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A Study Classifying with A Demonstration of the Effect of Detergent Testing On Ticks in Cattle in Suq- AL Shyuohk City – Thi – Qar 2015.

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Abstract:
A searching study in Suq- AL shyuohk city Thi – Qar Province explained the spread of the intruder ticks on the cattle for three months, the study found that the rate of infestation was (38 from 40) in the November and December the infestation rate was 35.29% from cattle were found infestation by ticks 1-5 animal is infested with ticks 43%this the highest rat and was infested 3% 21 – 25 ticks this the lowest rate. The study explained that the most infested places Testicle in the body of the animals were thigh area and the rate was 55% and the less infested places is the Neck and the testes the rate was 5%. Two species of ticks were isolated and classified. the *Hyalomma ssp* and dog's ticks *Rhipicephalus ssp* The rate of the first type was 50% from test tick's samples. The rate of the second tope was 64%. When the hard tick puts their larval stages with a dissolved liquid (1, 2, 3) and focus (5. 5, 10. 5, 20. 5) respectively, the females recorded the highest percentage of male deaths. The larvae stayed for short period while the nymphs showed ware resistance than the suffocated females because their cutical were more rigid.
Key word: Ticks**,** Rhipicephalu ssp, Hyalomma spp.

1-Intoduction:

Tick is one of the external parasites and the infection is important for human being and animal and it cause economical and health problems ، it's divided into two families from the healthy side The hard tick and soft tick (1). The study explained that the Ixodidae family has larval and humoral phases are blood – nourish hing. blood is the basic meal to eggs. from this comes the important of ticks soit causes humor by obsorbed large amounts of host blood, more over transfusion into blood vessels (2). The latest plays an important role in making Anemia due to red blood colls and endothelium. ticks are considered the main transporter for the leriasis. This disease is endemic disease Iraq specially in the warm areas and hot places (3). The study indicates that this parasite has great importance in terms of economic loses as a result of weight losing and reducing milk from 5 -6. Through the study and the comparison between the hard ticks the study found that the (Hsalomma anatomical needs two hosts to complete his life cycle (4). The ticks are dual family because the larva or nymph them spend their life on cows to fall on the ground and tarn into an adults tage then it parasite on another host cows (5). The tspe likes to change blood. While dog's tick needs three hosts (the tri – host ticks the ground and clings to its prey to snoop on the second host then fall into the ground to grow adult and snoop on the third host to complete its life cycle and sometimes two nosts (2). through the study it can be mono. This kind of ticks is considered the most dangerous because it moves from one host to an other. Through blood absorption the disease is transmitted from one host to another.

2-Materials and methods of working.

Forty tick sample were collected for both male and female. The number of males are 17and females number were 23. the cattle ages were between 6 months to 9 years.

The samples of ticks were taken from different are as in Suq - Al shyuohk for three months started from October to December 2015. The animals were examined carefully. The test was clinical one to observe the pressure of ticks and places of spread. on the animal's body. Samples were taken from ticks to test tand examine and classify it. Samples of each animal was kept separately in its own glass bottles containing the formalin Iiquid with concentration 10%. The total number of samples was 248 tick sample males and female's animals**.**

Aspecial form was made and it included the following in formatting:

a) The total number of tested animals and test date.

b) number of infected animals.

c) the age.

d) The place and the number of ticks on the single animal.

The ticks were classified depending on professor Mohamed khadhim who is working in the natural and historical museum of Baghdad University.

2-1 Method of preparation

Firegm was taken and three solutions were prepared with different concentrations.

1- Solution number (1) 5 grams were dissolved in (50) ml concentration 20.5.

2- Solution number (2) 5 grams were dissolved in (100) ml concentration 10.5

3- Solution number (3) 5 grams were dissolved in (150) ml concentration 5.5

The groups of ticks were placed in a petridish filled with (1, 2, 3) solutions to study the duration of loss by a time counter. 10 males, 10 females, 10 larvae, 10 nymphs,10Engorged of both sex esof the ticks in the petri dish separately. (**6**)

2-2 statistical analysis:

The results of the current study were subjected to the Chi-Square Tests (7) and Their suits of the study were analyzed according to the Model of the general experiments and the random design. The percentage of the losses were corrected according to the equation. (8)

Percent age the losses=percentage of losses in equation % - loss in control equation Percent age%
100- loss in control equation Percent age%

Less significant difference has been used and the level 0.05 to demonstrate the significance of the mesults. The parentages of depreciation were converted to angle values to beused in a statistical analysis.

