etal* ., 2017) . This fact obviously seen in Iraqi rural communities, the practice of animal slaughtering was usually performed in open spaces. Under these conditions, dogs would have free access to feed on livestock viscera, which may harbor hydatid cysts; the infective stage. Dogs play a critical role in the transition of echinococcosis (Maleki ,*et al*., 2018) . Urban abattoirs are insufficiently equipped and lack efficient veterinary control, efficient waste disposal facilities, and water, Abattoirs are also frequently accessibility to dogs, Lack of adequate health education and ignorance of transmission route of disease (Wyckliff and Chepkirui , 2017).

The hydatid disease was predominantly present among rural dwellers(55.8%) and urban was (44.2%) ,About 15% of cases had cysts more than (10cm) in diameter (Fadhil& Baiee,2018), this study agree with our results and agreement with Al-Rishawi& Al-Mayali (2019) respectively, According to residence urban or rural areas, the highest

prevalence was in rural areas (70%)and 30% in urban areas. But not a similarly with study Biranvand *et al* (2020) in Iran came different from our study , as his study showed Among the 20 CE patients, these, 13 (65.0 %) were from urban and 7 (35.0 %) were from rural areas of Khuzestan Province, southwestern Iran.

This explains the majority of patients were found to adopt poor hygienic practices and had a low level of understanding of how the disease was transmitted (El-Sherbini., *et al*, 2020).

Cystic hydatid disease is still a significant public health and economic problem among Iraqi cities, Previously many report in Iraq have been recorded variable indices of human morbidity due to the hydatid disease with the surgical case rate ranging between 1 and 20 patients per 100,000 inhabitants nationwide (Husain *et al*., 2018).

3.3- **Immunological parameters**:

To understand the association between cytokine response and the cyst developmental state, we first examined the serum levels of two type cytokines and their receptor (IL17 A ,IL17B ,IL17 RA and IL17RB) of the cystic echinococcosis patients presenting the active type of cyst compared cytokine levels with the normal controls.

3-3-1- Interleukin 17 A(IL17A) concentration

The result in the table (3-4) showed high levels of IL17A in age group (61-70) compared to healthy controls, the concentration was (251.78 ±25.3) and lower levels was (224.3±1 68.7) in patients of (20-30) age group .

Also levels of IL17A in age group (41-50)year was(241.51± 29.3)pm/ml increase compared to controls(133.6±26.8)pm/ml these results was significantly study of Mezioug & Touil-Boukoffa (2012), examine interleukin-17A (IL-17A) production in patients with cystic

echinococcosis (CE), and the role of IL-17A in the modulation of the immune response against the extracellular parasite, *E. granulosus*.

Cytokine production was measured from hydatid patients stimulated by a major parasitic antigen (antigen-5), The increased activity of IL-17A were observed in most serum samples from patients(Tilioua., *etal*,2020). In contrast, healthy controls showed only minor levels ,this similarly with our results show that IL-17A was produced during human cystic echinococcosis, and was involved in the host defense mechanisms against the extracellular parasite *E. granulosus.* And this suggest that IL-17A plays an immune protective role in this parasitic, helminth infection(Ajendra *etal* ,2020).

Table (3-4) : concentration levels of IL17A according to age of patients with hydatid cyst and controls .

Parameter	Age(years)		Concentration pg/ml Mean± SD	P value
IL17A	20-30	Patient	224.31± 68.7	0.03*
		control	186.9± 13.5	
	31-40	Patient	238.20± 34.1	0.01*
		control	156.7± 35.8	
	41-50	Patient	241.51± 29.3	0.02*
		control	133.6±26.8	
	51-60	Patient	244.39± 34.6	0.2 (NS)
		control	135.6±23.5	
	61-70	Patient	251.78± 25.3	0.00(HS)
		control	129.4±33.1	

*** Significant ≤ 0.05 .HS, higher Significant, NS, non Significant**

The six family members identified (IL17A-F) exert mostly pro-inflammatory activities (Iwakura *etal* .,2011). IL17A and IL17F,

mediators of the recently described pro inflammatory Th17-type immune responses, have been associated with inflammatory disorders like rheumatoid arthritis and inflammatory bowel disease (Seiderer *etal*.,2008) but also with protection against extracellular bacteria and fungi (Noda *etal* .,2011).

When analyzed levels of pro-inflammatory IL-17 members (IL-17A) as well as their soluble common receptors (IL-17RA) in clinically staged cystic echinococcosis patients, that is, cured, stable, and progressive CE, and in infection-free controls, The altered concentrations of IL-17A, Th17-type cytokine at distinct stages of cystic echinococcosis disease suggest that these pro-inflammatory cytokines may contribute to the clinical outcome of *E. granulosus* infection (Lechner, 2013).

The present study similar to study of (Li *et al*, 2020*)* when Revealed Nine cytokines including Th1-type IL-2, Th17-type IL-17A, IL-1βand IL-1Rα were significantly elevated in patients with hydatid disease when compared to the normal controls..

In vivo treatment of mice by a single intravenous injection of 200: l recombinant IL-17A at the optimal concentration of 125 pg/ml 2 weeks after *E. granulosus sensu stricto* infection decreased the infectivity rate by 2/3 and reduced metacestode growth by more than 90% (Labsi *etal*.,2018).

Also Herjan *etal* (2018) suggest that IL-17/IL-23 is a key element in inflammation and is involved in the immune responses to microbial and parasite infection and autoimmune disease .

IL-17A contributes to host protection against diverse infectious organisms during cystic echinococcosis while inducing hyper inflammation with detrimental outcomes for the host under certain conditions, Further investigation on the role of IL-17A and the interplay

with other immune factors needs to be conducted in clinical settings(Szabo e*tal*.,2017).

3.3.2- Interleukin 17 B (IL17B) concentration :

The present result show the levels of IL-17B were lower in healthy controls and were significantly increased in all cystic echinococcosis patient groups (Table 3-5). There significant difference between the cystic echinococcosis patients group, significant of IL-17B were detected in (20-30) of cystic echinococcosis , while highest significantly were observed in (31-40) age cases.

Also IL-17B was (31.59± 4.3) p g/ml in age (20-30)year while in control cases was (16.9±5.1) p g/ml of cystic echinococcosis , also highest concentrations were observed in (61-70) age(36.68± 11.5) pg/ ml of progressive cases.

Table (3-5) : concentration levels of IL17B according to age of patients with hydatid cyst and controls .

Parameter	Age(years)		Concentration pg/ml Mean ±SD	P value
IL17B	20-30	Patient	31.59± 4.3	0.04
		control	16.9±5.1	
	31-40	Patient	35.84± 10.2	0.00(H.S)
		control	19.6±6.4	
	41-50	Patient	34.69± 5.01	0.01*
		control	19.1±4.6	
	51-60	Patient	33.62± 3.1	0.06*
		control	16.8±6.1	
	61-70	Patient	36.68± 11.5	0.01
		control	17.9±3.8	

*** Significant ≤ 0.05 .HS, higher Significant, NS, non Significant**

In turn, other family members derive from different cellular sources and are associated with varying functions. IL-17A, IL-17F, IL-17C, and IL-17B function in host defense against pathogens and play various but not fully understood roles in mediating inflammation in autoimmune, allergic, and chronic inflammatory conditions Langley *et al*.(2014).

Ample evidence suggested that cytokines play a crucial role in the immune response process as both Th1 and Th2 cytokines are coexisting during hydatid disease(Biranvand *etal.*,2020) Published data indicate that Th1 cytokines are related to the protective immunity whereas Th2 cytokines are associated with the chronic stage, clinical complications and secondary episodes(Tamarozzi *etal*.,2016).

The role of IL-17B in host defense against intracellular protozoan parasites remains less well studied(Novoa *etal*.,2011). Infection studies demonstrate that Th17 cells mediate host defense against *Trypanosoma cruzi*, *Toxoplasma gondii*, *Leishmania braziliensis*, *Echinococcus granulosus* infections(Das&Khader,2017).

Increased IL-17B levels were detected in the PBMCs and tissue from leishmaniasis-infected patients and associated with enhanced neutrophil and macrophage-mediated destruction of the parasite(Gonzalez-Lombana *etal*.,2013).

Additionally, during echinococcosis, IL-17B plays a crucial immune protective role by regulating the Tregs which are associated with tolerance during infection (Pang *etal*.,2014) .

The present study is also inconsistent with Tuxun *et al* (2012) the results demonstrated that patients with cystic echinococcosis revealed significant increase in peripheral T regulated number, related cytokines (IL-10 and TGF-b1) and transcription factor (Foxp3) levels and moderate decrease in Th17 number, related cytokines (IL-17 and IL-23) and transcription factor (RORct) levels as compared with controls. Results

indicated that Th17 /Treg functional imbalance exists in patients with chronic cystic echinococcosis, suggesting a potential role for Th17 /Treg imbalance in the pathogenesis of immune evasion in echinococcosis.

Protective properties of IL-17B in disease have not yet been reported. In CE patients, plasma concentrations of IL-17B and its soluble receptor IL-17RB were strongly elevated and highest in those with progressive CE. The persistent exposure to growing *E. graneolosus* metacestodes may have triggered the release of IL-17B by cells of the gastrointestinal tract, leading to the recruitment of neutrophil granulocytes into peri-parasite lesions,While the effects of IL-17B are similar to those mediated by TNF-α, IL-17A, and IL-1β, its potency is limited (Yagi *etal*.,2007). The IL-17B-induced infiltration of neutrophils into the peritoneal cavity in rats required much higher concentrations compared to TNF-α and it was still considerably less effective than the cell migration induced by IL-17A (Zhu & Qian (2012), The elevated IL-17B production in CE patients disclosed a proinflammatory response triggered by *E. graneulosus* antigens, but potentially not strong enough to limit the progressive parasite growth.

3.3.3. interleukin 17recepter A (IL17RA) concentration :

Difference with regard to IL-17RA was revealed between patients of hydatid cyst and control show table (3-6) . The concentration of this interleukin a significant increasing ($P< 0.05$) in patients as compared to healthy control .was higher levels of IL -17RA concentration in (61-70) age (6939.26± 18.15) pg/ml of patient compared with(3833.29±21.3) controls significant ($P \leq 0.05$ value) .while recorded (20-30) and(31-40) year higher significant by concentration(6757.48± 16.75, 5941.45± 16.1) pg/ml for patients .

Table (3-6) : concentration levels of IL17RA according to age of patients

Parameter	Age(year)		Concentration pg/ml Mean ±SD	P value
IL17RA	20-30	Patient	6757.48± 16.75	0.00(H.S)
		control	4123±23.15	
	31-40	Patient	5941.45± 16.1	0.00(H.S)
		control	3112.15± 18.6	
	41-50	Patient	6493.59± 23.63	0.01
		control	3712.27±17.4	
	51-60	Patient	6651.30± 12.58	0.001(H.S)
		control	3425.18±23.8	
	61-70	Patient	6939.26± 18.15	0.02*
		control	3833.29±21.3	

*** Significant ≤ 0.05 .HS, higher Significant, NS, non Significant**

This explanation occur the interaction with IL-17 receptors A (IL-17RA) mediate host defenses while also contributing to inflammatory and autoimmune responses(Veldhoen , 2017) .IL-17A and IL-17F both preferentially engage a receptor complex containing one molecule of IL-17RA and one molecule of IL-17RC(Monin& Gaffen, 2018).

More generally, IL-17RA appears to be a shared receptor that pairs with other members of its family to allow signaling of different IL-17 cytokines because the crystal structures of homodimeric IL-17A and its complex with IL-17RA, Binding to IL-17RA at one side of the IL-17A molecule induces a conformational change in the second, symmetry-related receptor site of IL-17A (Goepfert *etal*.,2020).

Also found that Th17 development is dependent on IL-23 secretion (Harrington *et al*. 2006) and is characterized by release of IL- 17A and

IL-17F, which subsequently trigger the release of further pro-inflammatory cytokines and chemokines such as IL-8, TNF alpha and IL-17 (Pappu *et al.*, 2011). Both IL-17A and IL-17F induce neutrophil recruitment and production of antimicrobial peptides (Iwakura *et al*. 2011).

The a mixed between Th1/Th2-type response in infected hosts, and also regulatory mechanisms are likely to control both Th1 and Th2 parasite-killing effector mechanisms (Higuita *et al* ., 2016), A mixed Th1/Th2 cytokine response (Botezatu *et al*., 2018) with elevated levels of IL-10 and increased number of regulatory T cells (Treg) is suggested in cystic echinococcosis (CE) . It is also suggested that the production of IL-10 and TGF-β1 may be induced by the parasite in order to favor its establishment(La Hoz *et al*., 2019).

Correlation between IL17A and IL17 RA in the disease which IL-17R signaling is proposed to regulate neutrophil trafficking into the lung to maintain host immunity (Zhao *etal*.,2016), The addition of recombinant IL- 17A into the airway causes the production of chemokines that recruit inflammatory and immune cells .

3.3.4. interleukin 17recepter B (IL17RB) concentration :

The results in table (3 – 7) revealed a significant changes in serum IL-17RB level between the two different groups. There was highly significant increase in IL-17RB in patient with cystic echinococcosis between (20-40) age(26.33± 7.5)pg/ml compared with control group and lower concentration are in (41-50) age (23.28±15.1) pg/ml in patient with cystic echinococcosis for ($P<0.05$).

This study might be ascribed to the fact that the secretion of this cytokine important to the recruitment of innate and adaptive cell that control on CE infection , As study carried out by Keragala *et al*. (2018).

Table (3-7) : Concentration levels of IL17RB according to age of patients .

Parameter	Age		Concentration pg/ml	P value
IL17RB	20-30	Patient	26.33± 7.5	0.05*
		control	15.7±4.5	
	31-40	Patient	25.0± 3.2	0.01
		control	14.7±5.1	
	41-50	Patient	23.28±15.1	0.03*
		control	14.6±4.2	
	51-60	Patient	24.10± 1.2	0.04*
		control	15.5±3.9	
	61-70	Patient	25.61± 3.4	0.05*
		control	17.8±4.9	

*** Significant ≤ 0.05 .HS, higher Significant, NS, non Significant**

The distinct antigen-induced production levels of IL-17RB in different stages of disease sheds a new light on the role of L-17 family members in CE and could predispose these cytokines as possible markers for disease staging, The observed modulation of IL-17F levels suggests an role of pro inflammatory Th17 responses in CE.(Wakashin, *etal*,2008)

Th17 cells are increased under inflammatory events in the liver (Chang *et al*. 2012), an organ is mainly affected by chronic metacestode growth, and further IL-17 family members like IL-17B have been detected in the gastrointestinal tract, which is also afftected in CE this agree with our study.

The elevated serum levels of IL-17B and soluble receptor component IL-17RB observed in stable and progressive CE cases might signify rather innate immune responses, as IL-17B is expressed by cells of the intestine and stomach, and is a chemoattractant for neutrophil

granulocytes (Reynolds *etal*, 2010),which represent one of the first cell populations involved in inflammation.

IL-17B seems to drive early immune responses against various pathogens, and activation of neutrophils is a hallmark of early pro-inflammatory immune reactions(Isailovic *etal* .,2018).

Lechner's show (2013) The fact that progressive CE cases presented with highest levels of IL-17B and IL-17RB seems to support the concept of parasite-induced immune modulation and this is consistent with the current study.

Study of Lechner *et al* (2012), show significantly elevated levels of IL-17B and lL-17RB were observed, whilelL-17F and slL-17RA were reduced in patients with Alveolar Echinococosis . Similarly, the cellular production of lL-17F and slL-L7RA in response to *E. multilocularis* antigens was low in AE patients, while levels of lL-17RB were highly enhanced.

Recent studies demonstrated that *E. granulosus* secretes several molecules present in protoscoleces and in hydatid fluid that directly can modulate the immune responses thus altering the cytokine balance towards Th2 and favouring immune evasion and perpetuating parasite survival in the host (Bhutani& Kajal, 2018).

3.3.5. Correlation of IL-17A with IL-17RA and IL17B

There was positive correlation between IL-17A with IL-17RA (R =0.034, $p<0.05$) as shown in Figure (3-3) and (3-4),Also there Shows a significant difference between IL17A and IL17RA was higher by *P* value (0.03) and significant between IL17A and IL 17 B by recorded *P* value (0.01) in patients of Cystic echinococcosis.

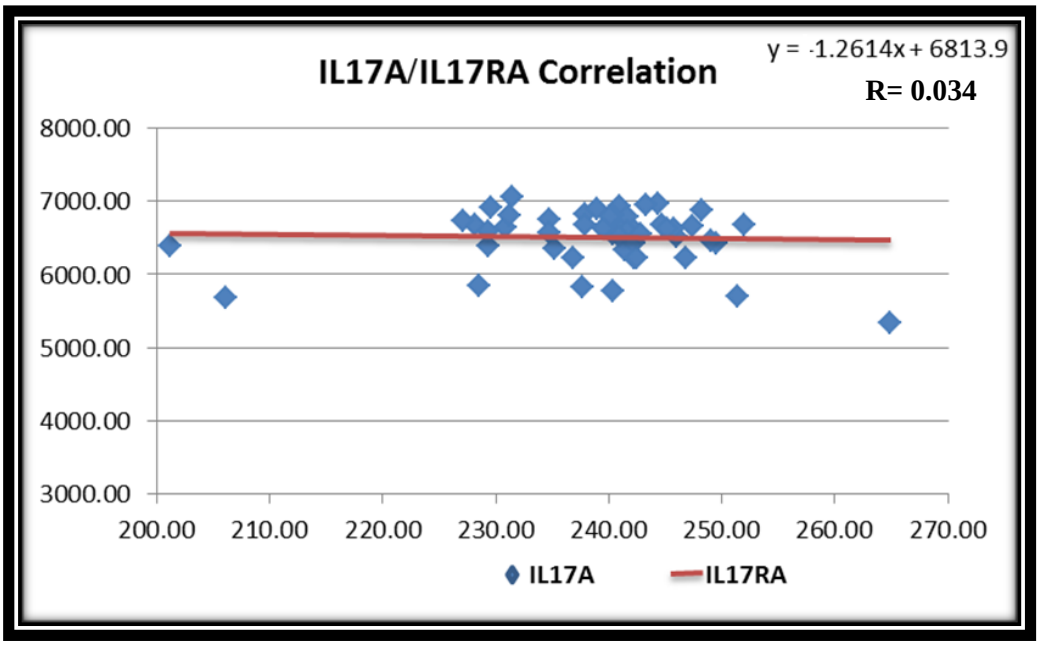


Figure (3-3): correlation between IL-17Awith IL17RA.

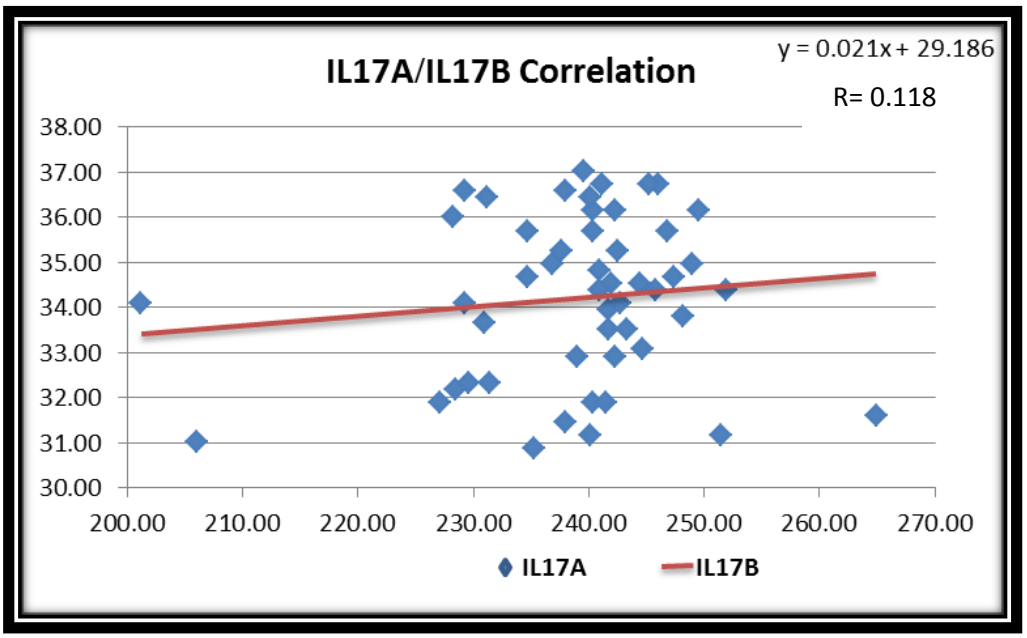


Figure (3-4): The correlation between IL-17Awith IL17B.

The cytokines IL-17A and IL-17 F are highly homologous and bind to the same receptor, and both contribute similarly to the progress of inflammation and to the host defense against pathogens (Reynolds *et al*. 2010).

Several studies have described the importance of cytokines and chemokines in the prevention and elimination of parasite infection,With the tapeworm *Echinococcus spp* infections, Th1-type cytokine IL-12 and IFN-gamma were identified to successfully kill the larval stages of the parasite (metacestode) at the initial stages of development, whereas Th2 immune responses, induced by IL-4, IL-5,IL17 family their receptor and IL-10, lead to a chronic course of disease (Vuitton & Gottstein 2010).

Elevated plasma levels of pro-inflammatory IL-17B and its soluble receptor IL-17RB were observed in stable and progressive CE patients,this significant explained highest reactivity being observed in patients with progressive CE(Huang *etal*.,2014) .

IL-17A is associated with elevated levels of pro inflammatory cytokines and accelerated tubular epithelial apoptosis in Acute kidney injury(AKI),and IL-17A induces neutrophil migration through CXCL5, a chemokine known to be associated with higher risk of renal damage(Luo *etal*.,2016).

The observed correlation between the increased serum concentrations of IL-17A and IL-17B indicates a link between them. IL-17A and IL-17B share a common source, lymphocytes. Therefore, they may play a role in immune, allergic, inflammatory and anti-infective responses(Robak *etal*.,2019), As these processes are involved in the pathogenesis of hydatid disease , and fibrosis predominates with time, it may be helpful to monitor the level of the two cytokines in the course of hydatid disease to assess the extent and staging of the disease.

3.3.5. Correlation of IL-17B with IL-17RB and IL17RB with IL17RA:

Analysis of circulating cytokine production in serum from hydatid patients showed the immune protective role of Th1 cytokines, especially Correlation of IL-17B with IL-17RA and IL17RB, and pathological role of Th17 cytokines during *E. granulosus* infection was positive correlation between of IL-17B with IL-17RA and IL17RB .The present study was focused on determining the role of IL-17B in the immune response against *E. granulosus* infection,We investigated IL17 B ,IL17RA and IL17RB production in serum from hydatic patients with liver and lung hydatid cysts was significant between them p value ≤0.05 between IL17 B and IL17RB and p value between was less significant by P value 0.05 , as show figure (3-5) and (3-6).

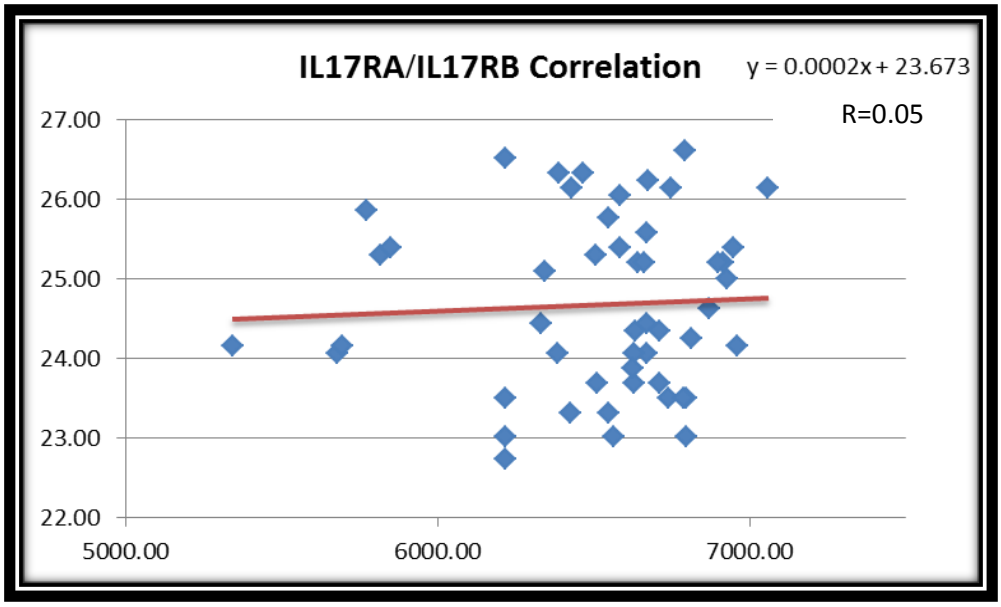


Figure (3-5): correlation between IL-17RAwith IL17RB.

