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INVENTION OR OTHERWISE COPYING AND REPRODUCTION IN HIGHER EDUCATION STUDIES: AN EDITORIAL

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EDITORIAL VIEW!

We confront advances and improvement in the medical sciences constantly the medical sciences and technology gained ground conceivable in medical practice, a part of the issues. That they were not resolved in advance are now effectively controlled as a result of the latest developments and improvements. Medical practice had advanced for a long time to achieve its present position. Significant pioneers of medication tackled a large part of our problems and cleared the street for us. His exceptional commitment to current practice is important. Undoubtedly a specialist is the person who continually develops and strives to do it the right way, clearly no great individual reliably does the right things that we call these bumpy deviations. Absolute reliability is required to use a moral framework for progress and to evaluate development in a fair and sincere manner. These developments raise such a large number of questions, were these progressions constantly carried out within the system of moral conduct? On the other hand were settled silent in inadequate danger only for the change? How does a specialist make another strategy? What internal questions should be asked before continuing? How much preparation is essential and from whom? How would we know if another advance is legitimate?

An audit of the recorded process gives answers about how we should continue later. In the event that we think of the antiquated days of therapeutic practice where exceptionally primitive methodologies were used to analyze or treat the ailment and contrast that days and late practice where MRI, CAT, insusceptible treatment or quality treatment is becoming In a file Sure, we can without much effort recognize how extraordinary our restoring parents and grandmothers were, and how impressive their work was. We are really fortunate to rehearse and appreciate the solution after its significant problems were taken into account. In any case, we must remember with certainty that such advances were impracticable without development and absolutely unimaginable if the insignificant reiteration of the visually impaired was minimized. Standard practice is the core or core for advancement and consequence. Advances and improvement must be customized by request and the presence of new restoration problems, the strong logical premise behind it is constantly mandatory. Changes are not made for change or simply imagining or establishing new thoughts as one may think. Advancement in therapeutic practice is important for the practice and life of the doctor and the patient, simple, safe and valuable as well. What is useful and great today or yesterday presumably will not remain so in the coming days. We must also remember that not every progress is fundamentally superior to previous practice; we need to sit tight for the judgment of time before we fight to perfect it. Dazzle the use of the latest advances can prompt a large number of entanglements; we must understand the reason or explanations of progressions or changes. The best way to understand progress is to make an intensive examination, by the amount that was maintained by the strong logical premise and by the possibility that it was absolutely protected,

productive and important. Little sudden assumption and individual vision can be harmful and unsafe if not reinforced with an archived logical reality. We have to sit aside towards the end of the week to re-process and ruminate our day-to-day sharpening so that we can respond to the variety of investigations; could we change it to a higher state? Can we offer bright lessons for our children in the coming days? Could we be far from our particular complexities? Could we make our vocation a true nectar and drainage? Will we establish another approach or thought? Can we demonstrate against an officially established certainty?

Confusions are the jolt for development, there is an unequivocal reason or purpose for the event of stormy and non-tempestuous complexities. In the event that the drawbacks continue as before and the confusion is returning once more, this certainly shows no improvement, but a visually impaired rehashed hone. Caring for problems and complexities can essentially be seen as a breakthrough. We really have to think about how to do what is annoying and tedious for us, simple and basic for supporters. With the possibility that we do not test the development, we are not reliable in the way the pioneers of the past in the drug were.

Tragically such a large number of couples had a long time however a monotonous sharpen for a long time, they used to consistently do the standard as instructed them in the old days, it is a real redundancy with visual impairment that is ruinous and destructive to Our dear vocation Another type of companions, made a great variation for themselves without distributing it, indicating some kind of mental narrowness. The two neglect to remember that each of us will remain alive for his logical commitment to the profession and not for his money that was left to him after his takeoff until the last objective.

In the event that we remembered how long it lasted it was extricated by the latest advances in therapeutic practice, and how the recipe became clearly protected and convincing through development we absolutely stop visual impairment redundancy and work as one with innovative work.