3- Results and discussion:

The result its of the study were shown in a table number (1) rate of infection with tick parasites 35.29% this rate is different from the results of. The rate was 72.9% in cows (9). The Anatolian ticks are more adoptive and energetic and active through all the seasons of the year. Whereas dogs tick the brown one was more sensitive and affected by seasons of the year specially in Autumn and winter. The (10) recorded the rate in cows 62% and sheeps 55%. goats 57% and explained the rate in calves was 43%. 9 the rates were closed through three Months from October to December. The (11) he noticed the rate was 21.7% This difference might be because of the differences in weather and the environment which plays aroleir the Morement and activity of the ticks. The nice air and plants have arole in activity and spreading the ticks. The (12) mentioned in ostudy that the white sheeps carry ticks more than brown sheep. In addition to the role of ruminants in the couserration of parasites and their trans mission to domestic animals as well

as the movement of agricultural projects and the removal of bush and brush which has clear and significant impact on the environment and therefore the prevalence and distribution of parasites. (13).

Table (1) Shows the incidence of ticks during the study period

Months	Total number of cattle	Numberof infected cattle	Percent age infected
October	17	6	٣٥,٢٩%
November	15	15	100%
December	8	8	100%
Total	40	38	

×=12.24 df=2 p-value=0.002 2

The results were shown in table (2) and in the study of the severity of infection
with tick's parasites in 400 of cattles were infected depending on the number of parasite of ticks found in the body of the animals. It turned out that cattle which carry number of from 5-1 ticks. The cattle were the highest rate and the rate was 43%. The larvae number on the animal was between 25-1 larvae / animal. These results are different from the results of (13). the first pointed to rate 20-10 ticks / animal and these results argree with number (14) between (25-1) larvae / animal number (15) pointed to rates 10 – 6 larval animal in summer and (7-5) larva / animal in spring. The result of this study larger and wider because of the difference in larva number to age of animal and the state of health and the type of the study and methods of raising and appropriate conditions for spreading parasites and the place of the study.

Tab (2) Shows the incidence andnumber infected animalsandseverity of infection

Number parasts	Number infected animals	Percentage
1-5	57	43%
6-10	62	38%
11-15	87	13%
16-20	97	3%
21-25	97	3%
Total	400	

×٢=92.5 df=4 p-value=0.00

The infected tick parasites were distributed to different areas of the body in different rates. the thighs area was fount the most infected areas and the rate was 55% where as testicle areas were and lower rate in neck it was 5% in the areas. It can be due to the different exposure of the body areas to friction more than other parts of the body. There is in equaility in hair distribution and degree of skin moisture and blood circulation in this area which help the parasites to be in the best area of the body.

Table (3) Shows numbers and distributed to different areas of the body

Parasitic of in body	Number infected animals	Percentage
Perineum	**87**	**13%**
Abdomen	**85**	**15%**
Ear	**89**	**11%**
Eye	**91**	**9%**
Neck	**95**	**5%**
Drosel	**89**	**11%**
Testicle	**95**	**55%**
Fount	**73**	**27%**
Total	**704**	