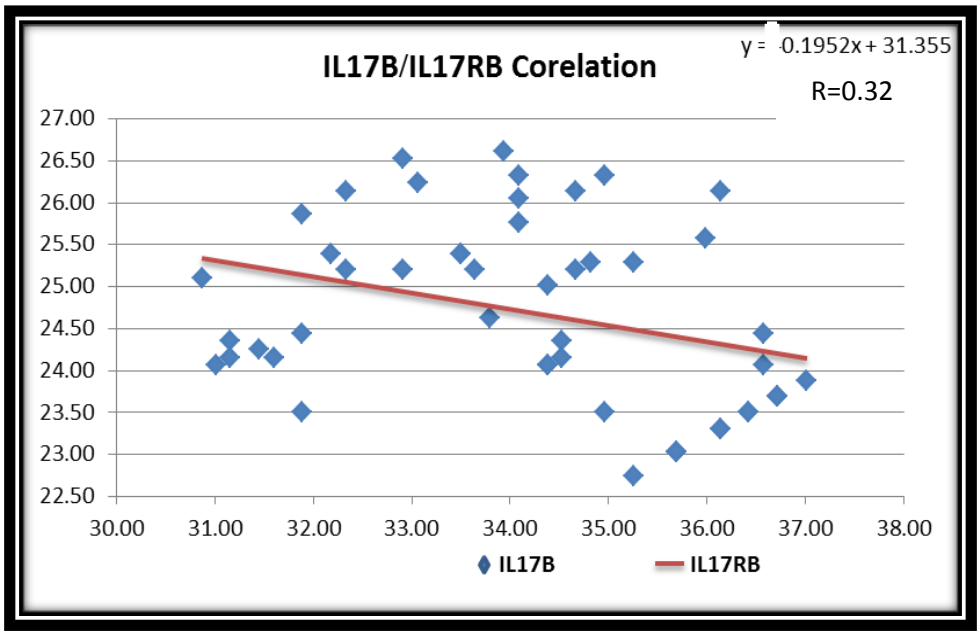


Figure (3-6): correlation between IL-17Bwith IL17RB

The present study show that negative correlation between IL17 B with IL17RA & IL17RB this observed production of certain pro-inflammatory cytokines (IL-17B) was enhanced, which seem to be too weak to induce successful clearance of infection and may rather mirror weak chronic inflammatory events during infection (Huber *et al*., 2012) The observed distinct parasite specific inducibility of IL-17 family members in different patient groups might render these cytokines useful as predictive markers in hydatid disease (Šnábel *etal*., 2016)

IL-17RB serves as receptor subunit for IL-17B and IL- 17E commonly expressed by cells of the intestine, but also by liver, pancreas, lung, and kidneys as well as on Th2 and Th9 cells (Gottstein *etal* ., 2017) ,also show that Plasma concentrations of soluble IL-17RB were highly elevated in CE with no significant differences between the patient groups.

The heightened pro inflammatory IL-17RB and RA responses in patients with progressive AE may primarily be induced by vesicle fluid components and germinal cells and engaging the IL- 17RB activation

pathway (Kern *etal*., 2017). Membrane bound and soluble IL-17RB are inducible in human antigen-presenting cells (APC) upon stimulation with Th2-type cytokines IL-4, IL- 10, IL-13 and TGF-*β* (Förster *etal*.,2019) and these cytokines are associated with progressive AE .

The higher concentrations of sIL-17RB in patients may be a direct consequence of this Th2 polarization associated with chronic CE (Naessens, 2012). The biological functions and importance of soluble IL-17 receptors in CE remain tentative; the soluble IL-17RB could act as decoy receptor for IL-17B (Rostami-Rad *et al* ., 2018).

Based on the results of the present study, we can explain that the shift from Th1 to Th2 reactivity may be associated with persistent of the disease because Th2 reactivity may be less effective than Th1 reactivity in countering the parasite.

These effects may be direct via widely detected IL-17Rs signaling through MAPK and NF-kB recruitment , IL-17 cytokines also act indirectly on immune and nonimmune peri tumoral cells' cytokine secretion.(Fabre *etal*.,2018).

The *E. granulosus* can use two mechanisms to subvert the host immune response: passive escape, in which the parasite, by developing into a hydatid cyst, avoids the damaging effects of an immune response, and immunomodulation through which the parasite actively interacts with the host immune system to reduce the impact of host response (Bhutani& Kajal, 2018).

3- Results and Discussion:

3.1. Percentage infection of Hydatid disease.

Hydatidosis is remain a main public health problem in our country as in some other parts of the world (Sarhan,2008) . A total of clinical samples in this study include 93(53 patients with Hydatid cyst attended to hospitals in AL- Hilla city(AL-Imam Al_Sadiq Hospital, AL- Hilla Teaching Hospital, and private hospitals) and 40 case of control . The results of this study showed that the total percentage of infection with *E. granulosus* was (56.9%) as show in figure (3-1).

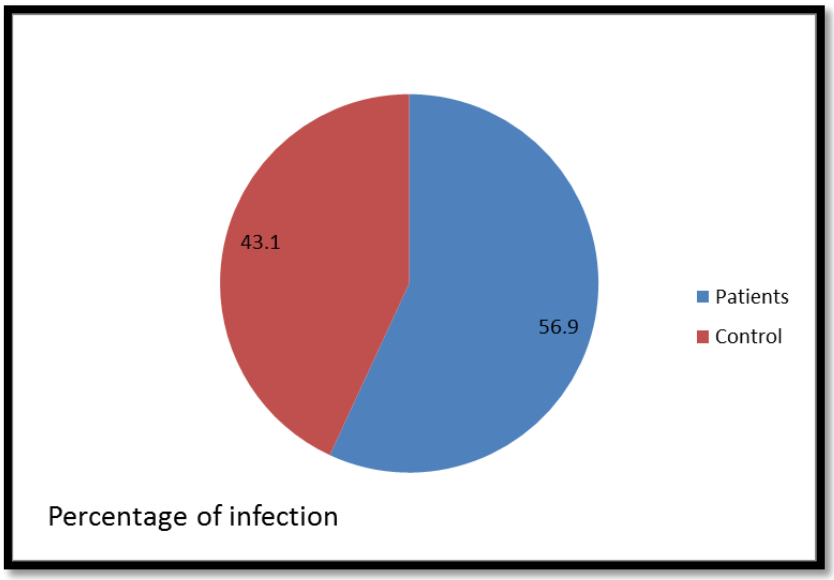


Figure (3-1) : The total percentage of infection with hydatid cyst.

A person becomes infected with this disease through accidental consumption of water, soil or food contaminated with the faeces of infected dogs, which is the most common type of method of infection(Al-Marsomy,2021).

A study of Saida & Nouraddin (2011), was disagreement with present study which confirm infection with hydatid cystic disease (HCD) in human and slaughtered animals in Erbil province. For humans a statistical analysis of documents, 149 cases have been recorded due to

cystic echinococcosis, and treated surgically in private and governorate hospitals in Erbil province. Among total of patients admitted to the surgical department, 0.846 % of which were found to be infected with cystic echinococcosis, and about 6.3 /100,000 persons among Erbil population. This different of our results explents that, low education of individuals on the disease leads to a high prevalence rate of infection while illegal home slaughtering of animals lead the infected organs to be at the access of the definitive hosts (stray dogs) for completing the life cycle of the parasite, These hosts act as a source of infection to the intermediate hosts by discharging huge numbers of eggs with feces.

Also, out of 480 cases, only 24 (5%) were positive with ELISA by Al-Mukhtar & Qasim,2017 in Mosul City, because this disease is usually detected late due to its silent growth and symptoms can appear years after infection when the internal organs are crushed by the cyst (Cappello *et al*. 2013).

This present result differs from many studies carried out in Iraq such as a study Obaid (2017),which Depending on the results of clinical and ultra sonographic, which show that 15.8 % of the patients were infected with cystic echinococcosis (CE), while 84.2% had simple cysts (SC), While the results of serological examination by ELISA revealed 14.1% for CE and 85.9% for simple cysts.

Another study which was carried out in Baghdad teaching hospital and in Al-Kut governorate by Al-Obaidi *et al* (2014), with liver hydatid cyst for elective surgery, The study found that patients with hydatid cyst represents 48 cases (80%) .

In both previous studies, This difference may be due to the variations of the sample sizes of these studies or it may be related to the early diagnosis of this disease in these countries due to more utilization of updated diagnostic tools, well functioned health facilities and high level

of awareness of public and health care providers about the epidemiological and clinical features of this health problem or due to the different inclusion criteria used for diagnosis.

Also study of Fadhil& Baiee (2018), Which included cross sectional was conducted by reviewing the clinical records of 208 hydatid cyst patients who were admitted to eight public hospitals in Babylon province and This study revealed that the average and the standard deviation of patients were (34.13±16.17).

Furthermore, our result differs than those of several countries study , such as, the study of (Khamesipour *etal*.,2021), in Iran, which included seropositive in terms of Hydatid cysts infection (an average of 7.08%, in Iran while the average of sero epidemiology of hydatid cysts in Jordan, 4.2%, and China 5.9 % have been reported of Hydatid cysts infection.

Also , the overall prevalence of echinococcosis in humans, are partially in accordance with findings of some workers who reported the prevalence rate ranged from 2.3 to 8.5 percent, and it's lower than findings of Al-Shaibani, *et al* (2015), Saida and Nouraddin (2011), AlShibani *et al*.(2012), Singh *et al*. (2013),and Abdulhameed,*etal* (2018) that his study included 748 cases of human with CE were diagnosed and operated in Basrah hospitals, equivalent to an annual clinical incidence of approximately 4.5 cases per 100 000 people.

3.2. Distribution of hydatid cyst:

3.2.1. Distribution of hydatid cyst infection according to patients sex :

The results of this study showed that the percentage of infection with *E. granulosus* was higher in female 37 (69.8%) than male it was 16 (31%), as shown in figure (3-2).

The observed percentage of higher in women because may be more frequently exposed to the infection than men due to being involved with

activities such as confined to house working and this will make them more exposed to the source of infection especially in rurual area. A higher occurrence of cystic echinococcosis in women has similarly been reported in other countries including Jordan, Tunisia, and Iran , Abdul hameed *et al*.(2018).

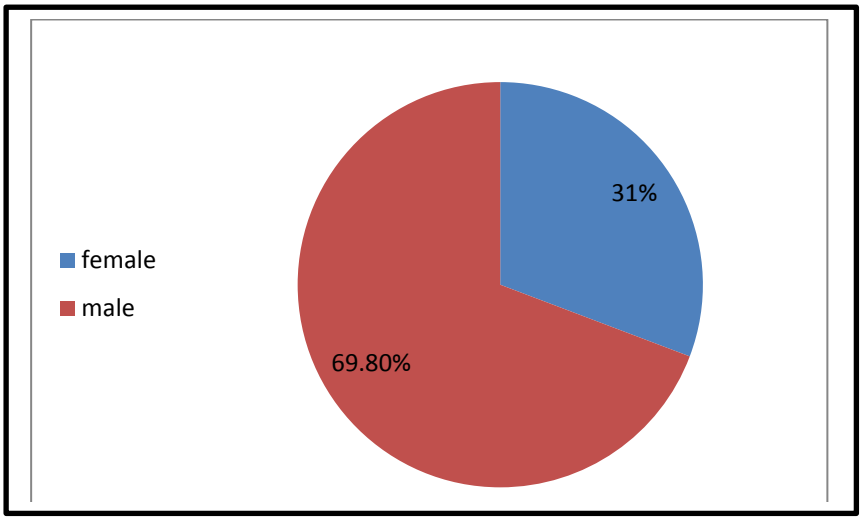


Figure (3-2): the percentage of infection in patients with hydatid cyst according to the sex .

Other study indicated that the rate of hydatid cyst in female is significantly higher than in male, or nearly equal in two sexes, confirming the findings of others (Khan *etal*.,2020), perhaps this because the females are more commonly involved in dealing with meat, contaminated food and vegetables, and with animals (Kadhim& Al-Mayali , 2021). In spite of this, investigational studies showed that the males were more prone to contract the disease than the females (Al-Fatlawi and Al-Mayali , 2021).

The findings of this study are corroborated by a number of prior studies (Moosazadeh *et al*., 2017), in which the percentage of males was lower (42.2%) than females (63.4%), also study by Rafiei *etal*. (2019) in Iran included 314 (77.7) % patients . females and males were 244(75%) and 70 (22.3%), respectively. And also study in Iraq by Hammad *et al* (2018).

Study of Fadhil& Baiee (2018) revealed that females (61.2) % were significantly ($p \leq 0.05$) more infected with hydatid disease than males(38.8)% .

This difference in sex distribution varies according to socioeconomic, traditional, and cultural factors (Tamarozzi *et al*., 2018). also differences between genders may be associated with occupation because considering that infection is frequently associated with living conditions, work with cattle and other domestic animals, in different countries there are different traditions ,it could also be important, and that supports these gender differences ,also In economically less developed regions the infection risk may be associated with contaminated water, including work with sewerage systems (Lymbery, 2017).

3.2.2. Distribution of hydatid cyst according to the location of organ infection :

The most organ which was infected with hydatid cyst in patients was the liver with the infection percentage is (75.4%). The second highest rate of infection was in the Abdominal cavity with percentage (9.4 %) of followed with infection for kidney (7.5 %) of infection as show in table (3-1).

Table (3-1) : Distribution of hydatid cyst infection among organs of patients .

Organs	Number of CE case	Percentage %	Sex (male case / female case)	P value
Liver	40	75.4	10/30 case	0.05*
Lungs	2	3.7	2/0 case	0.01
Abdominal cavity	5	9.4	1/4 case	0.04*
Kidney	4	7.5	1/3 case	0.01*
Brain	2	3.7	2/0 case	0.01
Total	53	100	53	

***Significant $p \leq 0.05$.**

The liver acts as the primary filter for the parasite, on the contrary, the lungs acts as the secondary filter but in few studies some researchers found that the lungs were the predominant site of hydatid disease this is probably because most frequently ,the oncosphere enter the portal vein in the liver and then to the first capillary filter ,if it passes the liver ,it reaches the lung and other distant foci (Husain *etal*.,2018).

The current study disagree with Ahmed (2013) who confirmed the majority of human infection in the lung 58.82%, while in the liver was the lowest rate 41.18%, while agreement with Saida (2019),who confirm that according the liver was highly infected 31(55.3%) and then followed by lungs 9(16.07)% in Sulaymania Province, also agreement with Al-Saeed & Al-Mufty (2016) study that the liver was the most common site of hydatid disease in 26 (54.2%) of cases .

During the years 1986-1990 in Iran 4.850 cases of hydatid disease were operated, the lung cysts was with a rate of 46.2% while liver cysts were 42%(Wen, *etal*.,2019)

In the present study as in usual in hydatid infection, the liver in both sexes was more frequently site involved than in other organs. However,

the Abdominal cavity is being the next. These findings are in agreement with results of other studies (Saida & Nouraddin ,2011) ,and in many cases, the resistances shown by the liver tissue surrounding the cyst determines a slow growth or even avoids the growth for many years. On the other hand, the lungs show lower resistance to the growth of the hydatid cyst due to their elasticity, This state allows an increase of the cyst size (Ali *etal*.,2020).

The study of Taha & Hassen(2018) confirm the number of hydatid cysts in any of the organs ranged the total number of organs containing a hydatid cyst or more was 58 (78.38%) for the lungs and 14 (18.92%). for the liver, 1 (0.8%) for the spleen, and 1 (0.8%) for the heart.

The reason for the variation in the number of cysts in the organs may be due to the number of embryos that reach the affected organs, and this may be due to the repeated eating of food contaminated with the parasite eggs as a result of the spread of the final hosts in the pastures in which these animals graze. Infested with eggs of the *E. granulosus*, or the cause may be attributed to variation in strains and geographic regions, different environmental conditions, or host immunity(Al-Mayali & Kadhim,2020).

3.2.3. Distribution of hydatid cyst infection according to Age :

In regarding to the age distribution of hydatid disease it was found the highest prevalence of infection was between the age 31-40(28.3%) year old ,and recorded lower infection in (61-70) age was(13.2)% also there significant between age (20-31)year and (61-70)year . As show in table(3-2).

Table(3-2) :-Distribution of hydatid cyst infection according to the age groups:

Age/years	Number of CE cases	Percentage %	Sex(male/ female cases	P value
20-30	12	22.6	4/8 case	0.01*
31-40	15	28.3	3/12case	0.00*(HS)
41-50	8	15.09	3/5case	0. 03
51-60	11	20.75	4/ 7case	0.02
61-70	7	13.2	2/5case	0.01*
Total	53	99.9	53 case	

*** Significant ≤ 0.05 .HS, higher Significant.**

These results are not consistent with Al-Yasari *etal*.(2013) , as his results show that the highest prevalence was between the age of 25-54 years old.

Also, the result was disagreement with the results obtained by Obaid (2019) during his study of infected people in Kirkuk, where he recorded the highest rate of infection in 56-64 year which were infected with the CE in male was with rate of 40%, followed by 20-28 with rate 18.2%, no positive sample were detected in each of (11-19), (47-55), (65-73) age group.

Study of Abdul hameed *et al*. (2018) show the age (41-50) year was higher infection percentage (42.5)% and lower in age (≤10) year it was (4) % infection .

The results of the present study were disagree with Mukhlisun (2007) who showed that there is a relationship between age and sex for the infection ,that the infection with this disease is the age between (21- 40) years in rate (26)% of patients .

This difference between the current results and previous studies is because high number of hydatid cyst acquired infections are in the period of the childhood but needs several years to patent themselves as harmful lesions.

The results of Al-Rishawi and Al-Mayali (2019) confirm that the most affected age groups were 16-30 years of age the highest (50%), followed by 31-45 years (30),this study was identical with the present study.

The finding that maximum prevalence is found among patients in the fourth decade of age is supported by several previous studies (Saida, 2019), though others have found high incidences in younger age groups (Cardona& Carmena,2013.; Hassan *etal*.,2017). Children are considered to be more exposed to infection from playing in the soil and having close contact with dogs, and since the clinical signs of hydatidosis may take 10–15 years to develop, their infections may not be detected until much later in life.

Also result the similar was to many studies carried out in Iraq such as study which was carried out in Baghdad teaching hospital and in Al-Kut governorate by Al-Obaidi *et al.*(2014), in both previous studies, the age of the majority of hydatid cyst patients were between(20-30) years, while results of (Mor, *et al.* 2015)in which the most affected age group was(16-30) and similar with study of Iran (31-40) years, (Vejdani, *et al.* 2013). This difference may be due to the variations of the sample.

This different between results may due to the type of social life, because this group is able to work outside the home and thus be more susceptible for infection, with noncompliance with public health rules and indifference to eat foods, as well as spend a long time outside the home, making them more compatible with the causes of infection. These results were similar to the previous results conducted by (Taher,2012) who

showed that they age range between (21- 40) years had the highest rate of disease incidence.

3.2.3. Distribution of hydatid cyst infection according to the residence .

The results of this study showed that the percentage of infection with *E. granulosus* in rural area was 40(75%) higher than urban area 13(24.5%), as seen in table(3-3):

Table (3-3): Distribution of hydatid cyst infection according to the residence area.

residence	*E. granulosus* infection		*P* value
	No of patients	Percentage(%)	
Urban	13case	24.5	0.02*
rural	40 case	75	0.001*
Total	53	100	

*** Significant ≤ 0.05**

The increasing distribution of hydatid disease in rural area in compare with urban area may be due to the many factors, including poor living conditions and lack of adequate health education in rural areas and economic instability and financial restrictions in control and prevention, This result is supported by many researches, (Mahmoudi *et al.*,2019) where they found that the infection rate in rural are higher than that in urban.

The study of Saeed *et al*. (2000)showed that the incidence rates were estimated to be 2 per 100,000 inhabitants this dis agreement with our study .

Saida & Nooraldeen, (2014) show the percentage of cystic hydatidosis among 149 patients , the result showed that, 82 (55.03%)

were in areas around or outside the city (rural) while the others 67 (44.97%) were urban, This result agree with a current study

The study done by Ahmadi& Badi (2011) show not Similarly, urban dwellers was also over-represented among the cases (87% urban vs. 13% rural; P<0.001) , also the investigation revealed(Rahi *et al* ., 2015) that the highest number of patients of 18 human hydatid cyst samples were collected from AL-Zahra'a and Al Karamah teaching hospital in Wasit province in iraq was in high infection appeared in urban than rural areas, this is not identical to the current study . This was not optimal since both of the studies were conducted in rural/urban , it appears that CE is being urbanized and can no longer be considered solely as a rural disease(Bait Almal., *et al*,2020) .

The human behavior also helps to perpetuate the domestic cycle of *E. granulosus* (Romig, *etal* ., 2017) . This fact obviously seen in Iraqi rural communities, the practice of animal slaughtering was usually performed in open spaces. Under these conditions, dogs would have free access to feed on livestock viscera, which may harbor hydatid cysts; the infective stage. Dogs play a critical role in the transition of echinococcosis (Maleki ,*et al*., 2018) . Urban abattoirs are insufficiently equipped and lack efficient veterinary control, efficient waste disposal facilities, and water, Abattoirs are also frequently accessibility to dogs, Lack of adequate health education and ignorance of transmission route of disease (Wyckliff and Chepkirui , 2017).

The hydatid disease was predominantly present among rural dwellers(55.8%) and urban was (44.2%) ,About 15% of cases had cysts more than (10cm) in diameter (Fadhil& Baiee,2018), this study agree with our results and agreement with Al-Rishawi& Al-Mayali (2019) respectively, According to residence urban or rural areas, the highest

prevalence was in rural areas (70%)and 30% in urban areas. But not a similarly with study Biranvand *et al* (2020) in Iran came different from our study , as his study showed Among the 20 CE patients, these, 13 (65.0 %) were from urban and 7 (35.0 %) were from rural areas of Khuzestan Province, southwestern Iran.

This explains the majority of patients were found to adopt poor hygienic practices and had a low level of understanding of how the disease was transmitted (El-Sherbini., *et al*, 2020).

Cystic hydatid disease is still a significant public health and economic problem among Iraqi cities, Previously many report in Iraq have been recorded variable indices of human morbidity due to the hydatid disease with the surgical case rate ranging between 1 and 20 patients per 100,000 inhabitants nationwide (Husain *et al*., 2018).

3.3- **Immunological parameters**:

To understand the association between cytokine response and the cyst developmental state, we first examined the serum levels of two type cytokines and their receptor (IL17 A ,IL17B ,IL17 RA and IL17RB) of the cystic echinococcosis patients presenting the active type of cyst compared cytokine levels with the normal controls.

3-3-1- Interleukin 17 A(IL17A) concentration

The result in the table (3-4) showed high levels of IL17A in age group (61-70) compared to healthy controls, the concentration was (251.78 ±25.3) and lower levels was (224.3±1 68.7) in patients of (20-30) age group .

Also levels of IL17A in age group (41-50)year was(241.51± 29.3)pm/ml increase compared to controls(133.6±26.8)pm/ml these results was significantly study of Mezioug & Touil-Boukoffa (2012), examine interleukin-17A (IL-17A) production in patients with cystic

echinococcosis (CE), and the role of IL-17A in the modulation of the immune response against the extracellular parasite, *E. granulosus*.

Cytokine production was measured from hydatid patients stimulated by a major parasitic antigen (antigen-5), The increased activity of IL-17A were observed in most serum samples from patients(Tilioua., *etal*,2020). In contrast, healthy controls showed only minor levels ,this similarly with our results show that IL-17A was produced during human cystic echinococcosis, and was involved in the host defense mechanisms against the extracellular parasite *E. granulosus.* And this suggest that IL-17A plays an immune protective role in this parasitic, helminth infection(Ajendra *etal* ,2020).

Table (3-4) : concentration levels of IL17A according to age of patients with hydatid cyst and controls .

Parameter	Age(years)		Concentration pg/ml Mean± SD	P value
IL17A	20-30	Patient	224.31± 68.7	0.03*
		control	186.9± 13.5	
	31-40	Patient	238.20± 34.1	0.01*
		control	156.7± 35.8	
	41-50	Patient	241.51± 29.3	0.02*
		control	133.6±26.8	
	51-60	Patient	244.39± 34.6	0.2 (NS)
		control	135.6±23.5	
	61-70	Patient	251.78± 25.3	0.00(HS)
		control	129.4±33.1	

*** Significant ≤ 0.05 .HS, higher Significant, NS, non Significant**

The six family members identified (IL17A-F) exert mostly pro-inflammatory activities (Iwakura *etal* .,2011). IL17A and IL17F,

mediators of the recently described pro inflammatory Th17-type immune responses, have been associated with inflammatory disorders like rheumatoid arthritis and inflammatory bowel disease (Seiderer *etal*.,2008) but also with protection against extracellular bacteria and fungi (Noda *etal* .,2011).

When analyzed levels of pro-inflammatory IL-17 members (IL-17A) as well as their soluble common receptors (IL-17RA) in clinically staged cystic echinococcosis patients, that is, cured, stable, and progressive CE, and in infection-free controls, The altered concentrations of IL-17A, Th17-type cytokine at distinct stages of cystic echinococcosis disease suggest that these pro-inflammatory cytokines may contribute to the clinical outcome of *E. granulosus* infection (Lechner, 2013).

The present study similar to study of (Li *et al*, 2020*)* when Revealed Nine cytokines including Th1-type IL-2, Th17-type IL-17A, IL-1βand IL-1Rα were significantly elevated in patients with hydatid disease when compared to the normal controls..

In vivo treatment of mice by a single intravenous injection of 200: l recombinant IL-17A at the optimal concentration of 125 pg/ml 2 weeks after *E. granulosus sensu stricto* infection decreased the infectivity rate by 2/3 and reduced metacestode growth by more than 90% (Labsi *etal*.,2018).

Also Herjan *etal* (2018) suggest that IL-17/IL-23 is a key element in inflammation and is involved in the immune responses to microbial and parasite infection and autoimmune disease .

IL-17A contributes to host protection against diverse infectious organisms during cystic echinococcosis while inducing hyper inflammation with detrimental outcomes for the host under certain conditions, Further investigation on the role of IL-17A and the interplay

with other immune factors needs to be conducted in clinical settings(Szabo e*tal*.,2017).

3.3.2- Interleukin 17 B (IL17B) concentration :

The present result show the levels of IL-17B were lower in healthy controls and were significantly increased in all cystic echinococcosis patient groups (Table 3-5). There significant difference between the cystic echinococcosis patients group, significant of IL-17B were detected in (20-30) of cystic echinococcosis , while highest significantly were observed in (31-40) age cases.

Also IL-17B was (31.59± 4.3) p g/ml in age (20-30)year while in control cases was (16.9±5.1) p g/ml of cystic echinococcosis , also highest concentrations were observed in (61-70) age(36.68± 11.5) pg/ ml of progressive cases.

Table (3-5) : concentration levels of IL17B according to age of patients with hydatid cyst and controls .