Our former partners and their anxious efforts at progress really deserve consideration and appreciation and we need to stay by their incredible efforts every day and do the same too. Ideally, our work will also keep us alive in the brains of our future partners in the way we remember the pioneers of the days outdated. To add, each of us needs progress, we need to move forward in innovative works that remain in the archived logical realities that emerge from our own private practice. Nothing is more terrible than a redundancy with visual impairments and a narrow-minded brain that does not recognize advancement, improvement, and personal conversation.

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EFFECTS OF OXIDATIVE STRESS ON FEMALE REPRODUCTIVE TRACT OF MICE INFECTED WITH *BRUCELLA MELITENSIS*

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ABSTRACT

In order to investigate the role of *Brucella melitensis* infection in inducing oxidative stress and the role of chitosan with and without Rev-1 vaccine in protective of female reproductive tract against *B.melitensis* infection and effects of oxidative stress. To achieve these goal, the experiment carried out on 60 white females mice that were divided randomly into five groups equally and treatment as following: 1st group (G1) was served as control positive second group (G2) immunized with *B.melitensis* Rev 1 vaccine S\C, two dose in two weeks interval, third group (G3) was vaccinated as 2nd group and fed diet supplement with 1g\kg diet of chitosan for the end of the experiment ,forth group (G4) was fed diet supplement with 1g\kg diet of chitosan for the end of the experiment ,5th group (G5) was administrated orally with PBS and served as negative control group. The result showed that LH and FSH of all female groups were decline post infection as compared with those values in non-infected immunized and positive control group but infected immunized chitosan group showed high levels of these gonadotropin hormones as compared with other groups. The conclusion from this research revealed that *Brucella melitensis* infection induced oxidative stress, has influence on fertility of females , vaccination by Rev 1 with chitosan improve the fertility of female mice and provide a good immune response against *B.melitensis* infection ;chitosan diminish production of oxidative stress and act as good antioxidant .

Keywords: *oxidative stress, female, reproductive, tract, Brucella, melitensis*

INTRODUCTION

Free radicals were formed by molecular oxygen via get electron and become highly reactive species consisting single unpaired electron in the outer orbital, these free radical called reactive oxygen species (ROS) which consist from the superoxide anion(SO,O_2-) which converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) and hydroxyl radical(OH-) form from reaction of H2O2 with transition metals including iron and cupper, other free radicals are Nitrogen reactive species (NRS) which intracellular production by reaction of nitric oxide (NO) and $O2^-$ and produce peroxynitrite ($ON00^-$) [1;2]. Low concentration of ROS and NRS, play an essential role in the cellular, physiological and metabolism process such as a cellular response to pathogens, cellular signals and immune

regulation in addition to regulate blood pressure [3]. The normal levels of ROS play essential role in oocyte maturation, steroidogenesis, folliculogenesis, ovulation and luteolysis, the source of ROS in the female reproductive tract were oocyte follicular fluid and cumulus mass cells [4]. Oxidative stress occurs through overproduction of ROS and depletion of cellular antioxidant molecules [5; 6]. Oxidative stress was associated with certain female reproductive system disease such as endometriosis [7], polycystic ovary, polycystic syndrome [8], unexplained infertility [9], miscarriage [10], other effects induced by oxidative stress, including intrauterine growth restriction [11], as well as spontaneous abortion due to damage of trophoblasts and degeneration syncytiotrophoblasts by ROS [12]. Numerous pathogens can induce oxidative stress directly or indirectly by induction of inflammatory mediators [13;14], oxidative stress play an important role in the bacterial pathogenesis [15; 16] , it's associated with impairment function of mitochondria and dead cells by apoptosis [17]. Brucellosis is one of the important zoonotic infectious disease associated with release of oxidative stress, its cause by *Salmonella* spp. that infected human and wide range of domestic and wild animals and cause important economic and public health problem worldwide. Reproductive disorders are the important problem in brucellosis in human and animals [18; 19]. Few studies about the influence of *B.melitensis* on female reproductive tract [20] but in Iraq, there is no researches about the influence of oxidative stress associated with *B. melitensis* on female reproductive tract, therefore the aims of the present study were to determine the influence of oxidative stress on female reproductive tract and to investigate the effects of both chitosan and Rev I vaccine on *B.melitensis* infection producing oxidative stress.