×=32.58 df=7 p-value=0.000 2

The results of table (4) showed the isolation and classification of two types of ticks. they are Anatolian tick and dog's ticks Both belong Oxydody family. The rate was 55% from testsamples of ticks (138) parasite of total (248). Female and 28 males. The rate of the secoud type was 64.5% (160) parasite to tal of (248) 20 female and lo males and 60 nymphs and these rates closed to the rates recorded by researcher (10). He recorded two types (*Rhipicephalus, Hyalomma* spp). The highest rate was Hyalmma ticks 33.5%. The researcher (14) recorded tick rate (Hyalomma *spp*) 43.7% and rescarcher (11) recorded types of *(Rhipicephalus* ssp, *Boophilus* spp, *Dermacenter* spp, *Hyalomma* spp). The Hyatomm as pp ratew as 24% this different from researcher (9) who recorded *Ixodes* spp 20%. This is due to different environmental conditions suitable for each sex and each type of ticks also to the difference in the host and it's descendants and his suitability to the parasite. The tick nymph was recorded for the sex (*Rhipicephalus* spp) 60% and (*Hyalmma* spp was 50% while *Ixodes* spp was 70% where as there is not any nymph for *Hyalomma* spp sex. The reason mighloe to difference in location of the study. The difference in environmental cordition affect on the time of parasitelife cycle forward and delay which lead to difference periods breeding and female congestion and males presense and larva spread and nymphs and other stages of development. Also the difference in samples sizes. More samples taken the percentage will increase and this a ffect the final result as show asfrom the result. The Hyalomm as pp sex shows oppressed females 10% and males 28% in this three months while (Rhipicepla spp sex noticed with all shapes prom the dead females and the rate was 20% and males 10% females 20%. (16) were found the best thermal deyrees for Hyalomma ticks and dog's ticks (17) with relative moisture (RH 80 – 70) through Autumn season. There ajon might be to the litteactivity with females from enzymes specially (Adenoce Tri-Phosphat) (6) this is different from (14) where he could not find oppressed famales and nymphs in Hyalomma spp sex only males and females. This is a clear evidence to the different behavior and growth needs and activity and reproduction between races of ticks. There ason is the different sexes recorded to different geographical locations and the environment and the agricultural projects in fluenced by races and animal breeds.

Table (4) Shows the number and types of isolated ticks and developmental stages

Tick stage	Rhipicephalu ssp.	R.Turanics	Hyalomma ssp.	H. scupens	H. Anaticum
Engorged	20	9	10	Nil	Nil
Female	70	4	50	2	12
Male	10	4	28	7	11
Nymph	60	Nil	50	Nil	5
Total	160	160	138	9	28

The study explained in table (5) the rote of infested was higher than females and the rate was (58). The male's injury was 43% female's infections were recorded higher the males in the two sexes *(Hyalomma spp, Rhipicephalu spp*) This emphasis the presence of more then on type of parasite and more thence sex on the single animal which gives to these infections importance than others in the one type. because of the abundance of parasites and its number and its type. It possible to be the most damgerous one for many reasons such as the pathogens transmitted by different species and the difference in host sensitivity for these types from parasitic ticks and the microbes transmitted.

Table (5) shows infected number both male and female.

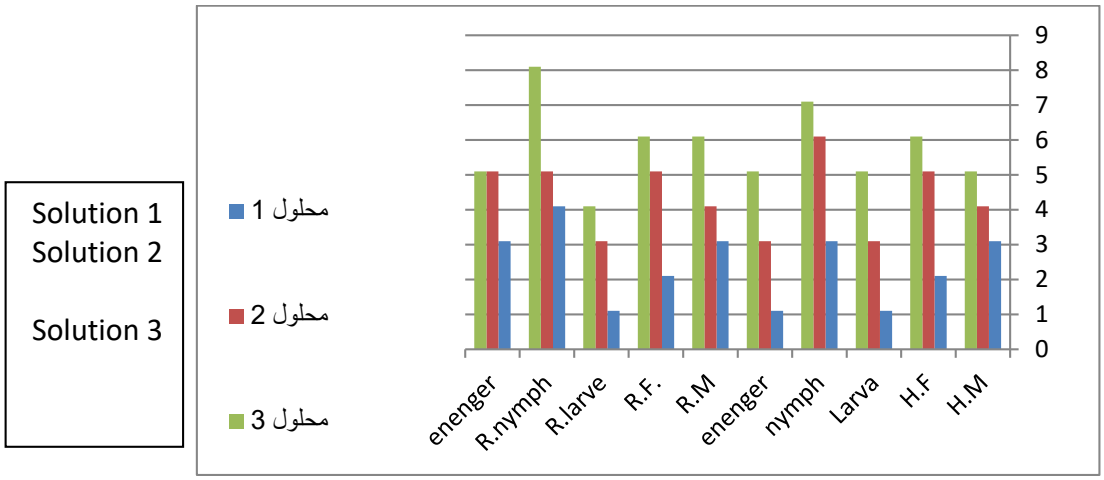
Sex	Number	Percentage
Mele	57	43%
Famele	42	58%
Total	40	