Parameter	Age(years)		Concentration pg/ml Mean ±SD	P value
IL17B	20-30	Patient	31.59± 4.3	0.04
		control	16.9±5.1	
	31-40	Patient	35.84± 10.2	0.00(H.S)
		control	19.6±6.4	
	41-50	Patient	34.69± 5.01	0.01*
		control	19.1±4.6	
	51-60	Patient	33.62± 3.1	0.06*
		control	16.8±6.1	
	61-70	Patient	36.68± 11.5	0.01
		control	17.9±3.8	

*** Significant ≤ 0.05 .HS, higher Significant, NS, non Significant**

In turn, other family members derive from different cellular sources and are associated with varying functions. IL-17A, IL-17F, IL-17C, and IL-17B function in host defense against pathogens and play various but not fully understood roles in mediating inflammation in autoimmune, allergic, and chronic inflammatory conditions Langley *et al*.(2014).

Ample evidence suggested that cytokines play a crucial role in the immune response process as both Th1 and Th2 cytokines are coexisting during hydatid disease(Biranvand *etal.*,2020) Published data indicate that Th1 cytokines are related to the protective immunity whereas Th2 cytokines are associated with the chronic stage, clinical complications and secondary episodes(Tamarozzi *etal*.,2016).

The role of IL-17B in host defense against intracellular protozoan parasites remains less well studied(Novoa *etal*.,2011). Infection studies demonstrate that Th17 cells mediate host defense against *Trypanosoma cruzi*, *Toxoplasma gondii*, *Leishmania braziliensis*, *Echinococcus granulosus* infections(Das&Khader,2017).

Increased IL-17B levels were detected in the PBMCs and tissue from leishmaniasis-infected patients and associated with enhanced neutrophil and macrophage-mediated destruction of the parasite(Gonzalez-Lombana *etal*.,2013).

Additionally, during echinococcosis, IL-17B plays a crucial immune protective role by regulating the Tregs which are associated with tolerance during infection (Pang *etal*.,2014) .

The present study is also inconsistent with Tuxun *et al* (2012) the results demonstrated that patients with cystic echinococcosis revealed significant increase in peripheral T regulated number, related cytokines (IL-10 and TGF-b1) and transcription factor (Foxp3) levels and moderate decrease in Th17 number, related cytokines (IL-17 and IL-23) and transcription factor (RORct) levels as compared with controls. Results

indicated that Th17 /Treg functional imbalance exists in patients with chronic cystic echinococcosis, suggesting a potential role for Th17 /Treg imbalance in the pathogenesis of immune evasion in echinococcosis.

Protective properties of IL-17B in disease have not yet been reported. In CE patients, plasma concentrations of IL-17B and its soluble receptor IL-17RB were strongly elevated and highest in those with progressive CE. The persistent exposure to growing *E. graneolosus* metacestodes may have triggered the release of IL-17B by cells of the gastrointestinal tract, leading to the recruitment of neutrophil granulocytes into peri-parasite lesions,While the effects of IL-17B are similar to those mediated by TNF-α, IL-17A, and IL-1β, its potency is limited (Yagi *etal*.,2007). The IL-17B-induced infiltration of neutrophils into the peritoneal cavity in rats required much higher concentrations compared to TNF-α and it was still considerably less effective than the cell migration induced by IL-17A (Zhu & Qian (2012), The elevated IL-17B production in CE patients disclosed a proinflammatory response triggered by *E. graneulosus* antigens, but potentially not strong enough to limit the progressive parasite growth.

3.3.3. interleukin 17recepter A (IL17RA) concentration :

Difference with regard to IL-17RA was revealed between patients of hydatid cyst and control show table (3-6) . The concentration of this interleukin a significant increasing ($P< 0.05$) in patients as compared to healthy control .was higher levels of IL -17RA concentration in (61-70) age (6939.26± 18.15) pg/ml of patient compared with(3833.29±21.3) controls significant ($P \leq 0.05$ value) .while recorded (20-30) and(31-40) year higher significant by concentration(6757.48± 16.75, 5941.45± 16.1) pg/ml for patients .

Table (3-6) : concentration levels of IL17RA according to age of patients

Parameter	Age(year)		Concentration pg/ml Mean ±SD	P value
IL17RA	20-30	Patient	6757.48± 16.75	0.00(H.S)
		control	4123±23.15	
	31-40	Patient	5941.45± 16.1	0.00(H.S)
		control	3112.15± 18.6	
	41-50	Patient	6493.59± 23.63	0.01
		control	3712.27±17.4	
	51-60	Patient	6651.30± 12.58	0.001(H.S)
		control	3425.18±23.8	
	61-70	Patient	6939.26± 18.15	0.02*
		control	3833.29±21.3	

*** Significant ≤ 0.05 .HS, higher Significant, NS, non Significant**

This explanation occur the interaction with IL-17 receptors A (IL-17RA) mediate host defenses while also contributing to inflammatory and autoimmune responses(Veldhoen , 2017) .IL-17A and IL-17F both preferentially engage a receptor complex containing one molecule of IL-17RA and one molecule of IL-17RC(Monin& Gaffen, 2018).

More generally, IL-17RA appears to be a shared receptor that pairs with other members of its family to allow signaling of different IL-17 cytokines because the crystal structures of homodimeric IL-17A and its complex with IL-17RA, Binding to IL-17RA at one side of the IL-17A molecule induces a conformational change in the second, symmetry-related receptor site of IL-17A (Goepfert *etal*.,2020).

Also found that Th17 development is dependent on IL-23 secretion (Harrington *et al*. 2006) and is characterized by release of IL- 17A and

IL-17F, which subsequently trigger the release of further pro-inflammatory cytokines and chemokines such as IL-8, TNF alpha and IL-17 (Pappu *et al.*, 2011). Both IL-17A and IL-17F induce neutrophil recruitment and production of antimicrobial peptides (Iwakura *et al*. 2011).

The a mixed between Th1/Th2-type response in infected hosts, and also regulatory mechanisms are likely to control both Th1 and Th2 parasite-killing effector mechanisms (Higuita *et al* ., 2016), A mixed Th1/Th2 cytokine response (Botezatu *et al*., 2018) with elevated levels of IL-10 and increased number of regulatory T cells (Treg) is suggested in cystic echinococcosis (CE) . It is also suggested that the production of IL-10 and TGF-β1 may be induced by the parasite in order to favor its establishment(La Hoz *et al*., 2019).

Correlation between IL17A and IL17 RA in the disease which IL-17R signaling is proposed to regulate neutrophil trafficking into the lung to maintain host immunity (Zhao *etal*.,2016), The addition of recombinant IL- 17A into the airway causes the production of chemokines that recruit inflammatory and immune cells .

3.3.4. interleukin 17recepter B (IL17RB) concentration :

The results in table (3 – 7) revealed a significant changes in serum IL-17RB level between the two different groups. There was highly significant increase in IL-17RB in patient with cystic echinococcosis between (20-40) age(26.33± 7.5)pg/ml compared with control group and lower concentration are in (41-50) age (23.28±15.1) pg/ml in patient with cystic echinococcosis for ($P<0.05$).

This study might be ascribed to the fact that the secretion of this cytokine important to the recruitment of innate and adaptive cell that control on CE infection , As study carried out by Keragala *et al*. (2018).

Table (3-7) : Concentration levels of IL17RB according to age of patients .

Parameter	Age		Concentration pg/ml	P value
IL17RB	20-30	Patient	26.33± 7.5	0.05*
		control	15.7±4.5	
	31-40	Patient	25.0± 3.2	0.01
		control	14.7±5.1	
	41-50	Patient	23.28±15.1	0.03*
		control	14.6±4.2	
	51-60	Patient	24.10± 1.2	0.04*
		control	15.5±3.9	
	61-70	Patient	25.61± 3.4	0.05*
		control	17.8±4.9	

*** Significant ≤ 0.05 .HS, higher Significant, NS, non Significant**

The distinct antigen-induced production levels of IL-17RB in different stages of disease sheds a new light on the role of L-17 family members in CE and could predispose these cytokines as possible markers for disease staging, The observed modulation of IL-17F levels suggests an role of pro inflammatory Th17 responses in CE.(Wakashin, *etal*,2008)

Th17 cells are increased under inflammatory events in the liver (Chang *et al*. 2012), an organ is mainly affected by chronic metacestode growth, and further IL-17 family members like IL-17B have been detected in the gastrointestinal tract, which is also afftected in CE this agree with our study.

The elevated serum levels of IL-17B and soluble receptor component IL-17RB observed in stable and progressive CE cases might signify rather innate immune responses, as IL-17B is expressed by cells of the intestine and stomach, and is a chemoattractant for neutrophil

granulocytes (Reynolds *etal*, 2010),which represent one of the first cell populations involved in inflammation.

IL-17B seems to drive early immune responses against various pathogens, and activation of neutrophils is a hallmark of early pro-inflammatory immune reactions(Isailovic *etal* .,2018).

Lechner's show (2013) The fact that progressive CE cases presented with highest levels of IL-17B and IL-17RB seems to support the concept of parasite-induced immune modulation and this is consistent with the current study.

Study of Lechner *et al* (2012), show significantly elevated levels of IL-17B and lL-17RB were observed, whilelL-17F and slL-17RA were reduced in patients with Alveolar Echinococosis . Similarly, the cellular production of lL-17F and slL-L7RA in response to *E. multilocularis* antigens was low in AE patients, while levels of lL-17RB were highly enhanced.

Recent studies demonstrated that *E. granulosus* secretes several molecules present in protoscoleces and in hydatid fluid that directly can modulate the immune responses thus altering the cytokine balance towards Th2 and favouring immune evasion and perpetuating parasite survival in the host (Bhutani& Kajal, 2018).

3.3.5. Correlation of IL-17A with IL-17RA and IL17B

There was positive correlation between IL-17A with IL-17RA (R =0.034, $p<0.05$) as shown in Figure (3-3) and (3-4),Also there Shows a significant difference between IL17A and IL17RA was higher by *P* value (0.03) and significant between IL17A and IL 17 B by recorded *P* value (0.01) in patients of Cystic echinococcosis.

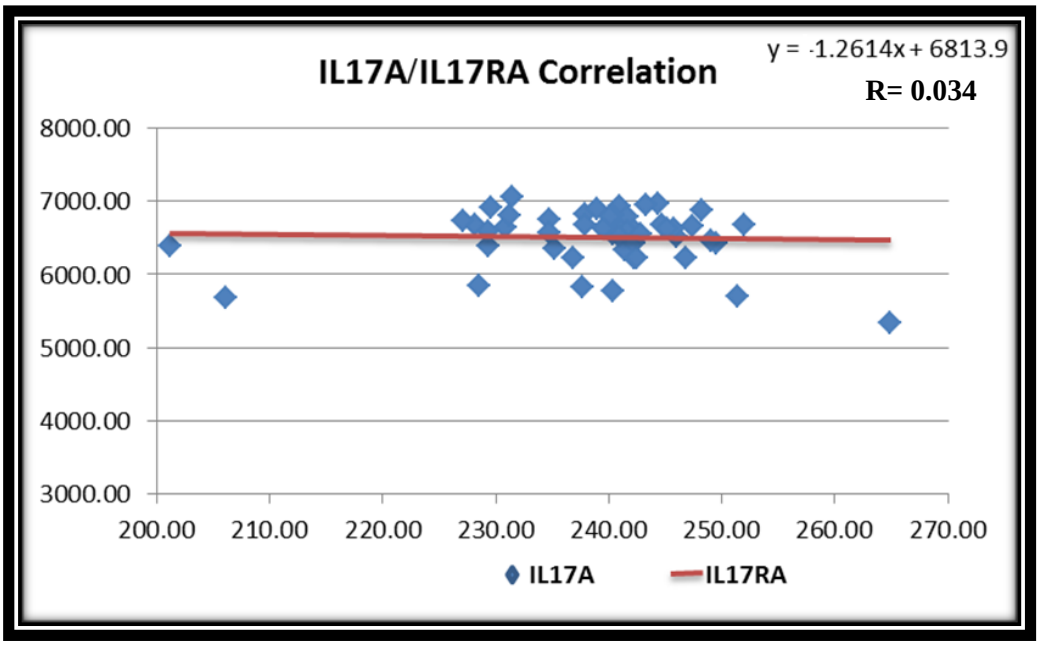


Figure (3-3): correlation between IL-17Awith IL17RA.

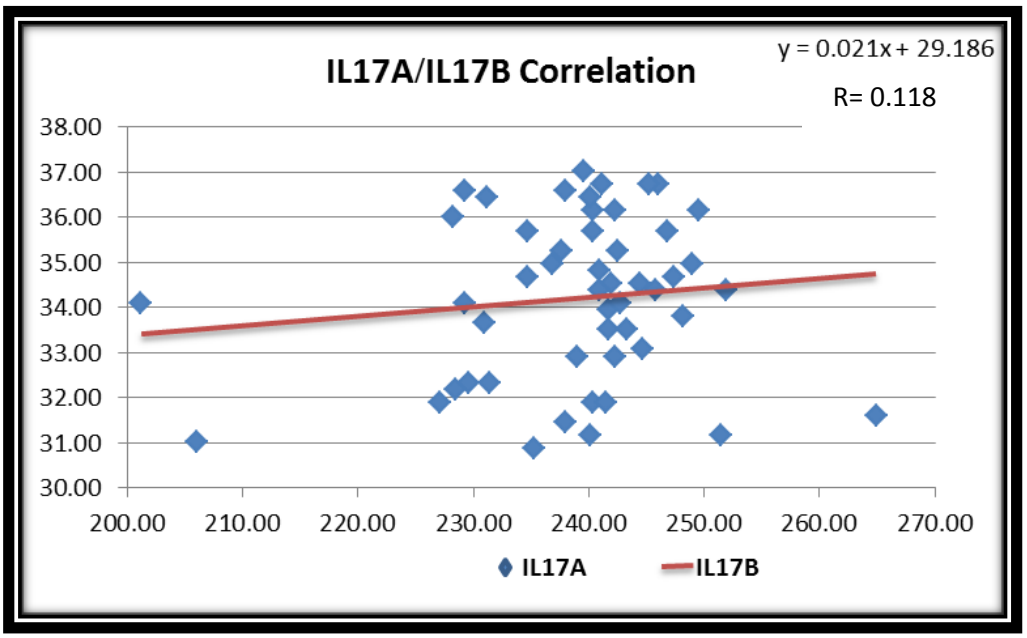


Figure (3-4): The correlation between IL-17Awith IL17B.

The cytokines IL-17A and IL-17 F are highly homologous and bind to the same receptor, and both contribute similarly to the progress of inflammation and to the host defense against pathogens (Reynolds *et al*. 2010).

Several studies have described the importance of cytokines and chemokines in the prevention and elimination of parasite infection,With the tapeworm *Echinococcus spp* infections, Th1-type cytokine IL-12 and IFN-gamma were identified to successfully kill the larval stages of the parasite (metacestode) at the initial stages of development, whereas Th2 immune responses, induced by IL-4, IL-5,IL17 family their receptor and IL-10, lead to a chronic course of disease (Vuitton & Gottstein 2010).

Elevated plasma levels of pro-inflammatory IL-17B and its soluble receptor IL-17RB were observed in stable and progressive CE patients,this significant explained highest reactivity being observed in patients with progressive CE(Huang *etal*.,2014) .

IL-17A is associated with elevated levels of pro inflammatory cytokines and accelerated tubular epithelial apoptosis in Acute kidney injury(AKI),and IL-17A induces neutrophil migration through CXCL5, a chemokine known to be associated with higher risk of renal damage(Luo *etal*.,2016).

The observed correlation between the increased serum concentrations of IL-17A and IL-17B indicates a link between them. IL-17A and IL-17B share a common source, lymphocytes. Therefore, they may play a role in immune, allergic, inflammatory and anti-infective responses(Robak *etal*.,2019), As these processes are involved in the pathogenesis of hydatid disease , and fibrosis predominates with time, it may be helpful to monitor the level of the two cytokines in the course of hydatid disease to assess the extent and staging of the disease.

3.3.5. Correlation of IL-17B with IL-17RB and IL17RB with IL17RA:

Analysis of circulating cytokine production in serum from hydatid patients showed the immune protective role of Th1 cytokines, especially Correlation of IL-17B with IL-17RA and IL17RB, and pathological role of Th17 cytokines during *E. granulosus* infection was positive correlation between of IL-17B with IL-17RA and IL17RB .The present study was focused on determining the role of IL-17B in the immune response against *E. granulosus* infection,We investigated IL17 B ,IL17RA and IL17RB production in serum from hydatic patients with liver and lung hydatid cysts was significant between them p value ≤0.05 between IL17 B and IL17RB and p value between was less significant by P value 0.05 , as show figure (3-5) and (3-6).

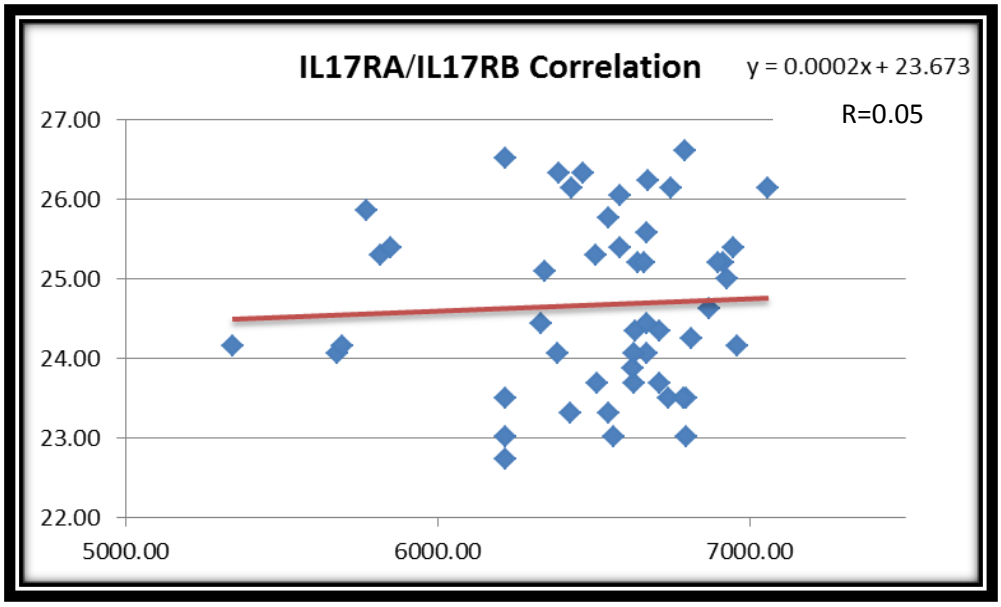


Figure (3-5): correlation between IL-17RAwith IL17RB.

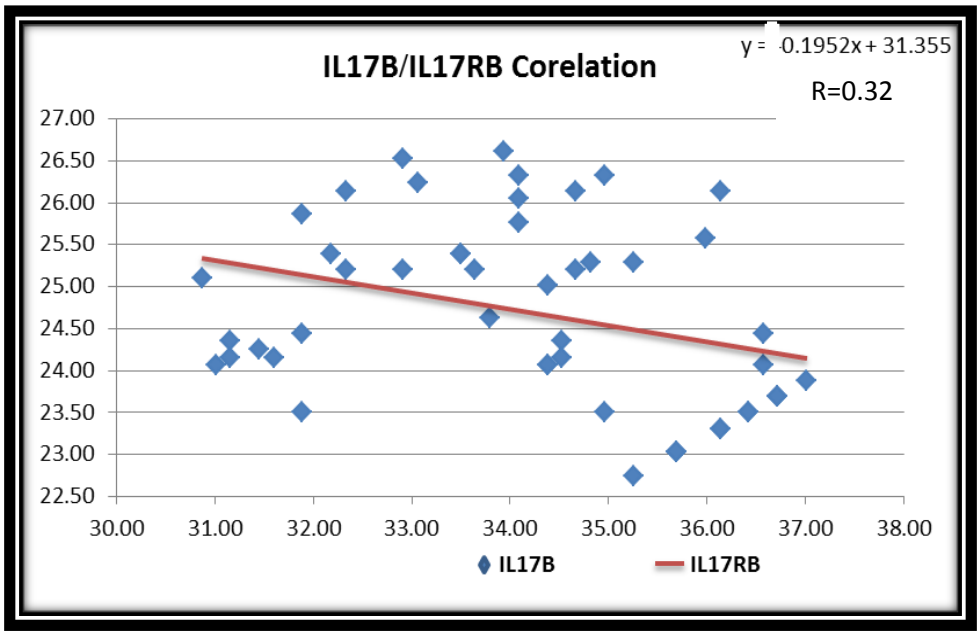


Figure (3-6): correlation between IL-17Bwith IL17RB

The present study show that negative correlation between IL17 B with IL17RA & IL17RB this observed production of certain pro-inflammatory cytokines (IL-17B) was enhanced, which seem to be too weak to induce successful clearance of infection and may rather mirror weak chronic inflammatory events during infection (Huber *et al*., 2012) The observed distinct parasite specific inducibility of IL-17 family members in different patient groups might render these cytokines useful as predictive markers in hydatid disease (Šnábel *etal*., 2016)

IL-17RB serves as receptor subunit for IL-17B and IL- 17E commonly expressed by cells of the intestine, but also by liver, pancreas, lung, and kidneys as well as on Th2 and Th9 cells (Gottstein *etal* ., 2017) ,also show that Plasma concentrations of soluble IL-17RB were highly elevated in CE with no significant differences between the patient groups.

The heightened pro inflammatory IL-17RB and RA responses in patients with progressive AE may primarily be induced by vesicle fluid components and germinal cells and engaging the IL- 17RB activation

pathway (Kern *etal*., 2017). Membrane bound and soluble IL-17RB are inducible in human antigen-presenting cells (APC) upon stimulation with Th2-type cytokines IL-4, IL- 10, IL-13 and TGF-*β* (Förster *etal*.,2019) and these cytokines are associated with progressive AE .

The higher concentrations of sIL-17RB in patients may be a direct consequence of this Th2 polarization associated with chronic CE (Naessens, 2012). The biological functions and importance of soluble IL-17 receptors in CE remain tentative; the soluble IL-17RB could act as decoy receptor for IL-17B (Rostami-Rad *et al* ., 2018).

Based on the results of the present study, we can explain that the shift from Th1 to Th2 reactivity may be associated with persistent of the disease because Th2 reactivity may be less effective than Th1 reactivity in countering the parasite.

These effects may be direct via widely detected IL-17Rs signaling through MAPK and NF-kB recruitment , IL-17 cytokines also act indirectly on immune and nonimmune peri tumoral cells' cytokine secretion.(Fabre *etal*.,2018).

The *E. granulosus* can use two mechanisms to subvert the host immune response: passive escape, in which the parasite, by developing into a hydatid cyst, avoids the damaging effects of an immune response, and immunomodulation through which the parasite actively interacts with the host immune system to reduce the impact of host response (Bhutani& Kajal, 2018).

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Decision of Examination Committee

We are the examiner committee, certify that we have read this thesis entitled (**Immunogenetic study of Interleukin - 17 Family from Hydatid Disease Patients in Babylon Province)** and have examined the student (**Inass Abbas Khiarulla**) in its content, and that in our opinion; it is accepted as a thesis for degree of **Doctor of Philosophy** in Microbiology with **Excellent** estimation.

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1- Introduction

Echinococcosis is the name given to a prominent silent chronic helminthes zoonotic infection caused by infection with the adult or larval stage of the dog tapeworm *Echicoccous granulosus*, which belongs to the Taeniidae family and genus *Echinococcus* (Larrieu *et al* ., 2019).

Echinococcosis an important pathogenic, zoonotic and parasitic infection (acquired from animals) of humans, following ingestion of tapeworm eggs excreted in the faeces of infected dogs. Hydatid disease is a major endemic health problem in certain areas of the world(Nunnari *et al* ., 2012; Rojas *etal*., 2018).

The two-major species of *Echinococcus* of medical and public health importance are *E. granulosus* and *E. multilocularis*, which cause cystic echinococcosis and alveolar echinococcosis, respectively in humans (McManus *et al.*, 2003; Agudelo *et al*, 2016). The disease can be life-threatening in humans and result in a significant economic impact to livestock producers (Azlaf & Dakkak, 2006). Although Cystic Echinococcosis is typically asymptomatic in affected livestock, the organs affected with cysts, which are detected on inspection at slaughter, are usually totally condemned resulting in financial loss for the producers. Severe cases in livestock may result in reduced productivity through interference of organ function (Torgerson &Heath, 2003; Scala *et al.*, 2006).

High incidences of infection by *E. granulosus* often coincides with rural grazing areas where dogs are able to ingest organs from infected wild and domestic animals, approximately 2-3 million human cases occur worldwide annually. In Africa, the prevalence is higher in the northern part such as in Sudan, Egypt and Ethiopia (Federer *etal*.,2016).

The World Health Organization (WHO) identifies human Cystic echinococcosis as one of the most important neglected zoonoses, as the disease continues to pose a serious socio-economic problem in many parts of the world (Budke *etal*., 2006). Cystic echinococcosis is highly endemic in most of the countries of the Mediterranean basin, including North Africa and the Middle East. The high endemicity of echinococcosis in the Mediterranean region has been attributed to many risk factors, such as a lack of adequate public health education, insufficient application of control programmes, and the common practice of home slaughter of small ruminants (Dakkak, 2010).

The host immune system against cystic echinococcosis is classically divided into the adaptive and innate response (Inclan & Siracusa ,2018). Innate immunity is the first line of defense against various parasites (Akira, *etal*.,2006) that can recognize pathogen-associated molecules patterns (PAMPs), via pattern-recognition receptors (PRRs), such as toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)- like receptors . These receptors are expressed by the host innate immune cells, including macrophages, neutrophils, endothelial cells, dendritic cells (DCs) and lymphocytes, which modulate immune responses through different mechanisms for host defense (Janeway& Medzhitov,2002).

The mechanisms involved in innate susceptibility/resistance to Cystic echinocosis /Alveolar echinocosis are mostly unknown (Gottstein& Hemphill,2008). Neutrophils and macrophages are the first responders to detect and eliminate parasites, but their natural activities can be prevented by parasite metabolites. Antigen B secreted by *E. granulosus* can interfere with neutrophil activity via the elastase secreted by

neutrophilic, granulocytes and enable the parasite to escape from thehost immune response (Virginio *etal*., 2007) .