MATERIALS AND METHODS

Bacterial isolate:

The virulent *Brucella melitensis* isolate was obtained from Al-Nahdha Veterinary Laboratories /Baghdad, the growth and biochemical tests were done to these isolates to confirm diagnosis according to [21]. It was used different culture media including Tryptic Soya Agar, Tryptic Soya Broth (TSB), and Blood Agar. These media were prepared according to manufactured instruction; the strain was confirmed diagnosis by gram stain and biochemical test according to [21].

Preparation of chitosan diet:

The commercial assorted pellets were grinded by food grinder and weighed , then 1gm of chitosan (fatsorb®) was added to each kilogram of grinded pellets mixed well and converted into paste which passed through meat grinder to mold the paste in to the original pellets form , then left exposed to dry in room temperature [22].

Determination of challenge dose of *B. melitensis*:

The suspension of bacteria made by harvested a culture plate by 2 ml of PBS pH 7.2 then centrifuged 3000 rpm for 5 minutes , finally 0.2 ml of suspension was injected in three mice to activate the bacteria , after 10 days of injection , a small pieces from internal organs (liver, spleen and lung) were cultured in TSA 37 Ċ for 72 hrs ,then the bacteria harvested by 2 ml of PBS pH7.2 and made a serial dilutions . The bacterial suspension was prepared according to method described by [23], by made serial dilution of bacterial suspension by added 1X of suspension to 9X of diluents. the dilutions made to 10^{-12} , then three plates were used for each dilution series , the surface of the plates were dry to allow a 20 µl drop to be absorbed in 15-20 minutes , the plates were divided in to equal

sectors (4per plate) , then the sectors were labeled with the dilutions. In each sector , 20 µl of the appropriate dilution was dropped on to the surface of the agar and the drop allowed to spread naturally , the plates were left upright on the bench to dry before inversion and incubation at 37 Ċ for 72 hrs . Each sector was observed for growth, high concentrations gave a confluent growth over the area of the drop, or a large number of small / merged colonies. The colonies were counted in the sector where the highest number of full- size discrete colonies can be seen (the sectors contained between 2-20 colonies were counted), and the following equation was used to calculate the number of colony forming units (CFU) per ml from the original sample:

CFU/ml = average number of colonies for a dilution X 50 X dilution factor. [23]. The challenge dose was $1x10^{-8}$ CFU /ml.

Experimental design

Sixty white female mice average age 7 to 10 weeks were divided randomly into five groups equally ,in addition to 3 males and treatment as following 1st group was considered control positive group(G1), 2nd group was immunized with *B. melitensis* Rev 1 vaccine S\C, two dose ,two weeks interval.(G2), 3rd group was vaccinated as 2nd group and fed diet supplement with 1g\kg diet of chitosan for the end of the experiment .(G3) 4th group was fed diet supplement with 1g\kg diet of chitosan for the end of the experiment.(G4), 5th group was administrated orally with PBS and served as control negative group. (G5). 30days post immunization, the animals of 1st ,2nd ,3rd ,and 4th groups were divided into two sub group A and B equally , subgroup A was infected I\P with o,3ml of bacterial suspension containing 1X10^8 CFU of viable virulence *B. melitensis* .subgroup b was persisting as control for subgroup A, at 30 days post infection, 6infected females of subgroup A were mated 3 normal males in ratio1:2 for 10 days , then both sexes will be separated and determine pregnancy percentage ;(pregnancy detected by observing pale mucus membrane of vagina according to [24]. After 60 day post infection, all animals were sacrificed, blood samples were taken for hormonal and determined the levels of oxidative stress, and tissue samples from, ovary, and oviduct and uterus were fixed in 10% neutral formalin to 72 hr for Immunohistochemical examination.