×=4.5 df=1 p-value=0.034 2

3-1-The effect detergent solution on tick loss

Table (6) explained the usage of solution (1, 2, 3) on the rate of destruction in the ticks for both types (*Hyalomma ssp, Rhicipcephalu ssp*) because of the existence of the hard envelops. The entrance of the cleaning solution through ventilators in the exit or through the wall of the body, the process takes many hours the oil normal (6). The result clarified the female distraction is short in comparison with males. It was (6 – 5) and (6 – 5 – 5) for both types respectively and this rate is proximitly with what has beer recorded (6) females loses was (5 – 4) males were (6 – 5). The hard layer doesnot cover all the females body. The larva was (3 – 2 – 5) for both tick sex. Was shorter then nymphs because of the light of couers and its leaver while the nymphs showed more resistance for cleaning solution males and the rate of destruction was (4 – 5 – 3) and (4 – 3 – 5) for both types respectively because it is harder. females recorded shirt period (3 – 5 – 2) and (3 – 2 – 5) for both types of ticks respectively because the covers are a bleto extend and porous because of the big size and it's filled with blood. These destruction's rate was for solution (1) which more focus and effect or females and larva. So individual differeuces appeared. The solution (1) was more effective on the destruction of hard ticks in both types and in different stayes of it's life. The researcher the effect tiveness of the pesticide depends on the speed of its permeability in side the body and the destruction and discharge from it. Figure (1) bshowed that nymphs for both types from ticks were less efective and the rate of destruction specially in solution (3). We cannot depend on detergents and using as exterminators for ticks because it has slow effect because of hard covers which covers which covers tick's body and it consists of two layers they are cementite and wax layer and the last one prevent

leakage of solution inside the body. These results Matches with (8) while solution was (7, 7, 11, 6, 23, 3) concentrations have effect on eggs and kill the prostitute and this is useful in cleaning walls and the floors of animal's hangars.



Shap (1) shows effect detergent solution (1,2,3) of percent losses ticks hard (*Hyalomma* ssp, *Rhicipcephalu* ssp) and laver stage.

Table (6) shows duration of loss ticks hard both types (*Hyalomma* ssp, *Rhicipcephalu*

Scientific name	stage	Solution 1	Solution2	Solution3
Hyalomma ssp	Mela	5-6	7-8.5	8-10
	Female	3-4	5-6	7-7.5
	Larve	2.5-3	3-4.5	4-5.5
	Nymph	3.5- 4	4.5-5	5-6
	Engorgd	2-3.5	4-5.5	6-6.5
Rhicipcephalu ssp	Mela	5.5-6	7-7.5	8-10
	Female	3-4.5	5-6	6-7.5
	Larve	2-3	4-4.5	5-5.5
	Nymph	3-4.5	4.5-5	5.5-6
	Engorgd	2.5-3	3.5-4	4.5-5

sp) accoeding to the number of hours

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Investigate The Relation Between Polymorphism of *(CTLA-4*) Gene and Asthma In Some Patients at Thi-Qar Province / IRAQ

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Abstract:

The present study was carried out at the Biology Department laboratories, for the period of (December 2017 to November 2018). The aim of the current study was to investigate polymorphism of CTLA-4 gene in patients with Asthma. The study subject consisted of patients with asthma (74 females) and (26 males) were their aged between (15-55) years, with (50 persons as control group. After DNA extraction, PCR was made by using specific primers for the *CTLA-4* gene, the study results explained the incidence of polymorphisms in a samples of 25 out of asthma patients when using the restriction enzyme (*BSTEII*). After analyzing the results by statistical analysis results showed that no association between the incidence of Asthma and appearance of mutation in *CTLA*-4 gene when compared to the control group.