Humans are accidental intermediate hosts and are infected by ingestion of tapeworm eggs shed in the feces of a definitive host (Casulli *etal*., 2019).

Echinococcus granulosus, genotype G1 has the most cosmopolitan distribution and is responsible for the great majority (almost 90%) of human CE, Its principal intermediate host appears to be sheep (Deplazes *etal*., 2017). Although considerably fewer (7%) infections have been attributed to *E. canadensis* genotype G6 than to *E. granulosus* , genotype G1, the former genotype was found to be the second most common cause of human CE worldwide ,Human infections with genotypes G5, G8 and G10 are rare and no cases of human Cystic echinococcosis caused by G4 have been described (Deplazes *etal*.,2017).

In Iraq, human CE is endemic, and the condition has been identified based on the number of individuals hospitalized to hospitals and surgically treated (Maktoof & AbuTabeekh, 2015). In Iraq's southern provinces, a higher number of cases of human CE have been reported (Abdul Ameer *etal*., 2013; Thweni & Yassen, 2015),and in particular Basrah province (Thamir *et al*., 2015). Despite the substantial burden of the disease, national surveillance programmes for Cyst Echinocosis do not exist in Iraq (Barnett-Vanes *et al*., 2016).

1-1- Aim of study :

To determine the some immunogenetic parameters in patients which infected with hydatid cyst and The role of IL-17 cytokines immune responses in CE disease is yet unexplored, This aim achieved by the following objectives.

1- Study the incidence of hydatid cyst infection in Babylon province

2- Study the role of hydatid cyst location (liver, lung, other) in intensity of cytokine induction.

3- study the role of interleukin-17 (IL-17) family (A, B) in patients with hydatid disease and control .

4- investigate the role of IL-17 receptor A and B (1L-17RA and IL-17RB) with Echinococcosis susceptibility.

5- investigate the correlation between IL-17 A,IL-17RA , IL-17B ,IL-17RB in hydatid disease patients and control .

6- Study the association of IL-17 family gene polymorphism with hydatid disease.

1-2- Literatures Review

1.2.1. Historical discoveries of Cystic Echinococcosis (CE):

Hydatid cyst have been known to humans for thousands of years with documentation of epidemic fever in the Ebers papyrus dating back to 1500 BC (Djuricic *etal*.,2010). Hippocrates (∽460-377 BC) was the first person to document information about hydatid cysts and indicated "In those whose water stuffed liver opens into the omentum, the belly is filled with water, and they die" (Eckert & Thompson, 2017). Al-Rhazes, a Persian physician, described hydatid cysts in the liver as watery balloons (Manouras *etal*.,2007). At that time it was believed that the cysts were eggs or embryos of an insect or a cystic tumour , More than 200 hundred years ago, researchers also reported the presence of cysts in the abdominal cavity of slaughtered goats and pigs, but probably were referring to *Cysticercus tenuicollis* rather than hydatid cysts (Ebrahimipour *etal* .,2019).

Philip Jacob Hartmann (1648-1707) had also described a Cysticercus tenuicollis with a scolex, the metacestode of *Taenia hydatigena* (Eckert &Thompson, 2017). For many years knowledge about hydatid cysts was lacking. Aelius Galenus 1821-1833 (cited in Larrieu, 2017) ,reported that "the liver is very much inclined to produce hydatid cysts in the surrounding fascia".

Hydatid cysts were diagnosed in several patients at that time, but the disease or its cause were not understood, and it was believed the cysts were due to dysfunction of the lymphatic glands (Fornaciari,*etal*.,2020).

In the 17th century many researchers described the features of hydatid cysts. For example, Peter Pallas divided hydatid cysts into adherent and non-adherent forms and named the cyst *Taenia hydatigena* (Malik&

Shams 2019). The protoscoleces in the hepatic cysts were described in detail by Goeze (Eckert & Thompson, 2017), and subsequently described the hydatid cysts in sheep and named them *Hydatigena granulosa*.

During the 18th century advancements were made on the understanding of the life cycle of the parasite in both the definitive and intermediate hosts(Brooks *etal*.,2019). Several experimental studies were performed by Carl Theodor von Siebold, Breslau, and Friedrich Küchenmeister to further the understanding of the life cycle of echinococcosis (Tappe *et al.*, 2010). They fed a group of dogs metacestodes of *E. veterinorum* isolated from slaughtered sheep, which led to the discovery of the strobilar stage. Subsequently Von Siebold named the adult worms *Taenia* (T.) echinococcus (Von Siebold, 1853 cited in Eckert & Thompson, 2017), However, a series of further experiments were undertaken to confirm the life cycle of the parasite.

The faeces of infected dogs were fed to a group of sheep resulting in the production of fertile liver cysts (Wen *etal*.,2019). Finally Rudolphi in 1801 suggested the genus name of *Echinococcus*, being derived from the Greek word meaning "spine and berry" (Al-Shabbani, 2014).

1.2.2. Classification of *Echinococcus granulosus*:

According to classified by Eckert *et al* (2002), the tapeworms whose larval stage causes hydatid cysts belong to the following classification :

Phylum: Platyhelminthes.

Superclass: Eucestoda.

Class: Cestoidea.

Subclass: Cestoda.

Order: Cyclophyllidea

Family: Taeniidae.

Sub family : Echinococcinae

Genus: *Echinococcus*

Species: *Echinococcus granulosus*

1.2.3. -Strains of *Echinococcus granulosus*

Echinococcus granulosus has been divided into several strains according to the host (Mario *et al*.,2011). *Echinococcus granulosus* has been researched extensively and discussed in detail as it is the species that is most widely distributed throughout the world, it has seven strains which have been identified as G1 genotype (common sheep strain), G2 genotype (Tasmanian sheep strain), G4 genotype (horse strain), G3, G5 genotypes (cattle strain), G6 genotype (camel strain), G7 genotype (pig strain)(Rahman *et al*.,2015).

These species can infect a specific type of animal or a range of animals: for example *E. felidis* is restricted to the lion (*Panthera leo*) (Hüttner *et al*., 2009); and *E. shiquicus*, discovered in 2005, is found in Tibetan foxes (*Vulpes ferrilata*) with the pika (*Ochotona curzoniae*) and voles (*Microtus limnophilus* and *Lasiopodomys fuscus)* from the Tibetan plateau being the predominant intermediate hosts (Boufana *et al.*, 2013; Wang *et al.*, 2019). This latter species is a "sister species" to *E. multilocularis* (Xiao *et al.*, 2006) and *E. felidis* has been shown to be phylogenetically closely related to *E. granulosus* (Hüttner *et al.*, 2009). In contrast to *E. felidis*, a wide range of hosts are susceptible to *E. granulosus*, including yaks, buffalo, camelids, pigs and equids (Eckert and Deplazes, 2004). The genotype G6/G7 of the pig strain has a wide

allopatric distribution, and has been assigned to the species *E. intermedius* and has been reported in pigs and camels as well as in other animals (goats, horses and humans) (Lymbery *et al.*, 2015).

1.2.4.Life cycle of *Echinococcus granulosus*

1- Final host

According to Lewall (Jenkins *et al* .,2015) the final hosts of *E. granulosus* are dogs, wolves, jackals, dingoes, coyotes and foxes, Feline species are seldom infected naturally, but the parasite has been reported in cats, wild cats and leopards, which can also serve as hosts, but with low efficiency.

2- Intermediate host

The common intermediate host of *E. granulosus* are many domesticated mammals such as human , sheep, cattle, pigs, goats and camels. Sheep, which harbor the most fertile hydatid cysts, are the most important intermediate host and represent the most important source of infection to dogs through the feeding of infected offal, (Rahman *et al.*, 2015).

3- **Development**

The intermediate host ingests eggs of *E. granulosus*, which are passed in the faeces of the final host and are immediately infective. They are resistant to external conditions and are capable of development even after months outside the body (Thompson & McManus, 2001). Following ingestion by an intermediate host, they hatch in the small intestine and the resulting oncospheres invade the blood vessels of the wall. The hatching process uses the disaggregation of the keratin-like blocks of the

embryophore by pepsin, pancreatic enzymes, etc., followed by the activation of the contained oncospheres (Khanfar, 2004).

The released muscular oncosphere attaches to the microvilli of the jejunal region of the small intestine with its six hooks, then enters the lymphatic or mesenteric venules and lodges in numerous organs, affecting the differential distribution of metacestodes between the liver and lungs, Within twelve hours after ingestion, it arrives at the liver, where if not destroyed by phagocytic cells, it develops into a hydatid cyst (Thompson & McManus, 2001).

Then settle in the liver, forming a respective cyst, which is the origin of an *Echinococcus*. However, the liver is not always the primary filtration site (Zarbaliyev *etal*., 2019). Inhaled eggs can also cause pulmonary hydatid disease as it has been shown that eggs administered to sheep by a tracheostomy develop into lung cysts (Torgerson *et al*., 2003). The larvae of *E. granulosus* may also develop in the heart, spleen, kidneys, brain, eyes and long bones (Ramos,*et al*., 2020). The speed of development of the embryo to the fully formed metacestoda is slow, and during the first 10-14 days, it involves cellular proliferation, degeneration of the oncosphere hooks, and formation of a central cavity and development of the laminated and germinal layers. For example, the time taken to produce the first brood capsules from eggs is reported to be not less than 7 months in mice (Ahmed *etal*., 2019) ,10-12 months in pigs and 10-48 months in sheep .

When a final host ingests these hydatid cysts, the protoscoleces project their heads and secure a firm hold among the villi of the small intestine and mature into adults in 40-50 days. Since each worm stays in a host for 5-29 months, the eggs of *E. granulosus* are liberated after

detachment of a gravid segment, which occurs every two weeks. Thus, the number of eggs produced by an adult is less than those for other cestodes (Hijjawi *et al*., 2018) In addition, the small intestine may finally become full of adults as a result of repeated infections or intake of a large number of cysts.

Each scolex that is ingested is capable of developing into an adult worm in the intestinal tract in about seven weeks (Thompson , 2017) figure (1-1). In heavily endemic areas, 50% of the dogs are infected with adult worms, and up to 90% of sheep and cattle and 100% of camels may be infected with hydatid cysts (Saheb *et al*., 2017), However, experimental evidence has been reported that echinococcosis can become established in the lungs of sheep from eggs without their prior ingestion through intra tracheal route.

The pathology caused by the presence of a single unilocular cyst depends very much on its site in the body. In the liver, the cyst may cause compression of the liver cells, leading to biliary stasis and cholangitis. Lung cysts are always intracapsular and their rupture may result in protoscoleces being coughed up in the sputum. Hydatid cysts in the brain or spinal cord are usually small and may cause symptoms earlier than in other sites. Renal cysts sometimes occur and, if they burst into the kidney pelvis, may become secondarily infected (AL-tameemi &Kabakli, 2019) .

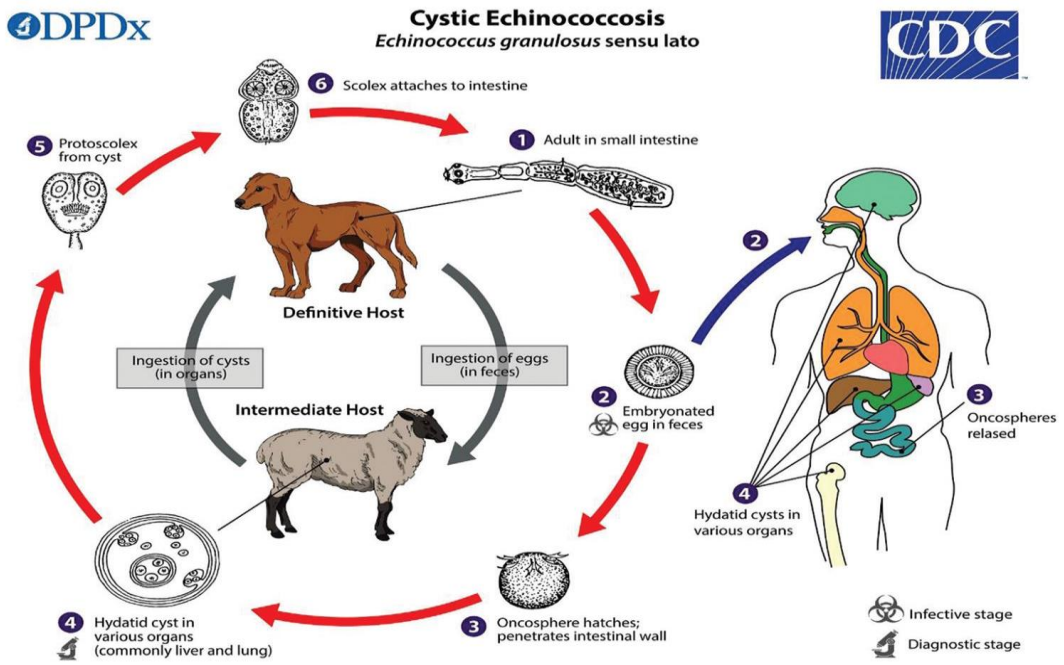
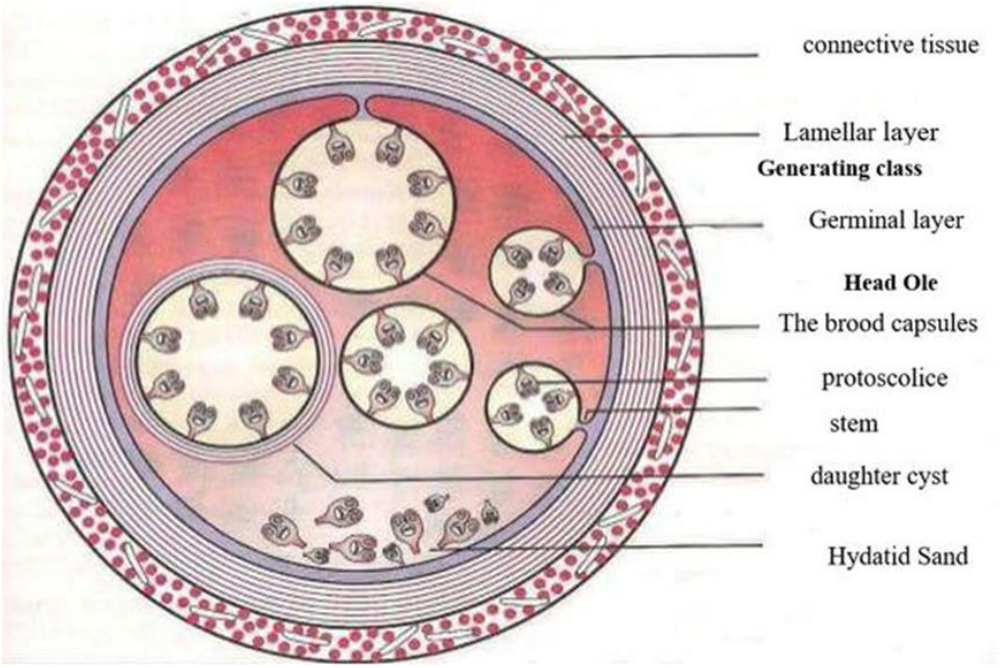


Figure (1.1): A typical domestic lifecycle of *Echinococcus granulosus*. Source:Centers for Disease Control and Prevention (Ndlovu *et al.*,2018).

The hydatid cyst has three layers: (*a*) the outerpericyst, composed of modified host cells that form a dense and fibrous protective zone; (*b*) the middle laminated membrane, which is acellularand allows the passage of nutrients; and (*c*) the inner germinal layer, where the scolices (the larval stage of the parasite) and the laminated membraneare produced. The middle laminated membraneand the germinal layer form the true wall of the cyst, usually referred to as the endocyst, although the acellular laminated membrane is occasionally referred to as the ectocyst (Malik, 2016;Hassan, 2017). Daughter vesicles (brood capsules) are small spheres that contain the protoscolices and are formed from rests of the germinal layer. Before becoming daughter cysts, these daughter vesicles are attached by a pedicle to the germinal layer of the mother cyst. At gross examination, the vesicles resemble a bunch of grapes (Fig 1-2). Daughter

cysts may grow through the wall of the mother cyst, particularly in bone disease (Osman, 2017).

Cyst fluid is clear or pale yellow, has a neutral pH, and contains sodium chloride, proteins, glucose, ions, lipids, and polysaccharides. The fluid is antigenic and may also contain scolices and hooklets. When vesicles rupture within the cyst, scolices pass into the cyst fluid and form a white sediment known as hydatid sand (Subramanyam *et al*., 2015).



Figure(1.2) :structure of hydatid cyst (Al-Khalidi *et al.*,2020)

1.2.5. Distribution of Cystic Echinococcosis

1.2.5.1. Global distribution of Cystic Echinococcosis

Anthropogenic influences, including increased globalization of animals and animal products, and altered human/animal interfaces are thought to have played a vital role in the global emergence of this pathogenic cestode (Davidson *et al.*, 2012; Deplazes *etal*.,2017). Expansion of the human population, resulting in a shrinkage of natural habitats and the associated increased urbanization of some wild carnivore

species, have resulted in the spread of *Echinococcus* spp. from the traditional rural environs (Carmena & Cardona, 2014). In some parts of the world, such as Central Europe, the Baltic States and the Scandinavian countries, hydatid cysts are less prevalent in humans and other animals (Dakkak, 2010).

In contrast a high prevalence has been reported in Russia (Torgerson *et al.*, 2010), and CE is considered to be re-emerging in Wales (Buishi *et al.*, 2005) . Re-emergence in some countries may be as a result of failure of existing control campaigns or socio-economic changes resulting from the collapse of previous political systems ,Transmission of CE in Europe relies primarily on dogs serving as the definitive hosts and domestic ungulates, including sheep, goats, cattle, buffaloes, horses and pigs, acting as the intermediate hosts(Cardona & Carmena, 2013).

Human CE remains endemic in Europe with regular recording of sporadic cases (Torgerson, 2017). In Germany CE cases are considered autochthonous with an annual incidence of approximately 0.05 per 100,000 population, being reported from 2001 to 2013. In Austria, the annual incidence of CE has been estimated to be 0.4 per 100,000 inhabitants (Schneider *et al.*, 2010).

Hydatid cysts are endemic in livestock and humans in Africa (Dakkak , 2010). Although some countries of Africa have undertaken studies to determine the prevalence of hydatid cysts (Mohammed , 2016), others have not and it is likely that the real burden of infection has been underestimated (Cardona & Carmena , 2013).

Molecular studies of CE have been carried out on cysts from production animals in African countries (Algeria, Mauritania, Ethiopia, and Sudan), revealing the presence of *E. granulosus*, *E. ortleppi*, *E.*

canadensis and *E. equinus* (Omer *et al.*, 2010). In Uganda and Kenya, *E. felidis* has been detected in lions (*Panthera leo*) and warthogs (*Phacochoerus* spp.), although no evidence of infection in humans has been reported (Hüttner *et al*., 2009).

In contrast, a genetic survey of cysts isolated from human patients in Mauritania and Kenya have detected the sheep strain G1, as well as the camel strain G6 (Salem *et al.*, 2011). In Egypt, *E. granulosus* have been isolated from sheep and *E. canadensis* from camels and sheep (Amer *et al.*, 2015).

In South America, CE is a common disease with around 5,000 new human cases reported annually in the five countries of Argentina, Brazil, Chile, Peru and Uruguay (Pavletic *et al.*, 2017). The life cycle of CE is maintained by domestic cycles of transmission involving dogs and herbivores (sheep, swine, cattle, goats, horses and camelids) and multiple species/genotypes including *E. granulosus* (G-G3), *E. ortleppi* (G5) and *E. intermedius* (G6/7) (Deplazes *et al*., 2017). In Argentina the annual incidence was reported as 0.95 per 100,000 inhabitants between 2005 and 2010 (Bingham *et al.*, 2014).

1.2.5.2. Distribution of Cystic Echinococcosis in the Middle East

Echinococcosis has been reported to be a public health concern in Iran, Iraq, Jordan, Egypt and Turkey as evidenced by the large number of hospitalised cases (Sadjjadi, 2006). A number of factors have been identified contributing to the increase in the disease's prevalence in humans in the Middle East, including poor slaughter-house hygiene, low general public awareness of the disease, inefficient veterinary services and large numbers of free-roaming stray dogs (Dakkak, 2010).

The home slaughter of animals (small ruminants particularly) is still common in a number of Middle Eastern countries, and large numbers of animals are often slaughtered for specific festivals such as the Muslim Eid (Othieno *et al*., 2016). Free roaming dogs have been shown to have high infection rates of *Echinococcus* with a prevalence of 7.9 to 14.3% and 14.2% observed in Palestine (Abdel-Hafez and Kamhawi, 1997). *Echinococcus granulosus* has been reported to be the most dominant species in dogs in Egypt, Jordan and Iran (Shariatzadeh *et al*., 2015).

In Iraq, cystic echinococcosis is one of the main health concerns (Al-Rishawi & Al-Mayali, 2019), which is endemic and a major health problem in the country, It is even more complicated because of that CE didn't have a real systematic national surveillance and control program in Iraq (Athmar & Ban-Abbas, 2014). The largest number of human cases were reported in the central and southern governorates of Iraq including Basrah, Dhi Qar and Al-Muthana (Abdulhameed *et al*., 2019).

1.2.6. Pathology of cystic echinococcosis

Despite carrying a massive parasite burden, definitive hosts do not normally show any symptom while being infected by adult worms on contrary, the larval stage of *Echinococcus granulosus* induces significant pathology in the,intermediate host (Torgerson& Budke, 2011). Indeed, the pathogenicity of hydatid cyst differs from host to host and depends on many factors such as age, sex, genetic traits, physiological condition and, species of the host (Cappello *etal*., 2013). Besides, severity of clinical symptoms is closely correlated to the size, number and localization of evolved cysts (Deplazes *etal*., 2017).

Almost in all intermediate hosts, hydatid cyst is principally located in the liver with a frequency of about 70%, although can be found in

other organs such as the lungs (20%), kidneys, spleen, brain, heart and bones with less frequency (Zhang *et al* ., 2012). About 20–40% of human patients have multiple cysts or multiple organ involvement. After an undefined incubation period which may last months or years, the exerted pressure on adjacent tissue by a grown cyst may cause symptoms and can be followed by other pathologic events(Craig *etal*., 2017). As hydatid cysts grow slowly, the host often tolerates it remarkably well and therefore hydatid patients may come to clinical attention only when the normal function of the infected organs is interfered by the mechanical pressure of the cyst (Chai *et al*., 2021). Other clinical signs such as allergic reactions, eosinophilia or accidental cyst rupture which triggers acute hypersensitivity responses can also indicate the existence of the infection, cysts or a cystic mass may also be discovered by chance during body scanning or surgery for other clinical complications (Nunnari *et al* . , 2012).

High temperature and desiccation are limiting factors for human infection with hydatid disease (Kwa, 2018), Other factors are type of the soil, wind dispersal, and vegetation cover also persons of both sexes appear equally susceptible but sex ratios of patients vary in different regions and host factors are human behavior such as widespread use of dogs and the habit of feeding on viscera of home butchered sheep or other livestock,Personal hygiene and cleanliness ,socioeconomic and cultural characteristics are among the risk factors for human infection (Caldas *et al* ., 2008).

1.2.6.1. Cystic echinococcosis of the liver

Once the onchosphere passes through the intestinal wall, it is carried by the portal venous or lymphatic system to the the liver as the first line of defense. That is why the liver is the most frequently involved organ. Most cysts tend to be harbored in the right lobe (Kanan & chain ,2006). Natural history of the hydatid cyst can be divided into two phases (Stojkovic & Junghanss , 2013).

1- During the first phase, continuous growth and the enlargement of the cyst can cause increased compression on the surrounding parenchyma and may result in upper abdominal pain and other non-specific signs. While hydatid cyst is growing, the cyst wall may lose its resistance against the pressure of the hydatid fluid, thus cyst rupture occurs. As well, this condition can happen due to a trauma or even surgical intervention. In general, symptoms such as acute allergic reactions, obstructive jaundice and emesis can be detectable as consequences of the cyst rupture.

2- If protoscolices and daughter cysts are overproduced during the first phase, the hydatid fluid will be replaced by these components which results in stiffness of the cyst cavity and is followed by the calcification of the cyst wall. In this phase, cyst growth usually halts and the ectocyst is detached from the fibrous capsule, Partial calcification of the cyst does not always indicate the death of the parasite; nevertheless, densely calcified cysts may be assumed to be inactive (Kanan & chain ,2006) .

Secondary infection (i.e. bacterial, fungal) of the hydatid lesion is the most common complication and can be somewhat symptomatic. Infection occurs only after communicating and direct rupture when both the

pericyst and endocyst fracture, which allows pathogens to pass easily into the cyst, in 5%–8% of cases (Lin *et al*., 2014). The evolvement of an infected hydatid cyst is usually dormant, sub-acute and is clinically identified by pain in the right hypochondrium, hepatic abscess, and fever (Mnati,*etal* ., 2020)

Biliary rupture may occur through a small fissure or bile duct fistula (Yilmaz *et al*., 2012). A wide perforation allows the access of hydatid membranes to the main biliary ducts, which can cause symptoms simulating choledocholithiasis (Bricault, 2012). Intrabiliary rupture of a hepatic cyst can be indicated as an occult drainage of hydatid fluid into the biliary tree and is observed in 10-37% of patients mainly in centrally localized cysts (Ramia *et al*., 2012; Touma,*et al*., 2013).

The increased pressure of the hydatid fluid can be also a prompting factor of the rupture usually in the right hepatic ducts, although the left hepatic ducts are sometimes involved, More severe complication can be detected due to an overt passage of intra-cystic material to the biliary tract in 3-17% patients (Abdelraouf *etal*.,2015), Perforation into the gallbladder can be detected in 5-6% of cases.