Blood collection for hormonal assay:

Blood was collected immediately after anesthesia, directly from the heart by using insulin syringe 1 ml, blood samples directly transferred in to a Eppendrof tubes ,after that kept in refrigerator for period of time in stand position then centrifuged at 1500 rpm for 3 minutes , the serum was stored in the freeze at -20 Ċ until hormonal analysis for LH kit and FSH kit by using ELISA technique .

Measurement of total antioxidant status (TAS):

Serum TAS was determined using an automated measurement method developed by [25]. This method utilizes the hydroxyl radical, the most potent biological radical. In the assay, a ferrous ion solution, which is present in reagent 1, is mixed with hydrogen peroxide, which is present in reagent 2. Other potent radicals are produced, such as brown dianisidinyl radical cation, which is produced by the hydroxyl radicals. This method measures the antioxidant effects of the sample against the potent free radical reactions initiated by the hydroxyl radical. The assay has excellent precision values of $< 3\%$. The results are expressed as millimoles of Trolox equivalent per liter (mmol Trolox equiv./l). Serum malondialdehyde (MDA) concentration was done according to [26]. Immunohistochemistry was done according to [27].

RESULTS

The current study demonstrated that immunized animal feed diet supplement chitosan expressed high levels of serum LH (3.365 ± 0.02) and FSH (0.309 ± 0.024) as compared with those values in immunized animals only (3.004 ± 0.003) (0.213 ± 0.001) that similar to those values in control negative group (3.004 ± 0.003) table (1) and (2)

Table 1. Effect of vaccinated, vaccinated + chitosan, chitosan on LH hormones of females mice at 60 post days

Traits	Vaccinated Mean ± SE	Vaccinated+ chitosan Mean ± SE	Chitosan Mean ± SE	C-	C+
(non -infection) **	3.016 ± 0.001 C	3.598 ± 0.027 A	3.354 ± 0.032 B	3.003 ± 0.0	
(post- infection)**	3.004 ± 0.003 C	3.365 ± 0.021 A	3.111 ± 0.024 B	3.003 ± 0.0	2.389 ± 0.051

The trait means which carried different letters horizontally indicates high significant differences at probability level 0.01.

Table 2. Effect of vaccinated, vaccinated + chitosan, chitosan on FSH hormones of females mice

Traits	Immunized Mean ± SE	Immunized+ chitosan Mean ± SE	Chitosan Mean ± SE	C-	C+
Non infection) **	0.227 ± 0.001 C	0.352 ± 0.006 A	0.315 ± 0.002 B	0.230 ± 0.006 A	0.157 ± 0.019 A
(post- infection)**	0.213 ± 0.001 B	0.309 ± 0.024 A	0.281 ± 0.003 A		

The trait means which carried different letters horizontally indicates high significant differences at probability level 0.01.

Measurement of total antioxidant concentration level in serum

The result show elevated level of antioxidant concentration in groups that fed on diet supplement of chitosan with or without infection (4.878 ±0.06A, 5.607±0.04A) respectively, comparing with those of immunized groups with or without infection (3.034±0.03D, 3.645± 0.03 C) respectively (Table 3).

Table 3. Mean of serum levels of antioxidant in non-infected and infected immunized females animals at 60 days post infection.

trait	Immunized	Immunized+ chitosan	chitosan	C-	C+
Non- Infection**	3. 645± 0.03 C	4.946 ± 0.01 B	5.607± 0.04 A	3.232± 0.01 A	
Post- Infection **	3.034± 0.03 D	3.733± 0.13 C	4.878 ± 0.06 A	3.232± 0.01A	2.174± 0.01 B

**The trait means which carried different letters horizontally indicates high significant differences at probability level 0.01.