Key words: *CTLA*-4, polymorphism, Asthma**.**

1-Introduction:

Asthma is a general complex disorder that is characterized by airflow obstruction, airway hyper-reactivity, persistent inflammation of the respiratory system and certain stimulants induce these signs, such as infection, exercises, occupational exposures, and allergens (Lambrecht& Hammad,2012). Asthma affects more than 23 million adults in the United State only, and females than in males (Kynyk *et al*. ,2011). Symptoms of asthma are versatile and consist of out of breath, coughing, hyperpnea, and feeling of repression in the chest (Moorman *et al.*,2012). In the previous study available evidence recommended that both genetic variations and environmental components may contribute to asthma susceptibility (Moorman *et al.*,2012). In recent times, the relations between gene polymorphisms asthma have become an important part of studies that has make essential insights into the pathogenesis of asthma (Temesi,2014). Many studies have recommended that the pathogenesis of asthma caused by interaction between environmental factors and different vulnerability genes, include air pollution, genetic variation, and allergens (Bouzigon *et al*. ,2015). T cells play essential function in asthma pathogenesis when TH1-type response to allergens and cytokines generated (Cao *et al* .,2011).Several researches have indicated that *CTLA4* acts an essential function in asthma pathogenesis (Munthe-Kaas *et al* .,2004),(Botturi *et al* .,2011) .*CTLA-4* located on chromosome 2q33 and expressed on activated T cells, polymorphism in this gene has been established to influence the development of asthma (Kawayama *et al* .,2013).*CTLA-4* plays an important role in the negative regulation of the immune ,and it is associated with TH2 cell differentiation and activation (Wang *et al* .,2015).Many researchers have identified numerous of SNP, and have documented a relationship between asthma and these polymorphisms (Yang *et al* .,2006) . SNP in *CTLA4*+49A/G linked with asthma severity and high levels of IgE which is a main factor in asthma,

and increasing the transcript *of CTLA4*, which possibly will play a role in advanced bronchoconstriction (Hizawa et *al.*, 2001) & (Lee *et al.*, 2002).

2-Materials & Methods

A total of 100 (26 males &74 females) Asthma patients their age between (15-55) years and (50) individuals as a healthy group. These samples were obtained from venous blood and put it in special tubes (EDTA), after that used to extract DNA.

DNA was extracted by using a special kit (Geneaid Biotech) according to the manufacturers protocol. Polymorphisms *in CTLA-4*+49A/G analyzed by using PCR-RFLP technique followed by RFLP method. The primers were used to amplifying in Table 1

Table (1): Oligonucleotide primer sequences used for *CTLA-4* gene amplification.

Primers	Primer sequences'
Reverse	5'-C T G C T G A A A C A A A T G A A A C C C-3
Forward	5'-A A G G C T C A G C T G A A C C T G GT-3

DNA template prepared as the follow:
- Primers (Bioneer company).
- Master Mix (Bioneer company).
- Sterilized D.W, PCR carried out with many cycles as inTable 2, after that 5U of the restriction enzyme *BstEII* incubated with 10 Ml of the pcr product (60 C^{o}) for 4 hours then run on a 3% agarose gel.

Table (2): PCR condition.

No. of Steps	Steps	Temperature	Time
1	Denaturation 1	94	5 min
2	Denaturation 2		
3	Annealing	58	30sec
4	Extension	72	30sec
5	Final Extension	72	10 min

Detecting of Deoxyribonucleic acid (DNA):

Technique is used(Electrophoresis) according to (Sambrook *et al.*,1989). The PCR products were separated on 1.5% agarose gel electrophoresis and visualized by ultraviolet light (302nm) after staining with Ethidium bromide stain. The 152 bp product was digested for 4 h at 60°C with 5 U of the restriction enzyme *BstEII*. The digested products were separated on 3.0% agarose gel.