Hepatic cyst rupture to the gastrointestinal tract is very rare (Vidoura *etal*., 2017), On the other hand, released protoscolices settle other visceral organs and every one of them can potentially evolve to a hydatid cyst. This condition is termed as secondary cystic echinococcosis and may occur either spontaneously or after trauma. Formation of secondary cysts has been also observed as a complication after inattentive surgery(Sokouti *etal*.,2017). Involvement of the pulmonary parenchyma or peritoneum is usually the most frequent trait of secondary cystic

echinococcosis. Nonetheless, primary infection of the peritoneum has been also reported (Panteleyev ,2018) .

1.2.6.2. Cystic echinococcosis of other organs

In human hosts, the lungs are the second most frequent sites of infection in adults, while the involvement of the lungs is the most common feature of cystic echinococcosis in children (Vatankhah ,2016). In organs such as the lungs and the brain, hydatid cysts may grow faster and achieve larger size more likely due to the softness of the tissues which is easy to compress, Calcification in pulmonary cysts is very rare (0.7% of cases)(Kern *etal*.,2017), although it may be seen in pericardial, pleural, and mediastinal cysts (Al-Yasari *et al*.,2013). Expectoration of the fluid or other materials of the cyst and its rupture into the pleural cavity may also occur. Bacterial infection of the cyst is the most serious complication commonly seen after rupture (Pezeshki *et al*.,2012).

The prevalence of renal infection is 3% and the involvement of the kidneys usually remains asymptomatic for many years, although symptoms such as flank mass, pain and dysuria can be commonly seen (Othieno,*et al*.,2016). Several round masses may be seen in the excretory system due to daughter cysts (Murtaza *et al*.,2017).

The splenic involvement in human hydatid disease has been reported in 8% to 9% of cases (Della *et al*.,2015). The metacestode can be harbored in the spleen majorly after systemic or inter-peritoneal dissemination of protoscolices due to the rupture of hepatic cysts. Consequently, the spleen is usually considered as the third most frequent site of infection in humans. Clinical symptoms such as abdominal pain, splenomegaly and fever are often observed in patients with splenic hydatid infection (Akbulut *etal*.,2013).

The osseous involvement in hydatid disease is most commonly seen in the spine and pelvis, followed by the femur, tibia, humerus, skull, and ribs with a frequency ranged between 0.5-4% (Pakala,2016). The absence of the cellular infiltrate and fibrosis (pericyst) around the cysts in the skelet al system allows them to grow vastly in an irregular manner and they may produce subsidiary branches that penetrate through less resistant compartments of the tissue, especially in the bone canals (Craig *etal*.,2019).

Hydatid disease affects the central nervous system in 1% of cases and is usually diagnosed during childhood, The hemispheres are the most common locations of the cerebral cysts, particularly around the middle cerebral artery, although the meta cestode may be harbored anywhere in the brain(Maraby-Salgado *et al* .,2017).

1.2.7. Clinical manifestations

The *Echinococcus* species are of medical and veterinary importance, since infection with the metacestode can cause severe illness (even death of the intermediate host) and livestock associated production losses (Miran *etal*.,2017). Clinical manifestations of the disease in intermediate hosts primarily depend on the size, location and number of cysts (Abo-Aziza *etal*.,2019). Patients can remain asymptomatic for months, years, and even longer, with clinical symptoms showing from below one year to over 75 years of age in all sexes (Seid& Melese ,2018). The clinical complications are therefore a result of mechanical pressure exerted on surrounding vital organs by the growing cyst, Accidental rupture of cysts can also happen, releasing fluid filled with protoscolices and possibly leading to anaphylactic reactions and secondary multiple cyst infections

(as protoscolices can develop into secondary cysts within the intermediate host) (Teshome *et al* ., 2017)

Lung infection may present as chronic cough, pneumothorax, pleuritis, lung abscess and parasitic embolism (Díaz , 2017). Heart involvement can present as a tumour, complete heart block and sudden death (Baumann *etal*.,2019),The effects in the brain are usually headache and mass effects with neurological symptoms, and patients with eye infection can present with pain, ptosis and visual disturbances (Baumann *et al*.,2019).

The tapeworm cysts in humans can be found mostly in the liver (62 to 70%), lungs (20%), and other organs (brain, wall of the heart, kidneys, spleen, orbit of the eye, marrow cavity of bones) can be involved in 10% of the cases (Yuan *et al*.,2017), Multiple cysts or multiple organ involvement can also be seen in 20% to 40% of patients .

The clinical manifestations of cystic echinococcosis can also be complicated by other factors such as co-infection with human immunodeficiency virus (HIV) (Lozneanu *etal*.,2019). It was found that the profound immunosuppression of the patient can result in extensive CE disease and can interfere with immunodiagnostic tests, leading to false-negative results (Lozneanu *etal*.,2019). The adult tapeworms are considered to be rather harmless to the definitive host, except when they occur in large numbers, when they might cause severe enteritis (Al-Khalidi *et al*.,2020).

1.2.8. Diagnosis of cystic echinococcosis

Due to the bad prognosis of developed infection, early diagnosis is an essential part of the treatment and control procedure in cystic echinococcosis, Considerably long incubation period of the infection

during which clinical manifestations are usually absent, is an important challenge to plan an efficient strategy for early diagnosis of the infection (Minaev *et al*.,2018). Parasite larvae show the highest susceptibility to chemotherapeutic compounds during the pre-encystment phase or before maturation of the established cyst (Hijjawi *et al*.,2018).

Because of the distinctive natural history of the infection, patients are usually found accidentally or when physical damage to the harboring organ and cyst rupture has occurred. A reliable diagnosis requires combination of physical examination, imaging techniques and serology methods. Upper abdominal discomfort, loss of appetite and pain are of the major complaint that along with results of physical examinations such as hepatomegaly, presence of abdominal palpable mass and abdominal distention may lead clinicians to consider potential occurrence of cystic echinococcosis (Khatonaki *et al*.,2020).

1.2.8.1. Imaging techniques

Generally used imaging techniques include ultrasonography, CT-scanning and magnetic resonance imaging (MRI). Ultrasonography is the most common imaging method to identify hydatid lesion and is useful to determine the number and size of hydatid cysts in almost all anatomical sites (Orsten *et al*.,2018), This technique can be used in field surveys by applying portable machines . Ultrasonography can only visualize cysts with at least partial calcification where echogenic regions (either hyperechogenic or hypoechogenic) are scattered throughout the lesion. Regarding this trait, it has been tried to introduce an internationally accepted system to standardize the hydatid cyst diagnosis based on classification of its ultrasonographic features (Turgut,*et al*.,2007). In 2003, the World Health Organization (Abdulhameed *et al*.,2019)

published a new standard system which was actually an amended version of Gharbi‟s classification method introducing six categories: cystic lesion (a unilocular cyst with unknown origin which is to undergo more investigation), CE1 (unilocular fertile cysts with visible wall), CE2 (multivesicular septated fertile cysts), CE3 (laminated layer is detached from the cyst wall which makes „water lily‟), CE4 (cysts with scattered hypo- and hyperechoic degenerative contents and no visible daughter cysts), and CE5 (cysts with thick wall showing partial or complete calcification) (Brunetti *et al.*,2018).

1.2.8.2. Immunological and molecular diagnosis

Although imaging techniques provide valuable information about physical appearance, dimension and anatomical site of hydatid cysts, diagnosis can be sometimes presumptive (Stojkovic *et al*.,2009).

Serology of hydatid cyst is normally based on tracing specific antibodies, parasite circulating antigens and circulating immune complexes in serum samples (Siles-Lucas *et al*.,2017). So far, various serological methods have been tested for diagnosis of the infection. Due to the lack of sensitivity and presence of non- specific results, early methods such as Cassoni intradermal test and complement- fixation test were replaced by more sensitive examinations. Routine laboratory tests include indirect hemagglutination (Nunnari *et al*.,2012) , enzyme- linked immunosorbent assay (ELISA), latex agglutination (LA), indirect immunofluorescence antibody test (IFA), immune- electrophoresis (IEP), and immunoblotting (IB) (Carmena *et al*.,2006), Immunoassays have appeared to be expedient not only for detection of the infection and follow- up analyses but also for screening studies in endemic areas.

Recent advances of molecular genetics have suggested application of more accurate methods such as polymerase chain reaction (PCR) for detection of hydatid antigens or expression of specific antibodies, although performing these methods requires high- tech equipment and well- trained laboratory staff and other conditions which are often difficult to provide particularly in endemic areas of the Third- world or developing countries(Zheng *et al*.,2013).

Molecular techniques have provided much more valid methods for identification and characterization of parasite (Craig *et al*.,2007). Recently analysis of DNA has been used to categorise variants of *E. granulosus* into distinct genotypic strains, only 10 genotypes (G1-10) have been identified (Sharbatkhori *etal*.,2016) .

Genetic studies have principally confirmed the concept of strain diversity within the species of *E.granulosus*, previously based on morophological, biochemical and epidemiological studies, This variation may reflect variable in characters which affect the life cycle pattern, host specificity, development rate, pathogenicity, sensitivity to chemotherapeutic agents, transmission dynamic, epidemiology and control of echinococcosis (Barazesh *etal* .,2019).

1.2.9. Treatment

Cystic echinococcosis treatment is based on surgery, chemotherapy or both, Cyst removal followed by anthelminthic medication is often proposed as the best choice for the disease management Schantz (Lupia *et al*.,2021).

Surgical operation is normally associated with many risk factors and may not be very cost- effective, although seems to be the best option for precise depletion of the parasite (Schantz, 2006). Furthermore, clinical

status of the patient and presence of other health complications as well as cyst indications (such as size, number and location which may make it surgically inaccessible) and presence of well- experienced medical team can restrict surgery (Monteiro *et al*.,2010). The most common techniques are used for hydatid cyst surgery include open procedure, laparoscopy and percutaneous treatment. Cyst removal by surgery is divided into radical (when pericyst and parasitic content of the lesion are entirely excised or cystectomy) and conservative (when only parasite cyst is removed or hydatidectomy) methods (Bayrak & Altıntas, 2019).

Chemotherapy has recently received more attention for treatment of cystic echinococcosis, Benzimidazole and albendazole are current chemotherapeutic choices for the disease, although albendazole is more recommended because of its better absorption (Muhammedoglu *et al*.,2021) . Although anti-hydatid drugs have had some success, adverse medication responses, non-responsiveness to treatment, resurgence of infection, parasite resistance to the administered drug, and in some cases severe illnesses have been reported in a significant number of patients (Khalkhali, *et al*.,2018)

1.2.10. Immunity response to the *E.granulosus* :

The host immunity plays an important role in determining the relationship between the host and the parasite. Parasite produces excretory compounds, which influence the immune-competent cells in the human host and stimulate pro-inflammatory immune responses, releasing antibodies, and activate T-cells in the body (Slimane *et al*.,2018).

Continuing presence of parasites in the body indicates that they have developed some of the evasion mechanisms from host immune mechanisms to preserve their development. *E. granulosus* can use two

mechanisms to reduce the host immune response: (Al-Tameemi & Kabakli,2019) Passive escape by developing into a hydatid cyst so avoiding the ruining effects of an immune response and, immunomodulation through which *E. granulosus* interacts with the host immune system to reduce the efficiency of the host response (Mohamed .,*et al*.,2017) Recent studies showed that *E. granulosus* secretes molecules that can modulate the immune responses so changing the cytokine balance toward Th2.

Antibodies play a major role in parasite killing as a protective immune response against *E. granulosus* involving antibody-dependent cell-mediated cytotoxicity reactions (Profumo *et al*.,2014), Cystic echinococcosis (CE) induces two (Th1 and Th2) cytokine secretion patterns in the active and inactive stages of hydatid disease. Early Th1 cytokine production (which kills the metacestode at the initial stages) changes to a predominant Th2 cytokine as a response in the chronic stage of *E. granulosus* and *E. multilocularis* infections (Zhang *et al*.,2012). Th2 cells produce interleukin (IL)-4, IL-5, IL-6, and IL-10 which are associated with susceptibility to the disease, whereas Th1 cells express IL-2 and interferon-gamma and they are well related to protective immunity. Some of the studies showed an increase in the production of some cytokines such as gamma interferon, IL-4, and IL-5(Craig *et al*.,2017).

The six family members identified (IL17A-F) exert mostly pro-inflammatory activities (Noda *etal*.,2011). IL17A and IL17F, mediators of the recently described proinflammatory Th17-type immune responses, have been associated with inflammatory disorders like rheumatoid arthritis and inflammatory bowel disease (Robert& Miossec, 2019).

1.2.10.1. Host- parasite immune interaction in cystic echinococcosis

Existing data about host immune reactions to cystic echinococcosis is still insufficient,Many studies have been carried out to unveil the association of immune responses to the host- parasite relationship in different stages of the infection, although a definitive picture to show the clear-cut traits of hydatid- induced immune mechanisms cannot be concluded by the achieved results (Yang *et al*.,2012). It probably reflects the impact of the intermediate host phenotype on immune reactions, as a wide range of mammalian species is involved in the parasite life cycle. Furthermore, genotypic variability within a certain species is likely determinative to the immunity against cystic echinococcosis (Shen *et al*.,2014). The main problem is raised by taking into account that these results are primarily derived from studies on laboratory animals, so cannot be a precise indication of immunity against the parasite in naturally infected intermediate hosts(Gottstein *et al*.,2017). Immunoserological assays have also provided indirect evidence to characterize parasite- induced immune reactions in human patients and other mammalian intermediate hosts(Mourglia-Ettlin *et al*.,2016). Nevertheless, such findings seem inadequate to thoroughly illustrate the real feature of host- parasite immune interaction, particularly in human(Mourglia-Ettlin *et al*.,2016).

Immunopathology of the infection can be studied during two distinctive phases: the establishment (pre-encystment) phase and established (encystment) phase (Farid& El Amir,2015), however this concept has appeared to be controversial as the *Echinococcus* species are multicellular organisms shown to bear a large number of antigenic compounds that may frequently change during their life span (Mnati *et al*.,2020).

1.2.10.2. Immune responses during the pre-encystment phase

Little is known about innate immune mechanisms are primed against early stages of hydatid infection, study has suggested the innate immune responses have a crucial role in host susceptibility/resistance to the infection (Vuitton& Gottstein2010). Evidently, various effector mechanisms corresponding to innate and adaptive immune functions are induced against experimentally produced hydatid infection either with eggs (primary infection) or with active onchospheres (secondary infection) in laboratory animals. Perhaps activation of complement system, particularly through its alternative pathway, associates with the host resistance against the infection during the pre-encystment phase. It has been implied that C5- mediated complement reaction followed by activation of inflammatory cells may cause pathologic changes when the embryo is trapped in the harboring organ (Kendall,2014). Infiltration of leukocytes such as eosinophils, neutrophils and macrophages is a hallmark of cellular reaction against invasive larvae of tissue-dwelling helminthes and is detectable after 3-5 days post-infection in mice (Rinaldi *et al*.,2014). Eosinophils seem not to have participation in the host defense against adult *Echinococcus granulosus* (Mezioug & Touil-Boukoffa, 2012).

Both humoral and cellular schemes of adaptive immune system are shown to contribute to the host resistance against developing hydatid cyst, Detectable levels of immunoglobulin (Ig) G are produced against primary infection after 2 and 11 days in mice and sheep, respectively (Wang *et al*.,2019). Activation of neutrophils and macrophages upon presence of IgM and IgG suggests that the antibody-dependent cell-mediated cytotoxicity (ADCC) has likely a pivotal role in depletion of the parasite, as well conducts the pathologic changes during early stages of

the infection (Sutton *et al*.,2019). There is a lack of information about early cytokine production in primary infection of hydatid cyst is insufficient, although it is believed that T lymphocyte profile activated against developing cyst is initially polarized toward Th1 cytokines which is capable to deplete the parasite and provoke inflammatory changes in the tissue(He *etal*.,2021).

1.2.10.3. Immune response during the encystment phase

Host immune reaction to the established cyst has received more attention for investigation about immunopathology of cystic echinococcosis. As soon as the embryo is lodged in a suitable organ, it starts to develop into hydatid cyst with complete endocyst and ectocyst (Zhang *et al*.,2012). In both animal and human host, humoral immune reaction against the established cyst is characterized by presence of circulating IgM, IgG1 and IgG4, and IgE (Díaz *et al*.,2011). Animal models challenged with either parasite eggs or active protoscolices initially show lower levels of expressed IgG subclasses, however gradual increase in antibody response is observed along with the cyst growth (Feng *et al*.,2013). Expression of IgM, IgG1, and IgG3 may induce complement activation and suggests the role of innate immune mechanisms during the early stage of encystment phase,Although complement factors are not clearly shown to have contribution to the host defense against established cysts, their activation has been indirectly confirmed (Sharma *et al*.,2020). Complement effector molecules such as C3 and C4 were also measured in sera obtained from hydatid patient and remained detectable after surgery (Barrios *et al*.,2019).

The established cyst is thought to induce activation of different immune effector cells in the intermediate host (Weatherhead et al.,2020).

Cytokine profiling assays have demonstrated the presence of IL-2, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-4, IL-5, ,IL12 and IL-17 in the sera obtained from animals with the primary or secondary infection and from human patients (Magatti et al.,2018; (Fereig& Nishikawa,2020). This cytokine may associate with the parasite survival and its production reclines after successful treatment but remains high in patients who does not respond to chemotherapy (Naik *et al*.,2015).

1.2.11. *E. granulosus* and Cytokine Induction :

Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells (Cassatella *et al*.,2019). Cytokine is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes) (Cameron& Kelvin, 2013). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action)(Scully *et al*.,2017).

There are both pro inflammatory cytokines and anti-inflammatory cytokines, There is significant evidence showing that certain cytokines/chemokines are involved in not only the initiation but also the persistence of pathologic pain by directly activating nociceptive sensory neurons(Ramesh *et al*.,2013). Certain inflammatory cytokines are also involved in nerve-injury/inflammation-induced central sensitization, and are related to the development of contralateral hyperalgesia/allodynia. (Eguchi *et al*.,2018).

E. granulosus that encounter the immune system can influence the differentiation decision. Th1 and Th2 cells are not precommitted phenotypes but rather, represent endpoints of a multistep differentiate process, whereby a common precursor population acquires a distinct cytokine secretion profile (Jankovic *et al*.,2001). During CE, the evidence concerning antibody levels of IgG4 and IgE isotypes and frequent eosinophilia, suggested that the immune response to established *E. granulosus* infection is Th2 dominated and that *Echinococcus* antigens modulate polarized T cells (Siracusano *et al*.,2008).

The IL-17A is a key pro-inflammatory cytokine in the T helper 17 pathway and it plays a critical role in host defense and inflammation. Evidences highlighting crucial role of cytokines in the host-parasite relationship come from studies on parasite-driven cytokine production in a large number of albendazole-treated patients with CE (Mévélec *etal*.,2020)

The T cell lines from a patient with an inactive cyst had a Th1 profile whereas T cell lines derived from patients with active and transitional cyst had mixed Th1/Th2 and Th0 clones (Farid& Amir ,2015) . Since PBMC from seronegative patients produced no parasite antigen driven- IL-5 and scarce IL-4 and IL-10(Della Bella *et al.*,2017).

Study of Li *et al*(2020) indicated that in CE a strong Th2 response correlates with susceptibility to disease (active cyst) whereas a Th1 response correlates with protective immunity (inactive cyst) and that Th1 and Th2 responses coexist .

1.2.11.1. Discovery and expression of IL-17 cytokines.

1.2.11.1.1. IL-17 (IL-17A)

Interleukin 17 (IL–17) also referred to as IL-17A, was discovered in a search for T-cell-derived molecules with immune functions. It was cloned from a T cell hybridoma produced by fusion of a mouse cytotoxic

The human IL-17 gene was originally mapped on human chromosome 2q31 (Basu *et al*.,2013). however, it has also been located in a sequence from a chromosome 6p12 clone (Tsai *et al*.,2013). The gene encodes a 20–30 kDa protein of 155 amino acids (Matsuzaki & Umemura, 2018).

The IL-17 polypeptide comprises at least one N-glycosylation site and six cysteine residues that form intermolecular interactions during dimerization. It has a 19-amino-acid signal sequence followed by a 136-amino-acid mature portion (McGeachy *et al*., 2019).

Sources of IL-17 appear to be rather restricted; expression of IL-17 has been detected mainly in activated CD4+ and CD8+ T lymphocytes (predominantly of the memory CD45RO+ subset) , Later studies detected the presence of IL-17 messenger RNA (mRNA) transcripts also in neutrophils and eosinophils (McGeachy *et al.*, 2019).

The IL-17 family and its receptors, which share minimal homology with other cytokines or known proteins, have been recognized as a distinct cytokine-receptor family and is crucial for normal host immune responses; this family is associated with many human pathogeneses, including those of inflammation and cancer (Lin & Leonard, 2018).

IL-17A, the prototypic member of this family, was first identified in 1993 (Rouvier *et al*., 1993) and named CTLA8. It was subsequently

renamed IL-17, and more recently, IL-17A. it is a 35-kDa, disulfide-linked, homodimeric protein with variable glycosylation (Morita *et al* .,2015; Ghanemi *et al*.,2020).

1.2.11.1.2.Interleukin 17 B (IL-17 B)

Interleukin17B was originally identified as a proinflammatory mediator that accelerates neutrophil recruitment and migration (Ge *et al*., 2020) ,IL-17B inhibits IL-25 signaling and attenuates mucosal inflammation .Although low IL-17B mRNA is detected in several organs, its expression is high in chondrocytes and neurons (Alves *et al.*,2018). Like IL-17E, IL-17B binds to IL-17RB with lower affinity than IL-17E. (Chan , 2019); however, the signal transduction mechanisms of IL-17B-IL-17RB are unknown. Both IL-17B and IL-17C induce TNF and IL-1b expression from a monocytic cell line and cause neutrophil infiltration (Cao *et al*.,2019).IL-17B, IL-17C, and IL-17D may have similar activity to induce inflammatory mediators, and contribute to inflammatory responses like IL-17A and IL-17F. Future experiments using cytokine-blocking Abs or cytokine gene-targeted mice may help to understand the functions of these cytokines in immune responses(Hadian *et al*.,2019).

Th1 and Th17 cells may have roles in clearing the parasites; however, with the extension of infection time, particularly after 3 months, Th2-type cytokines may begin to inhibit Th1-cell proliferation and immune response (Zheng , 2013). Thus, Th1 cells were downregulated, and the high levels of IL-17 secreted by Th17 cells resulted in a strong immune pathological injury of the host liver(Monin& Gaffen,2018).

Lechner *et al* .(2012), described how proper immune regulation, in response to an CE infection, depends on IL-17 regulation in pro-inflammatory immune responses , This can promote parasite tissue

infiltration and long-term growth *in vivo*, and can result in immune escape in a host of echinococcosis.

1.2.11.3. IL-17 Receptor and Its Signal Transduction:

The IL-17 cytokines bind to five cytokine receptors (IL-17RA to IL17 E-) on target cells to drive their biological actions , IL-17R is expressed in a variety of cell populations, including keratinocytes, fibroblasts, mesothelial cells, epithelial cells, and leukocytes (Xiang *et al*.,2020). IL-17RA is a shared receptor for different IL-17 isoforms. IL-17 cytokines can trigger signals via an IL-17RA/IL-17RC receptor complex (Fabre *et al*., 2018).. IL-17RB and IL-17RE serve as the specific receptors for IL-17B and IL-17RA/IL-17RB heterodimeric complex, respectively (Stevens *et al*.,2018). IL-17A and IL-17F act through the same IL-17RA/IL-17RC receptor complex, Studies suggest that IL-17RD also drives IL-17-mediated signaling, but the ligand of IL-17RD remains unknown(Bosmann *etal*.,2013).

1.2.12. Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are mostly biallelic point mutations, present within a population in a frequency higher than 1%. SNPs are also believed to be the main source of variability among humans, especially when they influence gene expression or function depending on their location in the DNA sequence. Moreover, since SNPs are relatively easy to be detected, they are considered as one of the best biological markers in association or case-control studies. Therefore, a large number of SNPs in cytokine loci have been described and studied in complex illnesses like infectious and autoimmune diseases and cancer (Pacheco & Moraes, 2009).

Single-nucleotide polymorphisms (SNPs) and single-nucleotide mutations result from the substitution of only a single base. The SNP or mutation can be relevant to disease susceptibility, pathogenesis of disease, and efficacy of specific drugs. It is important to detect SNPs or mutations clinically (Matsuda, 2017).

The promoter region SNPs affect gene expression by altering promoter activity, transcription-factor binding, DNA methylation and histone modifications (Schirmer e*t al*, 2016). The exonal SNPs affect cancer susceptibility by suppressing gene transcription and translation (Griseri *et al*., 2014). SNPs in intron regions generate splice variants of transcripts and promote or disrupt binding and function of long non-coding RNAs (lncRNAs),(Xiong *et al*., 2015).

SNPs in genes that regulate DNA mismatch repair, cell cycle regulation, metabolism and immunity are associated with genetic susceptibility to cancer (Ulaganathan *et al*., 2015).

The PCR and DNA sequencing have subsequently been used to construct a phylogenetic tree of the genus *Echinococcus*, with intraspecific variation in *E. granulosus* being identified Based on molecular characteristics (Ahmed,2016) .

1.2.13. **Single Nucleotide Polymorphisms (SNPs) and gene expression of IL 17Family**

The gene for human IL-17 is located in the human chromosome and have 1874 base pairs long (Wang *et al*., 2014).

Single nucleotide polymorphisms (SNPs) serve as important mutations that can affect transcription and translation. Numerous studies have reported the associations of IL-17A and IL-17F polymorphisms and

susceptibility to digestive system neoplasms, however, the results were not consistent. The IL-17A rs2275913 and IL-17F rs763780 polymorphisms were associated with susceptibility to digestive system (Gao *et al*., 2019).

Several studies have reported that IL-17 levels triggered in autoimmune diseases and elevated significantly in patients with rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, and autoimmune hepatitis (Liu *et al*., 2018).

Since then, some studies have reported the association of variation in species/genotypes of *E*. *granulosus* are reflected in morphological and biological characteristics of the parasite and it can influence the life cycle pattern, host specificity, development rates, pathogenicity, treatment, transmission dynamics, epidemiology and finally control of CE (Li *et al.*, 2015).