Measurement of Malondialdehyde level in serum:

Level of MDA according to these results show elevation in infected groups comparing with non-infected groups , also the present study explained that significantly decline in the serum levels of MDA in infected animals feed diet supplement with chitosan only followed by immunized –chitosan infected group and immunized infected animals as compared with control infected group, table(4).

Table 4. Mean of serum levels of MDA in non-infected and infected immunized females animals at 60 days post infection.

trait	immunized	Immunized+ chitosan	chitosan	C-	C+
Non-Infection**	431.400 ±1.39A	67.779 ± 1.04 C	42.004 ± 0.91E	83.67± 41.67A	
Post-Infection**	553.230 ± 3.16 A	162.755 ± 12.47 D	108.062 ± 2.50 E	83.67± 41.67A	728.30 ± 0.01 A

Immunohistochemistry analysis

Table (5) showed that liver and spleen of the infected animals with *B. melitensis* expressed high number of cells revealed 8-hydroxydeoxyguanosine (4 and 4 respectively) as comparing with control negative group (1, 1 respectively), in immunized animals (3, 4 respectively), immunized +chitosan group (2, 1 respectively),while there is no any cells expressed of 8-hydroxydeoxyguanosine in animals fed diet supplement of chitosan .

Table 5. Immunohistochemical scoring of liver and spleen 8-hydroxydeoxyguanosine after 60 days of females infection

	Liver	spleen	
Group	Mean± SE	Mean± SE	Score
immunized	59.66 ±0.33 B	80.00 ± 0.58 B	4
Immunized+chitosan	46.00 ±0.55 C	19.00 ± 0.57 C	1
chitosan	0.00 ± 0.00 E	0.00 ± 0.00 E	0
Control+	821.00 ± 0.57A	788.00 ± 0.58 A	4
Control-	10.00 ± 0.54 D	23.00 ± 0.57 D	1

Letters vertically which indicates high significant differences at probability level 0.01.

DISCUSSION

The result showed that the mean serum levels of LH and FSH in animals fed diet supplement with chitosan were higher than those values in non-infected and post infected animals ,these ideas may indicated that vaccination wit Rev-1 vaccine does not improve fertility but chitosan may improvement fertility of female ,since these hormones are considered a basic factors in reproduction ,these idea was supported previously studies demonstrated that the LH

stimulated theca cells to produce androgen during folliculogenesis, FSH stimulated granulose cells to produce aromatase enzyme which converted androgen into estradiol in the granulose cells ,therefore both gonadotrophins (LH and FSH) play role in production of estradiol during folliculogenesis in addition follicular maturation was regulated by growth factors such as insulin growth factor I,II which appeared in both theca cells and granulose cells during folliculogenesis [28]. It was demonstrate in the present study that infection by *B.melitensis* lead to decrease in the levels of LH and FSH in all group particularly in control infected animals, these result may indicated that ROS associated with Brucella infection cause damage of anterior pituitary gland a main source of LH and FSH hormones or the Brucella infection induced damage in the ovary, since LH and FSH hormone ,glycoprotein in nature, produced by anterior pituitary gland under the influence of gonadotropin releasing hormone(GnRH) that release from the hypothalamus, these hormone were regulated by ovarian hormone, progesterone and estridiol (negative and positive feedback mechanism) [29]. In Post-infection ,it was reported that the decline in the serum levels of LH and FSH in animals feed diet supplement with chitosan with or without vaccination were less than those in control infected animal and vaccinated animals only these result may indicated that *B. melitensis* infection cause oxidative stress and chitosan act as antioxidant removed or prevent oxidative stress production which protected the pituitary gland and ovary from oxidative damage that lead to less lower in the levels of serum of LH and FSH, therefore Levels of LH and FSH may considered a marker of healthy condition of ovary and pituitary glands as well as folliculogenes and estrogen levels ,these evidence was correlation with previous observation explained that LH and FSH were considered important markers of defect in the pituitary and ovarian disorder [30]. and depression in the LH associated with decrease levels of estrogen that effect on reproductive tract due to LH stimulated theca cells and mature follicles to produced estrogen[, in non-infected animals, the current result showed that high levels of serum total antioxidant in animals fed diet supplement with chitosan as comparing with other groups and immunized animals expressed ,low levels of serum total antioxidant as compared with other group ,these result may indicated that chitosan act as antioxidant agent since the host commonly exposure to endogenous ROS in which chitosan may neutralized these ROS, the low levels of TAC in immunized animals in the present study may indicated that the immune production may associated with oxidant production since the levels of TAC was high in immunized chitosan group as compared with immunized and control negative group, these indicated also chitosan act as antioxidant