3-Results:

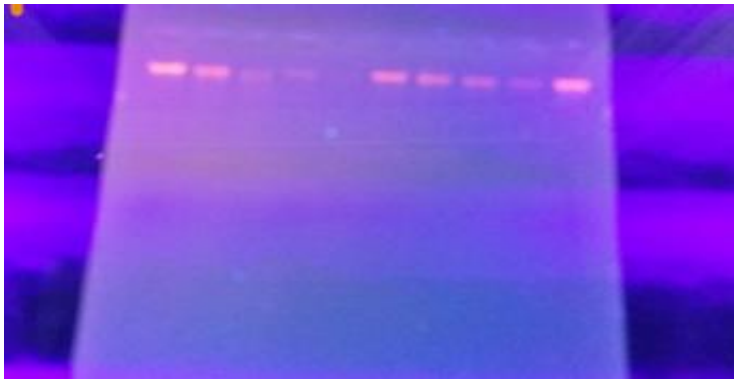

Figure (1): Eelectrophoresis of Extract DNA on a 0.8 % agarose gel.

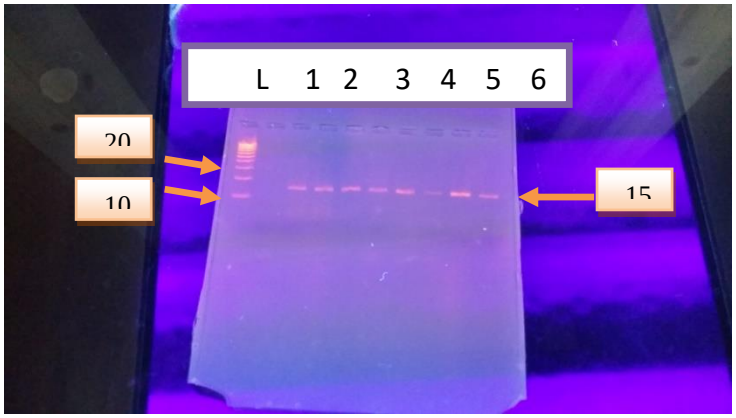

Figure (2): Electrophoresis for amplified *CTLA4* gene for asthmatic patients. Bands were fractionated on a 2 % agarose gel (80V/cm ,1 h., 1X Tris-acetic buffer), visualized under U.V after staining by ethidium bromide stain. (L:100 - 2000bp ladder); Lane 1-9 product band(152bp).

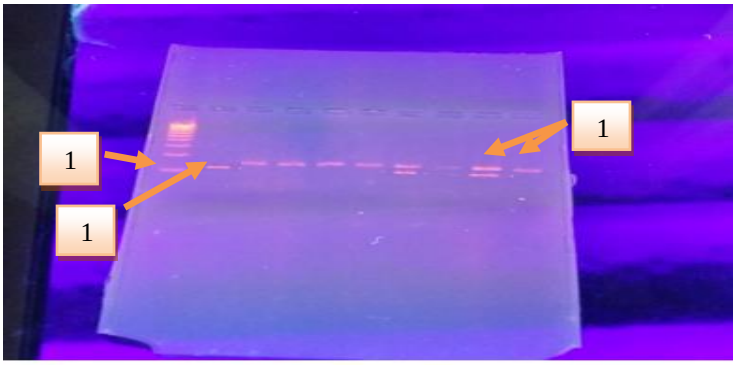

Figure (3) Electrophoresis of amplified *CTLA-4* + 49A\G gene of Asthma patients. Bands were separated on a 3% agarose gel (80V/cm ,1 h., 1X Tris-acetic) and visualized under U.V. after staining with ethidium bromide stain. (L1 :100 - 2000bp ladder) Lane: 1, 2,6,8,9 are PCR samples treated with restriction enzyme (BstEII); Lane: 3,4,5,7, Lane untreated samples Lane (6,8) represent a samples for A\G heterozygous genotype Lane (2,9) represent a samples for A\A homozygous genotype, Lane (1) represent a sample for G\G homozygous genotype.

1. **Frequency *of CTLA-4* gene genotypes.**

The results of the present study show no association between the genotypes of the *CTLA*-4gene and the incidence of Asthma, as the results showed no significant difference between healthy and patients group when genotype A\G (OR=0.9067). The genotype G\G also showed no significant difference between the two groups (OR=0.6951) as in table (3).