1.2.13.1. The types of SNPs

1-SNPs in non-coding regions :

Can manifest in a higher risk of cancer (Li *et al*., 2014), and may affect mRNA structure and disease susceptibility (lu *et al*., 2015). Non-coding SNPs can also alter the level of expression of a gene, as an eQTL (expression quantitative trait locus).

2-SNPs in coding regions:

A-synonymous substitutions by definition do not result in a change of amino acid in the protein, but still can affect its function in other ways. An example would be a seemingly silent mutation in the multidrug resistance gene 1 (MDR1), which codes for a cellular membrane pump that expels drugs from the cell, can slow down translation and allow the peptide chain to fold into an unusual conformation, causing the mutant

pump to be less functional (in MDR1 protein (Kimchi-Sarfaty *et al*., 2007).

B-nonsynonymous substitutions:

1-missense – single change in the base results in change in amino acid of protein and its malfunction which leads to disease (e.g. c.1580G>T SNP in LMNA gene – position 1580 (nt) in the DNA sequence (CGT codon) causing the guanine to be replaced with the thymine, yielding CTT codon in the DNA sequence, results at the protein level in the replacement of the arginine by the leucine in the position 527(Al-haggar *et al*., 2012) at the phenotype level this manifests in overlapping mandibuloacral dysplasia and progeria syndrome).

2-nonsense – point mutation in a sequence of DNA that results in a premature stop codon, or a nonsense codon in the transcribed mRNA, and in a truncated, incomplete, and usually nonfunctional protein product (e.g. Cystic fibrosis caused by the G542X mutation in the cystic fibrosis transmembrane conductance regulator gene) (Cordovado *et al*., 2012).

2. Materials and Methods

2.1.materials .

2.1.1. Laboratory Equipment and Instruments :

The Laboratory equipment and Instruments which used in this study are listed in the table (2-1).

Table (2-1): Laboratory Equipments and Instruments.

No.	**Equipments**	Company	Origin
1	Biological safety cabinet	Thermos scientific	Germany
3	Distillatory	Gallenkamp	England
4	ELISA reader	BioTek	USA
5	Gel electrophoresis	Cleaver	UK
6	Glass EDTA tubes 10 ml	Xinle	China
7	Glass gel tubes 10 ml	Xinle	China
8	Laboratory Centrifuge	Hettich	Germany
9	Medical cotton	Kardelen	Turkey
10	Medical injection syringes	MEDECO	UAE
11	Microcentrifuge tubes 1.5 ml	BIOBASIC	Canada
12	Microcentrifuges	Hettich	Germany
13	Micropipettes + tips	Slamed	Germany
14	PCR system/ Conventional	Cleaver	USA
15	Refrigerator	Kiriazi	Egypt
16	Thermocycler	Clever, BIO - RAD	UK, USA
17	Tourniquet for blood	Xinle	China

18	UV – trans-illuminator	Herolab	Germany
19	Vortex, Micro spin Centrifuge	My Fu gene	China
20	water bath	Memmert	Germany

2.1.2. kits :

The kits which used in this study with their companies and countries of origins were listed in table (2-2).

Table (2-2) :Commercial kits used in the present study

No.	Kits	Company	Country
1	**Genomic DNA extraction kit** - 2 ml collection tube -elution buffer -GS columns -GSB buffer -GST buffer -proteinase K -W1buffer -Washing buffer -Absolute Ethanol 99% (not supported by kit)	Geneaid	Taiwan
2	**ELISA kit included :** • IL17 A serum components and Quantity: • Standard Solution (1280ng/L) 0.5ml x1	Bioassay technology laboratory	China

	• Pre-coated ELISA • Plate 12 * 8 well strips x1 • Standard Diluent 3ml x1 • Streptavidin-HRP 6ml x1 • Stop Solution 6ml x1 • Substrate Solution A 6ml x1 • Substrate Solution B 6ml x1 • Wash Buffer Concentrate (25x) 20ml x1 • Biotinylated human IL-17A Antibody 1ml x1 • User Instruction 1 • Plate Sealer 2 pics • Zipper bag 1 pic		
3	- **IL17 RA serum components and Quantity :** • Standard Solution (9600ng/L) 0.5ml x1 • Pre-coated ELISA Plate 12 * 8 well strips x1 • Standard Diluent 3ml x1 • Streptavidin-HRP 6ml x1 • Stop Solution 6ml x1 • Substrate Solution A 6ml x1 • Substrate Solution B 6ml x1 • Wash Buffer Concentrate (25x)	Bioassay technology laboratory	China

	20ml x1 • Biotinylated human IL17RA Antibody 1ml x1 • User Instruction 1 • Plate Sealer 2 pics • Zipper bag 1 pic		
4	• **IL17B serum Components and Quantity:** • Standard Solution (160ng/L) 0.5ml x1 • Pre-coated ELISA Plate 12 X 8 well strips x1 • Standard Diluent 3ml x1 • Streptavidin-HRP 6ml x1 • Stop Solution 6ml x1 • Substrate Solution A 6ml x1 • Substrate Solution B 6ml x1 • Wash Buffer Concentrate (25x) 20ml x1 • Biotinylated Human IL-17B Antibody 1ml x1 • User Instruction 1 • Plate Sealer 2 pics • Zipper bag 1 pic	Bioassay technology laboratory	China
5	**IL17 RB serum :Components and Quantity** • Standard Solution (96ng/L)	Bioassay technology laboratory	china

	0.5ml x1 • Pre-coated ELISA Plate 12 * 8 well strips x1 • Standard Diluent 3ml x1 • Streptavidin-HRP 6ml x1 • Stop Solution 6ml x1 • Substrate Solution A 6ml x1 • Substrate Solution B 6ml x1 • Wash Buffer Concentrate (25x) 20ml x1 • Biotinylated Human IL17RB Antibody 1ml x1 • User Instruction 1 • Plate Sealer 2 pics • Zipper bag 1 pic		

2.1.3. Chemical materials:

The chemical substances that used in the present study were listed in table (2-3).

Table (2-3) : Chemical substance used in the study

No	Chemical substance	Company	Origin
1	Agarose	Bio Basic	China

2	Conventional PCR : PCR pre Mix reaction (master mix) consist of : • Top DNA polymerase (Taq) • Each: dNTPs(dATP, d GTP, d CTP, d TTP) • Tris-Hcl (PH 9.0) • Kcl • Mgcl2 • Stabilizer and tracking dye (pH 8.5)		
3	DNA sequencing for detection of IL-17A SNPs and gene mutation	Macogen	Korea
4	DNA ladder 100-4000bp (Promega) Consist of : • Loading dye has composition: • 15% ficoll, 0.03% bromophenol blue, 0.03% xylene cyanol, 0.4% orange G, 10Mm Tris-HCL (PH7.5) 50mm EDTA.	Bioneer	Korea
5	Ethidium bromide solution (5µg/ml)	Sigma	UK
6	Gel-casting platform and Gel comb	Cleaver Scientific	UK
7	Loading dye	Promega	USA

8	Tris EDTA buffer (TE)	Bio basic	Canada
9	Tris-Borate-EDTA Buffer (TBE)	Bio basic	Canada

2.2. Methods

2.2.1. Study information :

2.2.1.1. study population :

The current study was performed in Al Hilla Teaching Hospital, AL- Imam AL - Sadiq Hospital and private hospitals in Babylon province, during the period from first October 2019 till the end October 2020. This study consists of 93 participants , The age ranged from 20 to 70 years. The patients were divided into two categories, with the first group comprising 53 patient with hydatid cyst who diagnosis by surgion and the second group comprises 40 health peoples,the study's practical part was performed at the microbiology laboratory at the University of Babylon / Medical College.

2.2.1.2. Demographic information :

The demographic information of hydatid disease infected patients and controls, including age, sex ,location of infection , residence , family history of hydatid disease and severity levels by questioning method (Hou *et al*., 2015).

2.2.1.3. Inclusion and Exclusion criteria:

The inclusion criteria was all hydatid disease patients were previously diagnosed by the surgeon (based on the Ultrasound and CT scan repot in addition to clinical examination), while exclusion criteria

was any patients who had any other disease such as Asthma , diabetic Milletus , Any inflammatory diseases , autoimmune diseases and any parasitic infection .

2.2.1.4. Ethical approval :

Verbal consent was obtained from all patients before collection of samples .and its was approved by the committee publication ethics at college of medicine ,Babylon university ,also Scientific Committee approval of Hilla Teaching Hospital and Imam Sadiq Hospital in Babylon province .this study consider case – control study .

2.2.1.5.sample collection:

The blood samples were drawn from all individual by using disposable syringe (5 ml). Five mL of blood was obtained from each subject by vein puncture and slowly pushed into two tube (2.5 mL blood in EDTA- tube for genetic study and 2.5 ml blood in gel tube) . Gel tube of blood was centrifuged at 3000 - 10000 rpm for 10-15 minutes approximately ,then the serum had divided into three parts and stored at -20˚C until analysis.

2.2.2 .Immunological Examination

2.2.2.1. ELISA kit of IL-17A serum level:

1. Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human IL-17A antibody. IL-17A present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human IL-17A Antibody is added and binds to IL-17A in the sample. Then Streptavidin-HRP is added and

binds to the Biotinylated IL-17A antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human IL-17A. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm Figure (2-1).

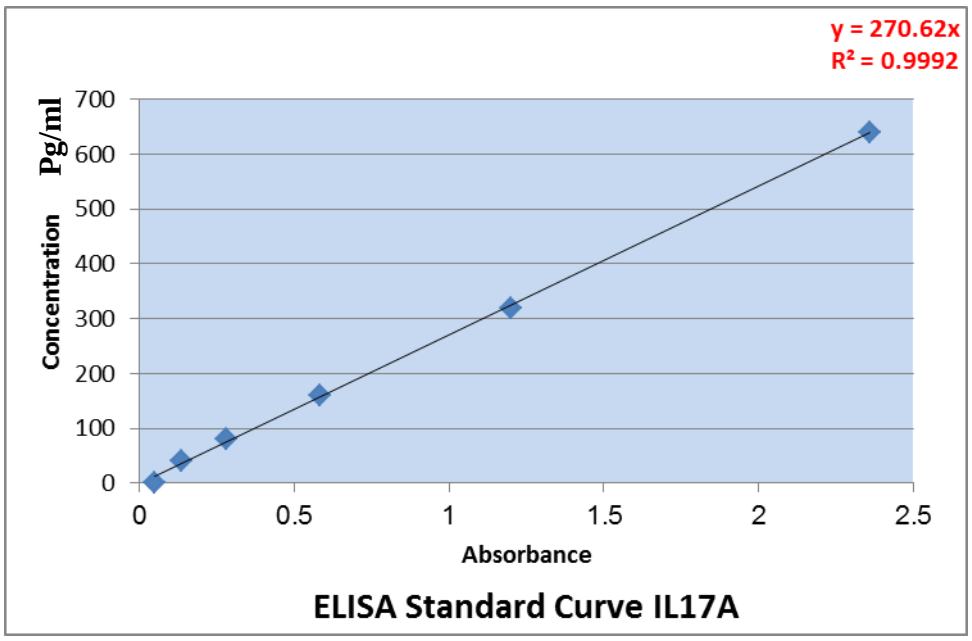


Figure (2-1): ELISA standard curve IL17A.

2.2.2.2. ELISA kit of IL-17RA serum level

The concentration of IL-17RA serum level for both patients and controls was evaluated by using ELISA to determine total IL17RA in the serum.

1. Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human IL17RA antibody. IL17RA present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human IL17RA Antibody is added and binds to IL17RA in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL17RA antibody. After incubation unbound Streptavidin-

HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human IL17RA. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm as in figure (2-2).

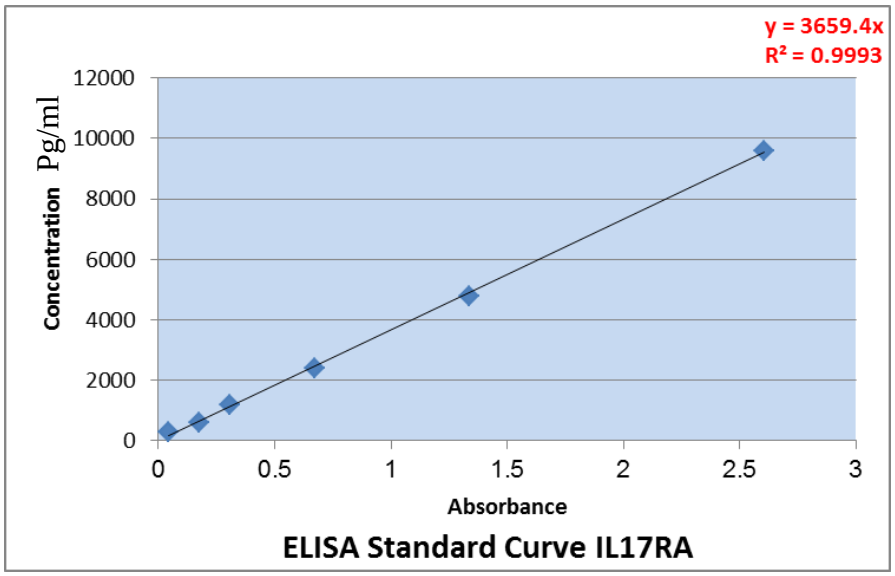


Figure (2-2) : ELISA standard curve IL17RA

2.2.2.3. ELISA kit of IL17 B serum level:

1. Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL-17B antibody. IL-17B present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL-17B Antibody is added and binds to IL-17B in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL-17Bantibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL-17B. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm, Figure (2-3).

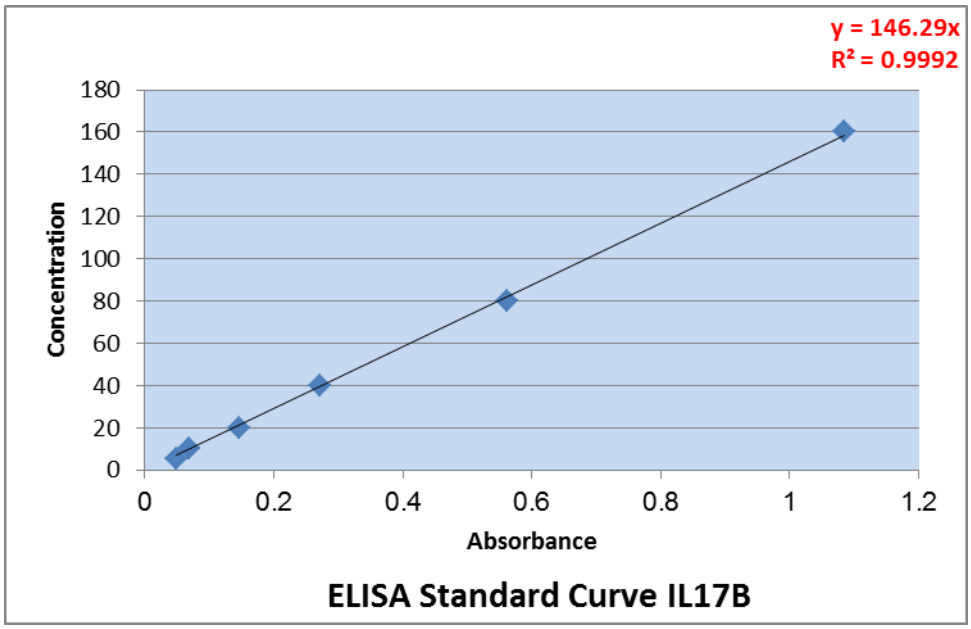


Figure (2-3) ELISA standard curve IL17B.

2.2.2.4 . ELISA kit of IL17 RB serum level: :

1. Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL17RB antibody. IL17RB present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL17RB Antibody is added and binds to IL17RB in the antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL17RB. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. Figure (2-4)

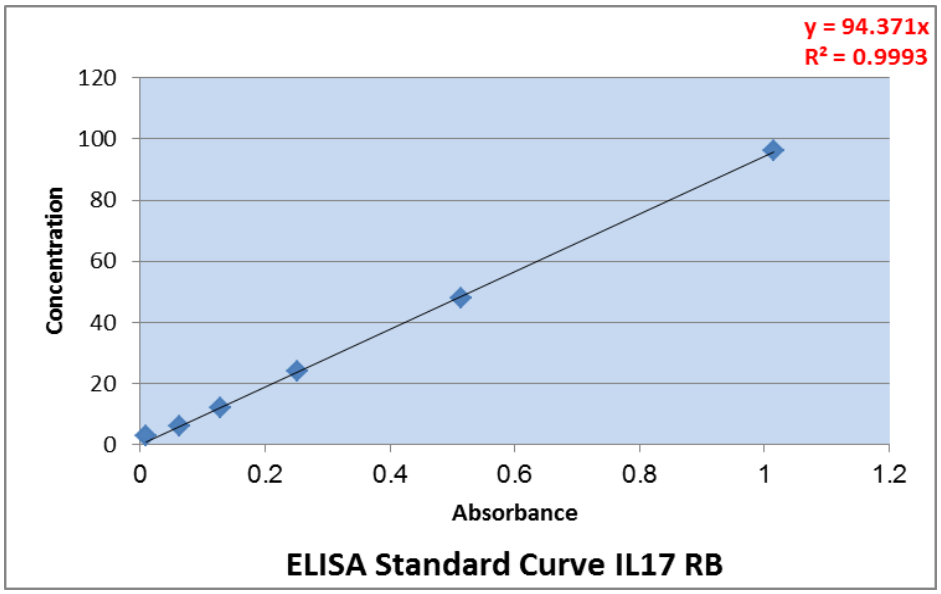


Figure (2-4) : ELISA stander curve IL17RB.

2.2.3 .Assay Procedure (IL17 A,B,RA,RB) kits:

The procedure was achieved according to the method recommended by the manufacturing company bioassay technology Procedure manual:

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips were stored at 2-8°C and add 50µl standard to standard well.

4. Add 40µl sample to sample wells and then add 10µl anti-IL17RB antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells . Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.

8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

2.2.4. Molecular study :

2.2.4.1. DNA Extraction from Human Blood Samples

Whole human blood is extracted according to method provided by the manufacturing company by using the gSYNC™ DNA Extraction Kit and the procedure was achieved according to the method recommended by the manufacturing company (Geneaid) in the user manual of Prep Genomic DNA Mini Kit general protocol for fresh blood samples and frozen blood samples as following :

1-The human blood was collected in an anticoagulant (EDTA) tube

2- Up to 200µl treated whole frozen blood was transferred to a 1.5ml micro centrifuge tube (Eppendorf tube). A volume of 20 µl of proteinase K was added and mix by pipetting. Incubate at 60 ºC for 5 minutes.

3-Avolume of 200 µl of GSB Buffer was added to mixture the mix by shaking vigorously (vortex) then incubated at 60 ºC for 5 minutes. During incubation, inverted the tube every 2 min.

4-Avolume of 200 µl of absolute ethanol was added to the sample lysate and mix immediately by vortex for 10 seconds then transfer all the mixture (including any insoluble precipitate) to the GS column that placed in a 2ml collection tube

5- The mixture Centrifuged at 14000 rpm for 1 minutes and was removed the supernatant completely. The 2ml collection tube was discarded and replaced with a new 2ml Collection tube.

6-The GS Column was washed with 400 µ l W1 Buffer and Centrifuged for 30 seconds at full speed (14,000 rpm) and discarded the flow-through.

7-The GS Column was placed back in the 2ml Collection tube and washed with 600µl Wash Buffer (ethanol added) and Centrifuged for 30 seconds at full speed (14,000 rpm) and discarded the flow-through.

8- The GS Column was placed back in the 2ml Collection tube. A Centrifugation was achieved for an additional 3 min at full speed (14,000 x g) to dry the column.

9-The dry GS Column was placed in a new 1.5ml microcentrifuge tube.

10- A volume of 50-100µl of Elution Buffer (Preheated at 60ºC) was added to the membrane center of GS Column and Stand for 3~5 min or until the buffer is absorbed by the membrane then Centrifuged for 30 seconds at full speed (14,000 x g) to elute the DNA

11-The DNA was stored at -20ºC until used for the PCR procedure .

2.2.4.2. Genomic DNA examination

The extracted DNA was checked by using Nanodrop spectrophotometer (THERMO.USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm).

2.2.4.3. The principle of Polymerase Chain Reaction (PCR).

Based on the *IL17A, IL17 B, IL17RA,* and *IL17 RB* genes in isolates, PCR method was used to detect and genotype *E.granulosus* hydatid cyst. The method outlined by Nikmanesh and Mirhendi (2014).was used to carry out this technique .

2.2.4.4.PCR Amplification

2.2.4.4. 1. Primer Design

The oligonucleotide primers for the studied SNPs (rs375208, rs879576 and rs1025689) were designed in this study according to an existing Gen Bank sequences for *IL17* genes at National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). These sequences used to design *SNP's* forward and reverse primers by the Primer 3 software (Rozen and Skaletsky, 2000). In exception of rs2275913, which obtained from previous study by Valverde-Villegas, *et al*., (2017). Oligonucleotide primers were synthesized by Macrogen company (Korea). All SNPs used in the present study were summarized in table (2-4). PCR amplification.

Table (2-4) primers upstream and downstream for each patients and controls DNA templates of IL17A,B and IL17RA,IL17RB gene

Gene	SNP ID	Dir.	Primer Sequence	PCR product (pb)	Reference
IL-17A	rs2275913	Forward Reverse	GCCAAGGAATCTGTGAGGAA TTCAGGGGTGACACCATTTT	328	Valverde-Villegas *etal*., 2017
IL-17B	rs375208	Forward Reverse	GCCCAGGAGTTGAAGATCAG CATCCTCCTTGCACCTTTGT	467	New design
IL17RA	rs879576	Forward Reverse	TGCAGTGACCCCAGTAAACA AGGTCTCCTTGTTGGGTGTG	549	New design
IL17RB	rs1025689	Forward Reverse	TTTGGCCTTAGCAGGAAGAA TTGGGCCTTATCTCTGATGG	465	New design

2.2.4.4.2. PCR master mix preparation.

PCR master mix was prepared by using (Maxime PCR PreMix Kit) and this master mix done according to company instructions as a, (Promega, USA).

Taq Green Master Mix Kit that containing all other components which needed to PCR reaction such as (Taq DNA Polymerase is supplied in 2X Green GoTaq Reaction Buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM MgCl2). Green GoTaq Reaction Buffer is a proprietary buffer containing a compound that increases sample density, and tracking dye(yellow and blue dyes), which function as loading dyes when reaction products are analyzed by agarose gel electrophoresis. The blue dye migrates at the same rate as 3–5kb DNA fragments, and the yellow dye migrates at a rate faster than primers.and this master mix has done according to company instructions as following: 3 µl of DNA template, 1.5 µl of gene forward primer, 1.5 µl of gene reveres primer and 13 µl of PCR water.

2.2.4.4.3 . PCR Thermocycler Conditions:

The PCR thermocycler conditions were done by using conventional PCR thermocycler system as following steps: the Initial Denaturation at 95 ºC for 5 minute and one cycle, (Denaturation at 94 ºC for 1 minute , Annealing at 55 ºC for 1 min (rs2275913), 56 °C for 1 min (rs375208), 58 °C for 1 min, (rs879576) 54 °C for 1 min , (rs1025689), Extension 72 ºC for 1 minute, and these 3 steps repeated 35 cycle, then the final extension at 72 ºC for 10 minute and one cycle, finally the hold steps at 4 ºC.

2.2.4.4.4. Assay procedures of conventional PCR

This procedure was carried out according to (Lorenz, 2012) as follow:

1- A volume of 12.5 µl of the Promega master mix was added to each reaction tube to reach final volume (25µl).

2- A volume of 1.5 µl of forwarding primer was added to the master mix tube.

3- A volume of 1.5 µl of reverse primer was added to the master mix tube.

4- A volume of 3 µl of genomic DNA was added to the master mix tube

5- A variable volume of free nuclease water was added to the master mix tube to reach final volume 25 µl.

6- All the components were mixed by spin the tubes to avoid the components attached to the wall of PCR tube.

7-The master mix tube was placed in a thermal cycler.

8- The PCR mixture tube was amplified for 35 cycles

9-The PCR product was removed from thermal cycler, a volume of 5 µl of PCR product was loaded on electrophoresis to ensure the presence of amplicons bands and the remaining 20 µl was sent to the Macrogen company in South Korea to read the DNA sequencing of amplicons.

2.2.4.5. IL-17 A,B,RA,RB genes amplification for PCR analysis:

The PCR amplification of DNA was carried out in final reaction mixture volume of 25 µl and within 35 cycles. Products of PCR amplification were electrophoresed by 1.5% agarose gel and then

visualized under UV-trans illuminator after staining with ethidium bromide at 100 v for 60 minutes. PCR mixtures and PCR conditions of this assay was summarized in Table (2-5).

Table (2-5): Uniplex PCR mixtures and conditions for identification of *IL17* SNPs.

PCR mixtures		PCR conditions		
Contents	Volume	Type of cycle	Condition	No. of cycles
Master Mix[a]	12.5 µl	Initialization	95 °C for 5 min	1
Forward Primer	1.5 µl	Denaturation	94 °C for 1 min	35
Reverse Primer	1.5 µl	Annealing	55 °C for 1 min (rs2275913) 56 °C for 1 min (rs375208) 58 °C for 1 min (rs879576) 54 °C for 1 min (rs1025689)	
Template DNA	3 µl	Extension	72 °C for 1 min	
Nuclase-Free Water	6.5 µl	Final Extension	72 °C for 10 min	1

a, GoTaq® Green Master Mix (Promega, USA)

2.2.6. Genotyping and SNP Selection

2.2.6.1. Agarose Gel Electrophoresis:

The PCR products of *IL17* genes were analyzed by agarose gel electrophoresis following steps:

1- 1.5% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then 3µl of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each combs well and 5µl of (100bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80 AM for 60 minutes.

5- PCR products were visualized by using UV trans illuminator

2. 2.7. Sequencing of PCR products:

All PCR products obtained above were purified and submitted for sequencing as follows. The PCR product was cleaned of amplification primer using the Gel/PCR DNA Fragments extraction kit (Geneaid, USA) as per manufacturer's instructions. Purified DNA was sequenced at Macrogen company (Korea) with the sequencing primers for each gene as outlined in Table 2-4. Sanger sequencing method was carried out on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Because of the DNA sequencing method consider the gold standard for SNPs detection in IL-17gene (Teräsjärvi *et al*., 2017).

2.2.8. Bio informatics and Statistical analysis:

The raw Sequence data was trimmed and aligned to the control sequences. The standard sequences for alignment were taken from GenBank sequences for IL17 genes at NCBI (http://www.ncbi.nlm.nih.gov). Multiple alignments were done by using Clustal W v2.0 (Thompson, *et al.* 1994) of Geneious Prime Software V2021.1 (Biomatters, Inc., North America) to identify SNPs, Allele frequency and genotypes. All other Bioinformatic and Statistical analysis were done according to Xavier (Solé, *et al.* 2006).

The statistical for serological IL17 family used Package of Social Sciences (SPSS) version 23 (Inc., Chicago, IL, USA) computer software was used for results analysis. Mean , stander error (SD) and correlation was used to calculate value significant. The level of statistical significance was set at alpha equal to 0.05 ($p = 0.05$).

3-4 . Molecular study :

As for any infectious disease, control of hydatid disease requires a clear species-level classification and genetic identification of the species, genetic factors and the immunologic response have been suggested to determine the susceptibility against the infection and the outcome of hydatid disease. In the present study, we analyzed four IL17 genetic variants (rs2275913, rs375208,rs879576 and rs1025689) regarding the predisposition to *E. granulosus* infection and the development of chronic hydatid disease in different patients .

3-4-1- Detection and genotyping of IL-17A (rs2275913) gene polymorphism :

From 53 positive case we selective (40) confirmed patients and the (30)control subject were submitted to PCR for the detection of IL-17A by using specific primers, the visualization of their amplicons on gel electrophoresis revealed that the product size was 328 bp as shown in figure (3-7).

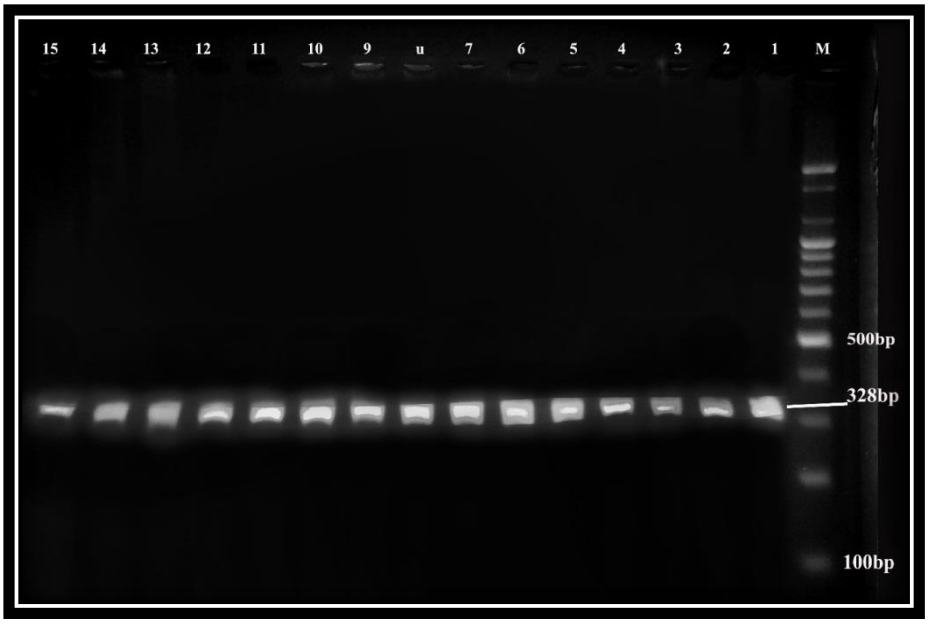


Figure (3-7): Agarose gel electrophoresis of PCR product obtained with rs2275913-specific primer(1.5% agarose gel, 328bp). lanes 1-15 represent the identified IL-17A genes, Lane M represent 100bp DNA ladder.

The results in this study showed that a total of (40) sample of patients and (30) sample of controls were positive for IL17A detection by PCR amplification method and all isolates having this gene by using PCR technique with specific primers when compared with ladder.

According to present study on interleukin-17 investigation, it was explained to us that the PCR technology has accuracy, specialized and high sensitivity as well as it considered the gold standard methods, and this is also indicated by previous studied (Michov, 2020).

In addition to environmental factors facilitating infection with the parasite, genetic constitution of hosts seems to play a crucial role in acquiring the infection and developing disease signs and symptoms, An appropriate example would be the exposure of many individuals to the parasite, with only some of them manifesting illness post exposure(Batool *et al*., 2017).

The results of genotyping for (40) patients and (30) controls subjects for the amplicon of the PCR product as achieved by sequencing for primers IL-17A,the genotype and allele frequencies IL17A(rs 2275913) polymorphism were compared between patients with hydatid disease and control group ,it shown in table (3-8). The *IL-17A* (rs2275913) gene polymorphism frequency of the G allele carriers was significantly increased in hydatid cyst patients than that in healthy controls (38 , 29%) respectively , These results suggested that G allele might play a protective role against hydatid cyst for IL-17A(rs 2275913).

Table (3-8): IL17 A SNP distribution frequencies in the screened population (Control and Patients).

SNP	Allele	Frequency	Controls	Patients	*OR (95% CI)
rs2275913 IL17 A	**G**	68 (0.96)	29 (0.97)	38 (0.95)	1.526 (0.132-17.663)
	A	2 (0.04)	1 (0.03)	2 (0.05)	
	P value	<0.0001*	<0.0001*	<0.0001*	
	Genotypes				
	G/G	32 (0.91)	14 (0.93)	18 (0.9)	1.00
	G/A	3 (0.09)	1 (0.07)	2 (0.1)	1.09 (0.08-14.660
	A/A	0 (0)	0 (0)	0 (0)	----
	P value	<0.0001*	<0.0001*	<0.0001*	

*** represent a significant difference at p<0.05.**

*** OR: Odd ratio, CI: Confidence interval**

The G and A alleles of rs 2275913 was more common in patients with hydatid disease than in healthy individual ($p \leq 0.0001$) also table (3-8) showed that the homozygous (GG) genotype have frequency (0.9) in hydatid disease patients in compared with (0.93) in healthy individual, whereas the heterozygous (GA) was found to be (0.1) in hydatid disease and (0.07) in control .this results showing the most patients and control were homozygous for the mutant type G allel ,however ,there were no significant differences in the genotype and allele frequencies between

patients and control (p= 0.095) .while there are significant difference between GG and GA in hydatid patients and control ($p \leq 0.05$).

According to current results showed that The IL-17A (allele A) was insignificantly difference associated with susceptibility to *E.granulosus* infection. Individuals with two A alleles (homozygous for the AA) were not significantly represented among the patients with *E.granulosus* 0(0%) p-value=0.0001, as compared with healthy control subjects, 3(8%) the Individuals with homozygous for the GG had a increased risk of developing hydatid cyst infection than other two genotypes.

The Polymorphisms in IL-17 cytokines alter the activity of interleukins and may alter cytokine function, thus, dysregulating IL-17 expression (Hussein& Ali , 2020).

Because IL-17 is assumed to be a key pro-inflammatory agent, several investigations of IL-17 SNPs and susceptibility have focused on inflammation (Bianchi & Rogge, 2019).

These studies have brought to the forefront many genes linked to signaling pathways that were not previously known to be involved in pathogenesis, pointing to new directions in the study of disease mechanisms, Genome-wide association studies also provided fundamental evidence for a key role of the immune system in the pathogenesis of these diseases, because many of the identified loci map to genes involved in different immune processes (Ellinghaus *etal* .,2016). However, the mechanisms by which disease-associated genetic variants act on disease development and the targeted cell populations remain poorly understood (Bianchi & Rogge,2019).

Various molecular investigations for genetic identification of *E. granulosus* in endemic places around the world have been done. Other study take , 32 human isolates were submitted to genetic identification using PCR-RFLP and sequencing of the 12s rRNA gene. The majority of the isolates [26/32] belonged *to E. granulosus* , while the G6/G7 and G5 geno-types represented five and four isolates, respectively. (Mogoye *etal*.,2013) , which near contradicts to the present findings.

This observed that IL-17 is a pleiotropic pro-inflammatory cytokine which enhances T cell priming and stimulates epithelial, endothelial and fibroblastic cells to produce multiple pro-inflammatory mediators, including IL-1, IL-6, TNF-α and chemokines(Kaur *etal*.,2018). IL-17 has been found to be associated with the pathogenesis of a wide range of inflammatory and autoimmune diseases , During inflammation, IL-17A are found to mediate pro-inflammatory responses (Zhong *et al*., 2021). Importantly, IL-17A must be considered within the context of the local microenvironment, because it acts synergistically or additively with other pro-inflammatory cytokines, including TNF5, Properties of IL-17 are largely dependent on the environment in which it is produced(Choi *et al*., 2019), In agreement with some previous studies our results indicated that polymorphism of *IL-17A*.

In particular, the rs2275913 SNP, produced by a substitution of the G by an A nucleotide base in the IL-17A gene promoter, is significantly associated with a vast number of diseases(Duan *et al*., 2014) It has been reported that allelic variants of the rs2275913 SNP differentially bind the transcription factor NFAT, leading to differences in IL-17A secretion.

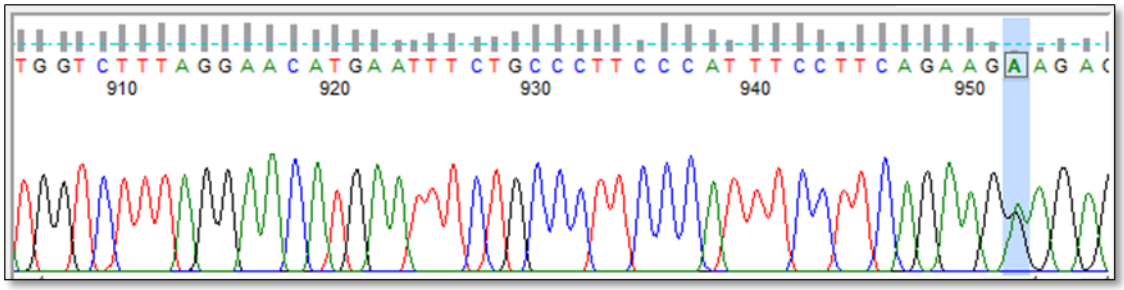


Figure (3-8): DNA sequencing chromatograms of(rs2275913) in the *IL17A* gene SNPs.

Observed that, within the hydatid disease population, individuals that carry the GG genotype displayed the highest levels of IL-17A in plasma, show in table (3-9) .

Table (3-9) rs2275913 SNP distribution frequencies in the screened population according to Sex of rs2275913. As shown in this table, hydatid disease patient carrying the GG + GA genotypes of this variation had a significantly higher in female than in male (38.90 ±0.61) for OR(odd ratio (95% CI). as compared with those with the AA genotype. In hydatid disease subjects, significant difference was P value (0.305) found in three parameters only GG and no significant between GA +AA genotypes of rs2275913.

Table (3-9): rs2275913 SNP distribution frequencies in the screened population (Control and Patients) according to Sex

Genotypes	Sex	Controls	Patients	*OR (95% CI)	P value
GG	Female	6 (0.43)	11 (38.9)	1.00	0.305
	Male	8 (0.57)	7(0.61)	0.48 (0.12-1.98)	
GA	Female	1(0.2)	2(0.4)	1.00	N.S
	Male	0	0	---	
AA	Female	0	0	---	N.S

	Male	0	0	---	

* **OR: Odd ratio, CI: Confidence interval**

This method concentrates on locating a correlation between hydatid disease and sequence variants in or near biologically defined candidate genes chosen for their known physiological function By comparing the frequency of these or other nearby variants in HC patients and control subjects, the significance of these or other nearby variants is determined, In the early stages of the infection, the IL-17A is a crucial cytokine secreted by a wide range cell types such as Th17, B cells, innate lymphoid cells, CD4+, CD8+, gamma-delta T and invariant NKT10-13,32. The rs2275913, which was associated with the risk to hydatid disease infection in Iraq population studied, is a functional polymorphism that modifies the binding of the transcriptional nuclear factor of activated T cells (NFAT) in the IL-17A promoter.(Strauss *etal*.,2020).

Several studies showed that IL-17A has an important immunomodulatory role in the chronic phase of the disease(Wen *etal*.,2019) IL-17 expression by Th17 cells and B cells were found in patients with cardiac involvement more frequently, compared to asymptomatic patients, correlating with worse cardiac function.

3-4-2: Detection and genotyping of IL-17RA (rs879576) gene polymorphism.

The results molecular detection of IL17RA showed that all samples had ***(rs879576)*** gene with 549 bp band compared with allelic ladder shown in Figure (3-9) .

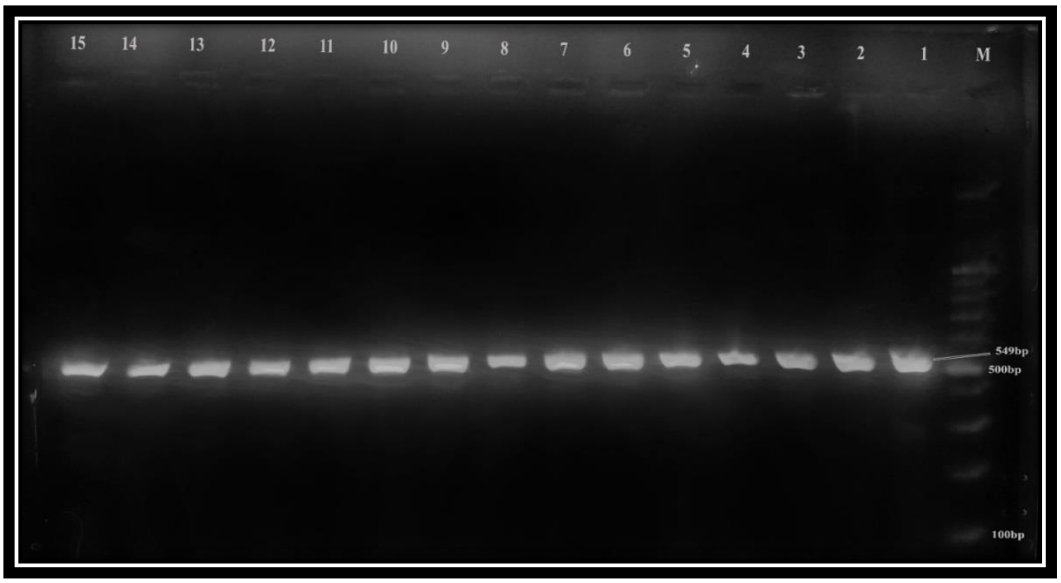


Figure (3-9): Agarose gel electrophoresis of PCR product obtained with rs879576-specific primer(1.5% agarose gel, volume of 549 pb) . (lanes 1-15 represent the identified IL-17RA genes, Lane M represent 100bp DNA ladder.

Originally, *Echinococcus* genotyping relied on the analysis of a short fragment (549 bp), that led to the identification of the *E. granulosus* G1–G3 genotypes (Bonelli *etal*.,2021).

Interleukin 17 Receptor A is a Protein Coding gene, Diseases associated with IL17RA include Immunodeficiency and Chronic Mucocutaneous Candidiasis. Among its related pathways are Cytokine production by Th17 cells in CF (Mouse model). Gene Ontology (GO) annotations related to this gene include interleukin-17 receptor activity.

The results genotype and alleles frequencies of rs879576 is shown in table (3-10) ,the G allele of the rs 8799576 was more common in patients with hydatid disease .than in health individuals (p =0.801 ,odd ratio 1.161,confidence interval CI 0.363-3.713)respectively .so this results revealed that subjects with G allele were more likely to get hydatid disease compared with those bearing the A allele , In addition, all

subjects carrying GG genotype have significantly higher risks of hydatid disease compared with GA genotype ($P < 0.05$).

Table (3-10): rs879576 SNP distribution frequencies in the screened population (Control and Patients).

SNP	Allele	Frequency	Controls	Patients	*OR (95% CI)
rs879576	**G**	55 (0.79)	24 (0.8)	31 (0.78)	1.161 (0.363-3.713)
IL17RA	**A**	15 (0.21)	6 (0.2)	9 (0.22)	
	P value	<0.0001*	0.001*	0.001*	
	Genotypes				
	G/G	20 (0.57)	9 (0.6)	11 (0.55)	1.00 1.19 (0.30-4.73)
	G/A	15 (0.43)	6 (0.4)	9 (0.45)	
	A/A	0 (0)	0 (0)	0 (0)	
	P value	0.398	0.439	0.655	

*** represent a significant difference at p<0.05. *OR: Odd ratio, CI: Confidence interval**

The present study is, the first study investigate a potential influence of IL17A and IL17RA,gene polymorphisms in hydatid disease patients, The action and expression of IL-17RA on the sex has not been evaluated yet. Therefore, expression of IL-17RA in the sex and its role in pathophysiology of hydatid disease should be investigated in the future. In this SNP, the GG, AG and AA genotype frequencies are reported respectively to be 20 (0.57), 15 (0.43) and 0(0) as in table(3-10), the current study that *IL17RA* polymorphisms have the possibilities of

determining the pathophysiology of hydatid disease or expression of hydatid disease phenotypes .Also the expression of rs879576 polymorphism of *IL17RA* gene can influence the onset time of hydatid disease .

Clinical characteristics of the patients and controls of *Echinococcosis granulosus* , plays a central role in inflammation and tissue injury., It also stimulates the production of acute-phase proteins by the liver. These proteins include CRP, serum amyloid A (SAA) and fibrinogen(Reis *et al* ., 2017)

Therefore, it is important to determine systemic markers which may reflect the inflammatory activity in the hydatid disease (Lima *et al*., 2018) According to these results, we think that elevated IL-17RA levels have anti-inflammatory effects by inhibiting the increased levels of pro-inflammatory cytokines in hydatid disease .

The results of the study also showed the clear difference in IL17 RA, in genotype frequency according to sex, rs 879576, which represents IL17 RA GG homozygous , had the highest frequency in patients (0.5 females and 0.2 males, while GA showed (0.4 for females and 0.1 for males), but AA did not It has an effect on both sexes

Table (3-11): IL17 RA SNP distribution frequencies in the screened population (Control and Patients) according to Sex

Genotypes	Sex	Controls	Patients	*OR (95% CI)	P value
G/G	Female	4(0.4)	7(0.5)	1.00	0.391
	Male	5(0.6)	4(0.2)	0.46 (0.08-2.76)	
G/A	Female	3(0.1)	6(0.4)	1.00	0.519
	Male	3(0.1)	3(0.1)	0.50 (0.06-4.15)	

A/A	Female	0	0	---	---
	Male	0	0	---	

* **OR: Odd ratio, CI: Confidence interval**

Dominant and recessive model also demonstrated the similar results that G allele of rs2275913 and rs 879576 increase the risk of hydatid disease .

Uncontrolled inflammation has been widely accepted as a hallmarker of hydatid disease ; however, the regulation of genes within the inflammatory pathways is not well understood yet, any changes in structure and expression, may be due to genetic variations, might affect cytokine production and hydatid disease development. A common type of genetic variation in the genome is single nucleotide polymorphism (SNP). IL-17 have many SNPs, among which rs879576(A/G) , rs 1025689(C/G) rs2275913 (G>A) and rs375208 (G/A) loci are located in 6p12.1 chromosome and have been shown to correlate with aggressive disease and poorer survival in recent series with inflammatory disease (Xie *et al*., 2019).

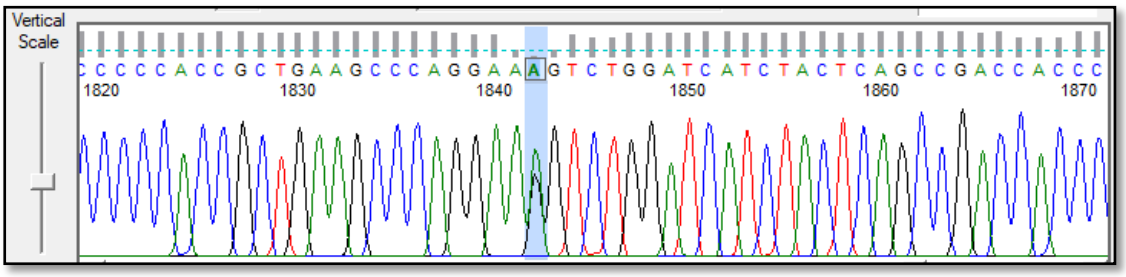


Figure (3-10): DNA sequencing chromatograms of(rs879576) in the *IL17RA* gene SNPs.

The genotypes were determined by direct sequencing. Genomic DNA was amplified using the following primers rs 879576 as in figure (3-10) .

Polymorphism in genes encoding cytokines may influence the level of cytokines production and, consequently, cause different immunological responses to different diseases, Previous studies show that genetic polymorphisms of IL17A and IL17F affect the production of IL-17A and F, respectively (Ding *et al*., 2017). Such polymorphisms have already been associated with autoimmune and inflammatory diseases, as rheumatoid arthritis , periodontitis , cancerand both gastric and breast cancer (Eskandari-Nasab *et al*., 2017).

3-4-3: Detection and genotyping of IL-17B (rs 375208) gene polymorphism.

The amplification of IL 17B gene at region rs375208 appear the present of gene amplicons(467 pb) on electrophoresis gel for all groups of study as shown in figure (3-11),the IL-17B (rs 375208) gene have been shown to association with hydatid disease .

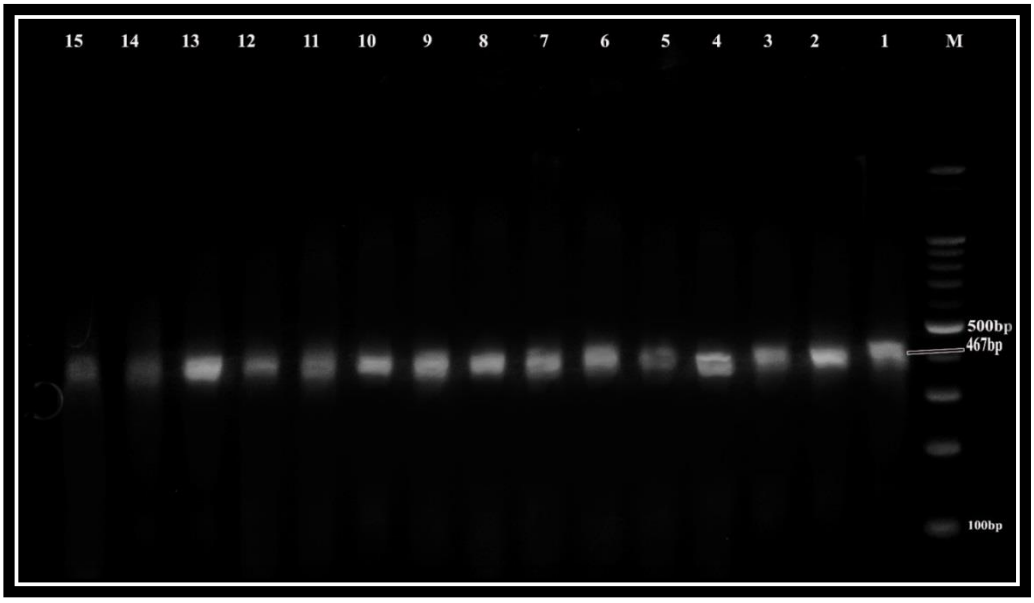


Figure (3-11): Agarose gel electrophoresis of PCR product obtained with *rs375208*-specific primer((1.5% agarose gel, size 467 pb). lanes 1-15 represent the identified IL-17B genes, Lane M represent 100bp DNA ladder.

The *Echinococcus* spp isolates harbour a high degree of internal variation with substantial genetic differences and inter-isolate variation in development in different geographical environments and different hosts. These differences cause variation in propagation dynamics, pathogenicity, antigen-antibody reactions, clinical manifestations and chemotherapy responses between hosts (Rojas *et al*., 2014) . There are a wide range of intermediate hosts showing adaptability to *E. granulosus* (Onac *et al*., 2015). In the long evolutionary process, variations also arise in the mutual adaptation of *Echinococcus* spp. Therefore, studies examining polymorphism in *Echinococcus* spp. might be directly related to the prevention of epidemics and treatment of local hydatid disease (Ma *et al*., 2015).

Genotype association and allele's frequency for patient and control were listed in table (3-12). In case of IL17B gene, since AA is predominant which may represent A allele the wild type and there was higher significant association between patient and control .

Table (3-12): rs375208 SNP distribution frequencies in the screened population (Control and Patients).

SNP	Allele	Frequency	Controls	Patients	*OR (95% CI)
rs375208	**A**	65 (0.93)	27(0.9)	38 (0.95)	0.474(0.074-3.031)
	G	5 (0.07)	3 (0.1)	2 (0.05)	
	P value	<0.0001*	<0.0001*	<0.0001*	
	Genotypes				
	A/A	30 (0.86)	12 (0.8)	18 (0.9)	1.00
	A/G	5 (0.14)	3 (0.2)	2 (0.1)	0.42(0.06-3.02)
	GG	0 (0.000)	0 (0)	0 (0)	
	P value	<0.0001*	0.02*	<0.0001*	

***represent a significant difference at p<0.05.**

* **OR: Odd ratio, CI: Confidence interval**

The identified *Echinococcus granulosus* genotypes isolates were submitted into of NCBI-GenBank, The genotype distribution and relative allele frequencies of rs375208 (A/A), (A/G), and (G/G) polymorphisms at the IL17 B gene in the study subjects are showed in (3-12). All three variants were significant difference at p<0.05.

Results showed the IL-17B genotype was rs375208 gene polymorphism frequency of the G allele carriers was significantly decreased in HC patients than that in healthy controls (2 (0.05)vs. 3 (0.1)%), as shown in Table (3-12). These results suggested that G allele might play a protective role against hydatid disease .