Table (3) : Distribution of *CTLA-4* gene genotypes for controls and patients .

Geno type	*Control*	Percentage %	Patients	percentage %	OR	95% CI
AA	34	68%	75	75%	1.0	
AG	1	2%	2	2%	0.9067	0.0795 to 10.3448
GG	15	30%	23	23%	0.6951	0.3230 to 1.4959
Total	50	100%	100	100%		

2. The distribution of *CTLA-4* gene genotypes in patient according to sex.

The results of the present study, showed there are no significant differences for sex and damage of Asthma, there were no significant difference in the A\G genotype (OR= 0.3273), while G\G genotype showed a significant difference (OR=1.7182), as seems in Table (4)

Table (**4**): Distribution of *CTLA-4* gene genotypes for the of patients group according to the sex.

Genotype	*Male*	%	*Female*	%	*OR*	*95% CI*
AA	18	18%	55	55%	1.0	
A\G	1	1%	1	1%	0.3273	0.0195 to 5.5043
G\G	4	4%	21	21%	1.7182	0.5204 to 5.6727

4. The distribution of *CTLA-4* gene genotypes of the patient according to smoking.

The results of the present study showed no correlation between genotypes in people smoking and the risk of Asthma in genotype A\G where there were not significant differences (OR=0.3273), While genotype G\G showed a significant difference (OR=1.7182), as in Table (5).

Table (5): Distribution of *CTLA-4* gene genotypes for the patients according to smoking.

Genotype	Smoking	%	Nonsmoking	%	OR	95% CI
A\A	2	2%	71	71%	1.0	
A\G	1	1%	1	1%	0.0282	0.0013 to 0.6302
G\G	2	2%	23	23%	0.3239	0.0432 to 2.4313

5. The distribution of *CTLA-4* gene genotypes in patient according to family history.
The results of the present study, showed a correlation between patients with Asthma who have a family history and genotype A\G and significant difference more than two times (OR=2.3333), While genotype G\G does not appear any significant difference(OR=1.0000) Table (6)

Table (6): Distribution of *CTLA-4* gene genotypes of patients according to the family history.

Genotype	Family history	%	Non – history	%	OR	95% CI
A\A	21	21%	39	39%	1.0	
A\G	7	7%	27	27%	2.3333	0.8311 to 6.5507
G\G	2	2%	4	4%	1.0000	0.1686 to 5.9314

4-Discussion

As Asthma is a multifactorial, polygenic disease, there are many factors involved in its incidence. These factors are both environmental and genetic. *CTLA-4* represents significant role in the T-cell regulation. In the current study demonstrated that the association between normal control group and asthmatic group as regard of the *CTLA*-4 (A/G 49 in exon 1) genotyping, there was no significant difference between control and patient's groups. This is in agreement with (Munthe-Kaas *et al.*,2004) who also did not find significant difference between control and patients groups. In addition, (Heinzmann *et al.*,2000) had denoted that SNPs in the *CTLA*-4 gene are not linked with atopy or asthma. Also (Nakao *et al.*,2000) established no relationship between atopic asthma and *CTLA*-4 gene polymorphisms in Japanese population. on the other hand, this result differs of the result of (Lee *et al.*,2002) who established that the *CTLA*-4 A/G 49 exon 1 polymorphism may has a disease-modifying in asthmatic airways.

As well as (Sohn *et al.*,2007) had found significant relations between atopic asthma and +49 A/G polymorphism in *CTLA*-4 in Korean children. *(*Yao *et al.*, 2015) suggested that *CTLA*-4 +49A/G in exon 1 polymorphism as risk factor for asthma susceptibility. Also this study showed no significant difference in genotypes of *CTLA4* gene between males and females in the genotype A\G, while genotype G\G showed significant difference between males and females. This result may be due to the subject of study that mostly females.

When we examination of the distribution of genotypes of patients according to smoking results shows statistically non-significant differences between smokers and non-smokers for individuals who have genotype G\G, also genotype A\G showed no significant difference, perhaps this result occurs because of the largest number of our subjects are female and our customs and traditions doesn't allow to the female to smoke or they hide a true information.

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