Further analysis showed that individuals carrying the AA genotype and A allele of rs375208 were more likely to have a significantly increased risk of hydatid disease when compared with the AG genotype and G allele. The OR (95% confidence interval) for the AA genotype and A allele of rs375208 were calculated as 0.474 (0.074-3.031) . However, no association was found between AA and GG+AG genotype of rs375208 with hydatid disease . Also, the distribution of genotypes according sex for AG and AA of this SNP were significantly higher in the hydatid patient than in the control, as show in table (3-13) .

Table (3-13): rs375208 SNP distribution frequencies in the screened population (Control and Patients) according to Sex.

Genotypes	Sex	Controls	Patients	*OR (95% CI)	P value
AA	Female	5(0.6)	12(0.86)	1	0.176
	Male	7(0.8)	6(0.43)	0.36 (0.08-1.62)	
AG	Female	2(0.05)	1(0.03)	1.00	0.709
	Male	1(0.03)	1(0.03)	2.00 (0.05-78.25)	
GG	Female	0	0	---	---
	Male	0	0	---	

* **OR: Odd ratio, CI: Confidence interval**

The IL17 B level was linked to the frequency allele A homozygous patients (AA) higher in both sex (0.86 female,0.43 male) Compared with control while GG homozygous no exist in patient for both sex and about (AG) heterozygous The frequency ratio is similar in both sexes(0.03) . The Female patient of the AA genotype of rs375208 variant showed a significantly increased risk for HC (OR = 1% p = 0.176). Compared with the control. As shown in this table, HC subjects carrying the GG genotypes of this variation had no significant difference in any variables as compared with those with the AG genotype.

According to preliminary studies (Dousti *etal*.,2013), G1 was discovered in all people isolated, which was further validated by a study (Spotin *etal*.,2017) in Iran's Ilam area to which all human isolates belonged G1genotype. These results are in disagreement. could be attributed to the sort of samples examined، Geographical differences and the strategy used in order to genotype(Rahi & Ali, 2016).

IL-17B was originally identified as a pro inflammatory mediator that accelerates neutrophil recruitment and migration , IL-17B inhibits IL-25

signaling and attenuates mucosal inflammation (Ge *et al*., 2020). IL-17B promotes the proliferation and survival of cancer cells in animal models (Brevi *etal*.,2020), and increased IL-17B levels are linked to poor outcome in patients with several types of cancers (e.g., breast, lung, and pancreatic) , These cytokines exert their activities through binding to IL-17 receptors (IL-17R, IL-17RA to IL-17RE) that function as homo- or heterodimeric complexes.

DNA sequencing method was performed for genotyping of some positive local *Echinococcus granulosus* hydatid cysts isolates as figure (3-12) for IL17 B gene.

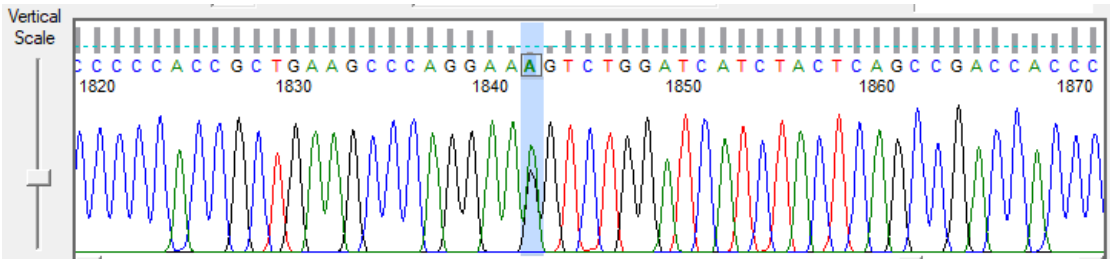


Figure (3-12): DNA sequencing chromatograms of(rs375208) in the *IL17B* gene SNPs.

3-4-4: Detection and genotyping of IL-17RB (rs1025689) gene polymorphism.

Figure (3-13) show the optimized PCR products of the designed primers pair which would be used in rs1025689 genotype , The optimized done by the Uniplex PCR mixtures at (54 °C) Annealing temperature .

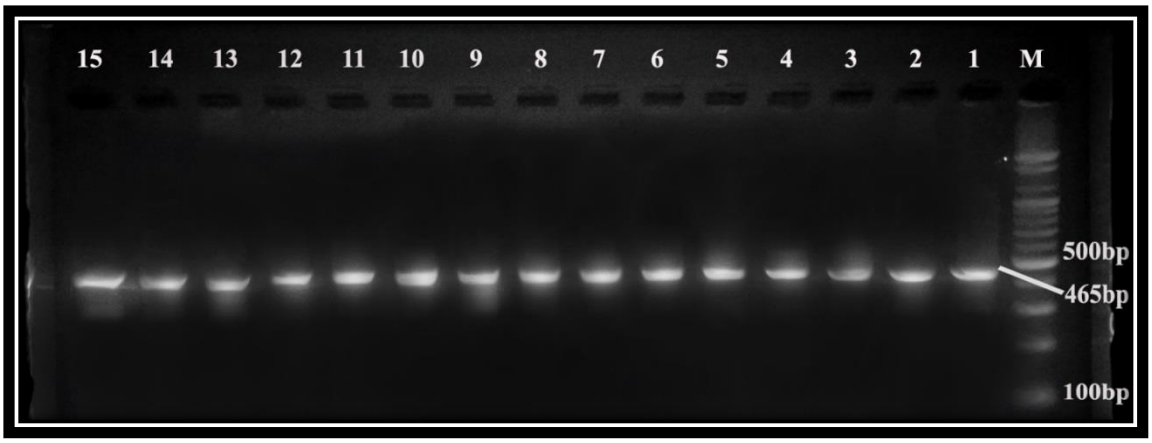


Figure (3-13): Agarose gel electrophoresis of PCR product obtained with *rs1025689*-specific primer(1.5% agarose gel, size 465 pb). lanes 1-15 represent the identified IL-17RB genes, Lane M represent 100bp DNA ladder.

In normal host immunological responses, the IL-17 cytokine family and its receptors play critical functions, Their abnormal expression has been linked to a variety of human diseases, including inflammation and cancer.

Sequence data were analyzed using sanger method as show in figure (3-14).

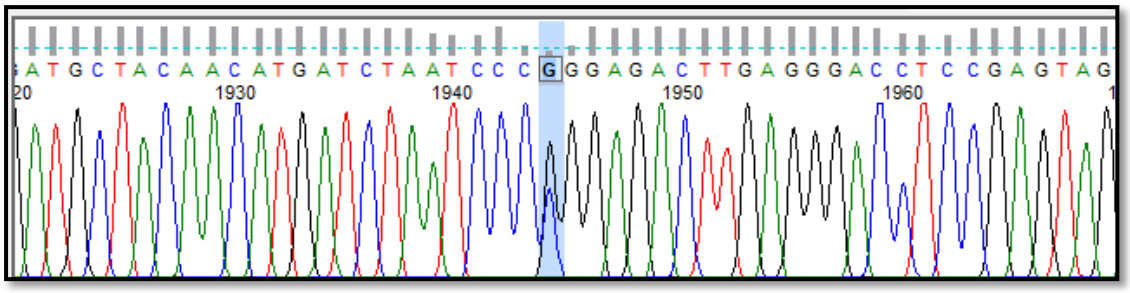


Figure (3-14): DNA sequencing chromatograms of (rs1025689)SNP for IL17RB gene .

The SNP (rs1025689) in the *IL17RB* gene showed significant difference between the hydatid disease patients group and the control group (Table 3-14).

Table (3-14): rs1025689 SNP distribution frequencies in the screened population (Control and Patients).

SNP	Allele	Frequency	Controls	Patients	*OR (95% CI)
rs1025689	**C**	44 (0.63)	21 (0.7)	23 (0.57)	1.725 (0.634-4.694)
	G	26 (0.37)	9 (0.3)	17 (0.42)	
	P value	0.031*	0.028*	0.343	
	Genotypes				
	C/C	9 (0.26)	6 (0.4)	3 (0.15)	1.00 3.49 (0.69-17.76)
	C/G	26 (0.74)	9 (0.6)	17 (0.85)	
	G/G	0 (0)	0 (0)	0 (0)	
	P value	0.004*	0.439	0.002*	

***represent a significant difference at p<0.05, OR: Odd ratio, CI: Confidence interval**

The polymorphisms within the IL17RB C allele confer an increased risk of susceptibility to extensive forms of hydatid disease , especially with an early onset of disease, In this SNP, the results appear than CG genotype were more frequent in patients , CC, CG and GG genotype frequencies are reported respectively to be 0.26, 0.74, and 0, The *IL17 RB* rs1025689 C allele effect was consistent in the three cohorts and the association improved after the meta-analysis, showing statistically significant results (P = 0.12, OR = 1.00, 95% CI, under a fixed-effects meta-analysis) after Bonferroni correction.

The action and expression of IL-17RB on the sex has rs 1025689 CG heterozygous had the highest frequency in patients (0.71) females than (0.29)males, while CC homozygous showed (0.33 females and 0.67 males), but GG homozygous did not show an effect on both sexes. as show table (3-15). Therefore, expression of IL-17RB in the sex and its role in pathophysiology of hydatid disease should be investigated in the future.

Table (3-15): rs1025689 SNP distribution frequencies in the screened population (Control and Patients) according to Sex.

Genotypes	Sex	Controls	Patients	*OR (95% CI)	P value
C/C	Female	3 (0.50)	1 (0.33)	1.00	0.635
	Male	3 (0.50)	2 (0.67)	2.00 (0.11-35.81)	
C/G	Female	4 (0.44)	12 (0.71)	1.00	0.192
	Male	5 (0.56)	5 (0.29)	0.33 (0.06-1.79)	
G/G	Female	0 (0)	0 (0)	--	
	Male	0 (0)	0 (0)	--	

* **OR: Odd ratio, CI: Confidence interval**

However, there have been conflicting findings regarding blood levels of IL-17RB, with the C allele being linked to higher, lower, or no significant amounts of protein transcription and synthesis(Eichler, 2019). We hypothesized in this study that individuals who have the C allele in the IL17RB gene are more susceptible to hydatid disease infection, and that probably due to a variation in the gene expression and therefore lower IL-17RB production, which would impede a rapid pro inflammatory activation of chemokines and cytokines for the resolution of hydatid disease infection (Rolandelli *et al* ., 20017). However, further

studies are required to understand the complexity of *IL17RB* gene polymorphism functional effect.

IL-17RB is found in endocrine tissues and epithelial cells throughout the body, including the kidney, liver, and mucosal tissues (Ramirez-Carrozzi *et al* ., 2019) ,Elevated IL-17RB expression is also found lung tissues from asthmatic patients and in skin lesions from patients with atopic dermatitis . IL-17RB expression in human innate type 2 lymphocytes, natural killer T (NKT) cells, and Th2 cells (Hadian *et al* ., 2019) suggests a potential role in immune cells. In these human cells IL-17B promotes IL-33-driven type 2 immune responses, a function shared with IL-17E, but not withIL-17A (Deng *et al* ., 2021).

In the chronic phase of the disease, several studies suggest that the clinical progression of hydatid disease involves the overexpression of IL-17 by Th17 cells and B cells(Eskandari-Nasab *et al* .,2018).

It is known that genetic variability and immunologic response influence the pathogenesis of the chronic phase of the disease, Associations were observed in several cytokine genes with the susceptibility or protection against the development or progression of the hydatid disease and/or its clinical forms(Zacarias *et al*., 2015) ,the IL-17 is a proinflammatory cytokine secreted by T cells activated and expressed in different tissues. This cytokine takes part in inflammatory responses mediated by T cells and plays an important role in the tissue homeostasis and diseases progression.

Study on the SNPs of IL-17 receptor suggested that the presence of the C allele at position 467 bp could cause an increased inflammatory response, such as the one we expecting atopic patients, This response is

more intense in the case of a homozygous genotype of the C allele (Settin& Salem , 2008).

Although there was no significant differences in IL17RB polymorphism between patients with hydatid cyst and healthy control, the result was showed that C allele was most predominant than G allele and the G allele appeared with low percentage, So that the source of (CC) genotype was come from (GC) genotype was come from the conversion of (GC) to (CC) and both genotype were associated with low production of IL 17 RB in serum of patients and controls this lead to make the patients having high chance of infection , while the control group become more susceptible to infection.

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Summary

Hydatid disease is a major endemic health problem in certain area of the world ,approximately two to three million human cases occur world wide annually .

Case - control descriptive study was done on patients with hydatid diseases attending Al-Hilla Teaching Hospitals, AL- Imam AL- Sadiq Hospital and private hospitals in Babylon province during the period from first of October 2019 to the end of October 2020 to study the immunological and molecular to hydatid disease .

Regarding the patients character, the total number of sample included in the study was 93(53 patients and 40 control) , the highest rate of infection was among females (69.9%)with the highest age range affecting the diseases was between 30-40 years old (28.3%) .

Most of the study cases were from the rural areas (75%) which higher than urban (24.5%) .

According to ultrasound technique and CT scan ,the highest rate of hydatid disease infection was in liver which mainly (75.4%) then abdominal cavity (9.4%) following by kidney (7.5%) while in brain and lung it was (3.7%) for each .

Enzyme – linked Immunosorbent Assay (ELISA) were performed to measure the level of IL-17family among patient and control ,there are significant increase in mean of serum level concentration of IL17A and IL17B , also there was increasing in concentration of their receptor's in patient of hydatid disease compared with control ($p \leq 0.05$), the present study found the highest concentration was in IL17RA with (61-70) year (6939.26± 18.15) pm/ml in patients of hydatid disease compared with control (3833.29±21.3) pm/ml by($p \leq 0.02$), in IL17 RB was increase rate concentration in (61-70) year (25.61± 3.4) pm/ml for ($p \leq 0.05$) and increase of concentration in (20-30) year (26.33± 7.5) pm/ml compared with control.

Summary

Also the results showed that high levels of concentration IL17B in age group (31-40 **)** year was**(** 35.84± 10.2) pm/ml compared to healthy controls comparted to IL17A level in (31-40) year by (238.20± 34.1) pm/ml . but these results was significantly . In the present study showed there was positive correlation between IL-17A with IL17 B (R2=0.0141 $p<0.05$) and their receptors.

Polymerase Chain Reaction -sequence were used to detect four SNPs rs2275913 IL17A , rs375208 IL17B, rs 879576 IL17RA and rs1025689 IL17RB for all samples cases.

The polymorphism of interleukin-17A gene at rs2275913 was detected for 40 cases and 30 control, rs2275913 the genotypes (GG ,GA, AA), The odd ratio (95% confidence interval) of the genotype GG was (1.00) that mean risk allele for patients compared with 1.09(0.08- 14.660), (0) for GA, AA respectively that mean protective allele for control group and . For rs375208 the genotype (AA, AG, GG). The odd ratio (95% confidence interval) of the genotype AA was (1.00) and compared with 0.24(0.06-3.02),(0) for AG,GG respectively that mean protective allele for patients and control group also rs 879576 the genotype GG,GA,AA was the odd ratio (95% confidence interval) of 1.00,1.19(0.30-4.73) and(0) respectively that mean AA genotype no signified for hydatid disease ,and finally rs 1025689 was the odd ratio (95% confidence interval) for CC,CG,GG (1.00,3.49(0.69-17.76),0) respectively that mean GG no signified by $p \geq 0.05$.

Moreover, in serum level of IL17-A was closely linked to the alleles according to sex that frequency allele A homozygous patients (AA) no effective when compared with patients carrying one or two copies of allele G (0.61 female ,38.9 male) for GG homozygous patients also GA allele was 2 female and not exist in male , Compared with the IL17 B level was linked to the frequency allele A homozygous patients (AA)

higher in both sex (0.86 female,0.43 male) Compared with control while GG homozygous no exist in patient for both sex and about (AG) heterozygous The frequency ratio is similar in both sexes(0.03).

The results of the study also showed the clear difference in IL17 RA, IL17 RB in genotype frequency according to sex, where rs 879576, which represents IL17 RA GG homozygous , had the highest frequency in patients (0.5 females and 0.2 males, while GA showed (0.4 for females and 0.1 for males), but AA did not It has an effect on both sexes ,while rs 1025689 represents IL17 RB CG heterozygous had the highest frequency in patients (0.71 females and 0.29 males, while CC homozygous showed (0.33 females and 0.67 males), but GG homozygous did not show an effect on both sexes.

The present study conclude that the rs 879576 IL17 RA gene and re 1025689 IL17RB gene was risk for infection of hydatid disease .

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Conclusions & recommendations

- Conclusions:

According to the results that obtained in present study , the following conclusions have been detected :

1. The percentage of infection with *E.granulosus* was higher in female than male . with highest percentage for age group (31-40)years .
2. The most frequent infection with *E.granulosus* was found in liver , and abdominal cavity and lower in lung .and distribution of *E.granulosus* higher in rural area than urban area.
3. High levels of IL-17 A and IL17 B in age group 31-40 year compared to healthy controls.

4. The result showed increasing in IL-17RA in (61-70) year in patients of hydatid disease compared with control also IL17RB was increase of concentration in (20-30) year compared with control.

5- The study showed There was positive correlation between IL-17A with IL17 B And between IL17 A with IL17RA and IL17RB**.**

6. The genotyping frequency of IL-17A of rs2275913 and IL-17RA of rs879576 showed the largest GG genotype and G allele frequency in patient compared with control in same gene site .and distribution of GG in female was higher than control while no appear in AA in both sex .
7. The AA genotype in IL-17RA of rs879576 was significantly in patients and control that suggest the implication of this polymorphism in *E .granulosus* infection incidence.
8. The genotyping frequency of IL-17B of rs375208 showed the largest AA genotype and A allele frequency in patient compared with control in same gene site.
9. The genotyping frequency of IL-17RB of rs1025689 showed the largest CC genotype and C allele frequency in patient compared with control in same gene site.

Conclusions & recommendations

- Recommendations:

1- Urging researchers to develop an effective vaccine against Hydatid cystic diseases, as well as treatments in the field for humans and animals, to help prevent or reduce the risk of infection, as well as to break the life cycle by preventing dogs from eating dead animals or by burying or burning infected animals slaughtered in abattoirs.

2- Educating people, especially butchers and residents of rural areas, about the parasite's life cycle and the role of dogs in it.

3- Study the polymorphism of IL-17family in other site of gene.

4- Additional studies are needed to detect the IL-17family gene expression with large sample of study population to establish an obvious idea about the prevalence of this polymorphism among Iraq hydatid disease patients .

5- Studying of other immunological parameters in order to evaluate the immune response of *E.graneolusus* .

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الخلاصه

يعد مرض الاكياس المائيه مشكلة صحية متوطنة رئيسية في مناطق معينة من العالم ، حيث تحدث ما يقرب من مليونين إلى ثلاثة ملايين حالة بشرية في جميع أنحاء العالم سنويًا.

قد أجريت هذه الدراسة على مرضى الاكياس المائيه في مستشفيات (الحلة التعليمي ومستشفى الإمام الصادق والمستشفيات الاهليه بمحافظة بابل) خلال الفترة من الأول من أكتوبر 2019 إلى نهاية أكتوبر 2020 كدراسة وبائيات ، مناعيه جزيئيه لمرض الاكياس المائيه .

بلغت إجمالي عدد المرضى المشمولين في الدراسة 93 حاله (53 مريضاً و 40 مجموعه سيطره) ، وكانت أعلى نسبة إصابة بين الإناث (56.9)ا لفئة العمرية بين 30-40 سنة بنسبه (28.3٪) وايضا سجلت حالات الدراسة من المناطق الريفية بنسبه (75٪) وهي أعلى من الحضريه (24.5٪).

وفقًا لتقنية الموجات فوق الصوتية والتصوير المقطعي ،سجلت أعلى معدل للإصابة بمرض الاكياس المائية كانت بشكل رئيسي في الكبد بنسبه (75.4٪) ثم تجويف البطن (9.4٪) وبعد ها الكلى بنسبه (7.5٪) بينما كان في الدماغ والرئة اقل نسبه اصابه حيث بلغت (3.7٪) بالنسبة للإصابة بالعدوى.

كذلك تم إجراء فحص الاليزا (ELISA) لقياس مستوى عائلة IL-17 بين المريض ومجموعه السيطره ، وكانت هناك زيادة كبيرة في متوسط تركيز مستوى المصل من IL17A و IL17B ، كما كان هناك زيادة في تركيز مستقبلاتهم بين مرضى الاكياس المائيه مع مجموعه السيطره عند مستوى احتماليه ($p\leq 0.05$) .

بينت الدراسة الحالية أن أعلى تركيز كان في IL17RA في الفئه العمريه (61-70) سنة بتركيز (6939.26 ± 18.15) pm/ml في مرضى الاكياس المائيه مقارنة مع مجموعه السيطرة بمقدار (3833.29 ± 21.3) pm/ml عند مستوى معنوي ($p\leq 0.02$) ، اما في IL17 RB كان زيادة معدل التركيز في الفئه العمريه (61-70) سنة (25.61 ± 3.4) pm/ml بمستوى معنوي ($p\leq 0.05$) كذلك سجلت الفئه العمريه(20-30) سنة زيادة بالتركيز (26.33 ± 7.5) pm/ml مقارنة بالتحكم.

كما أظهرت النتائج أن المستويات المرتفعة لتركيز IL17B في الفئة العمرية (31-40) سنة كانت (35.84 ± 10.2) pm/ml مقارنة بمجموعه السيطره ومقارنه إلى مستوى

IL17A في الفئه العمريه (31-40) سنة بمقدار (34.1 ± 238.20) pm/ml . لكن هذه النتائج كانت غير معنويه ، و ايضا أظهرت الدراسة الحالية وجود علاقة إيجابية بين IL-17A و IL17 B (R2 = 0.0141 p <0.05) ومستقبلاتها.

تم استخدام تسلسل تفاعل البلمره (PCR) للكشف عن أربعة جينات SNPs rs2275913 IL17A ، rs375208 IL17B، rs 879576 IL17RA و rs1025689 IL17RB لجميع حالات العينات.

و تم الكشف عن النمط الوراثي لجين إنترلوكين A -17 في الموقع rs2275913 لـ (40 مرضى و 30 مجموعه سيطره) ، بالنسبه rs2275913 الأنماط الجينية (GG، GA، AA) ، كانت النسبة الفردية (95 ٪ فاصل الثقة) للنمط الجيني(1.00) GG مما يعني ان أليل G يمثل الاليل الخطر للمرض مقارنة بالانماط الجينيه GA، AA بنسبه (فاصل الثقه 95) 1.09 (0.08- 14.660) ، (0) على التوالي والتي تعني ان الاليل A أليلًا وقائيًا لمجموعة السيطره والمرضى .

اما بالنسبة لـ جين IL17 B في الموقع rs375208 ، فإن التركيب الوراثي (AA، AG ، GG). كانت النسبة الفردية (فاصل الثقة 95 ٪) للنمط الجيني (1.00) AA ومقارنتها بـ 0.24 (3.02-0.06) ، (0) لـ AG، GG على التوالي والتي تعني أليلًا وقائيًا للمرضى والمجموعة السيطره ، أيضًا الموقع rs 879576 من النمط الجيني IL17RA كان التركيب الوراثي GG ، GA، AA وكانت النسبة الفردية (95٪ فاصل الثقة) 1.19،1.00 (0.30-4.73) و (0) على التوالي التي تعني أن النمط الجيني AA لا يدل على اي تغيير جيني على مرض الاكياس المائيه ، وأخيراً الموقع rs 1025689 للنمط الجيني IL17RB كانت النسبة الفردية (95٪ للفاصل الثقه) لـ تركيب الوراثي (CC، CG، GG) (1.00 ، 0.69-17.76) 3.49، 0) على التوالي وهذا يشير GG الى عدم وجود اي تغيير جيني في هذا الموقع عند كلا المجموعتين بمستوى معنوي $p \geq 0.05$.

علاوة على ذلك ، في مستوى المصل في جين IL17-A كان مرتبطًا ارتباطًا وثيقًا بالأليلات وفقًا للجنس ، فإن الأليل المتكرر A لمرضى متماثل (AA) ليس فعالًا عند مقارنته بالمرضى الذين يحملون نسخة أو نسختين من الأليلات G (0.61 أنثى ، 38.9 ذكر) لـ GG مرضى متماثلون الزيكوت، أيضًا سجل GA allele كان (2 أنثى ولا يوجد في الذكور)، مقارنة بمستوى IL17 B كان مرتبطًا بأليل التردد A للمرضى متماثلي الزيكوت (AA) أعلى

في كلا الجنسين (0.86 أنثى ، 0.43 ذكر) مقارنةً بمجموعه السيطره بينما GG متماثل الزيجوت لا توجد في المريض لكلا الجنسين واما الاليل (AG) متغاير الزيكوت كانت نسبة التردد متشابهة في كلا الجنسين (0.03).

أظهرت نتائج الدراسة أيضًا اختلافًا واضحًا في IL17 RA و IL17 RB في تردد النمط الجيني وفقًا للجنس ، حيث كان الموقع rs 879576 ، الذي يمثل جين IL17 RA للاليل GG متماثل الزيكوت ، أعلى تردد في المرضى (0.5 إناث)و(0.2 ذكور) ، بينما أظهر الاليل GA (0.4) للإناث و (0.1)للذكور ، لكن AA لم يكن لها تأثيراً جيني على كلا الجنسين .

بينما الموقع rs 1025689 للجين IL17 RB اظهر الاليل CG متغاير الزيكوت أعلى تردد في المرضى (0.71 إناث) و(0.29) ذكور ،و الاليل CC متماثل الزيكوت (0.33 إناث) و(0.67 ذكور) ، لكن الاليل GG متماثل الزيكوت لم يظهر أي تأثير على كلا الجنسين.

ويستنتج من الدراسة الحالية أن الجين 879576 IL17 RA و الجين IL17RB 1025689 يمثلان خطرًا للإصابة بمرض الاكياس المائيه .