The low levels of serum total antioxidant in immunized animals may indicated that over stress resulting from active immune cells produced ROS, these idea was agreement with [31] who showed that increased ROS in the plasma of immunized bats , the same result was observed by [32] in the birds ,also [32] recorded that increase in the levels of ROS with decrease of antioxidant post immunization. Increasing in the levels of ROS during immune response as a result of elevated of the host metabolic rate [33] that associated with increased activity of the mitochondria and consequently mediated ROS production [34] , also the immune response cause elevated number of WBCs that produced ROS in order to kill the pathogen [35]. The current result showed that ,post infection, decline in the levels of TAC in all groups but the lowest levels of serum total antioxidant in non-immunized infected animals followed by immunized infected animals ,but these levels were high in immunized chitosan group followed by chitosan group as comparing with other groups, ,these result also may confirmed idea that Brucellosis was associated with oxidative stress that consumption of antioxidant, these idea was agreement with [36], who found diminished in the levels of TAC in the serum of human suffering from brucellosis , previously [37] demonstrated that ROS can produced by natural infection agents ,also [38] found that the

pathogenesis of chronic viral hepatitis was associated with oxidative and nitrosative stress, Imbalance between production of ROS and removal them by antioxidant called oxidative stress that cause differences pathological changes [39 ; 40 and 41) such as modified protein ,lipid peroxidation and DNA damage [42]. In non-infected animals ,The present study demonstrated that the lowest serum levels of MDA in the animals fed diet supplement with chitosan followed by immunized chitosan group as compared to immunized animals which expressed high levels of MDA, post infection, the highest levels of MDA were seen in control positive group, these result may indicated that *brucella melitensis* was associated with oxidative stress , these observation also supported above idea that Brucella infection mediated release of free radical that attach polyunsaturated fatty acid of cellular membrane cause lipid peroxidation ,these evidence was agreement with [36] who found that significantly increase in the levels of Plasma total peroxide and malondialdehyde in patients with brucellosis as compared with healthy controls and they suggested that Oxidative stress was increased in patients with brucellosis.

The present finding recorded that ,post infection, lower levels of MDA in the animal fed diet supplement with chitosan followed by immunized chitosan group ,but immunized animals expressed high levels of MDA as compared to immunized group fed on diet supplement of chitosan, chitosan supplement group and control negative group ,these result may indicated that vaccination program may protected the animals against infection but may be it not protective the immunized host against over production of ROS as a result of Brucella infection, it was suggested that the levels of TAC and MDA may give indicator of releasing of ROS in the Brucella infection due to these parameter are a good sensitive indicators of oxidant – antioxidant condition of the host body and they are used to evaluate the oxidative stress status but these parameters are not specific for specific disease due to using technique, sampling hr in the day physiological and pathological condition of the body are influence in these parameters however, these parameters are a better indicators of oxidative damage particularly DNA damage [43]. According to the present result, it was suggested that *B.melitensis* infection was induced oxidative stress that decrease antioxidant enzymes and increase levels of MDA, however, high levels of MDA indicated lipid peroxidation, essential marker of oxidative damage and insufficiently antioxidant mechanism to prevent oxidative stress [44; 45; 46]. Decrease of MDA in animals feed chitosan post infection, in present study may be indicated that chitosan scavenger of free radicals due to its antioxidant features an or inhibited lipid peroxidation through antilipidemic activity [47], also [48] found that chitosan have highly activity against certain highly toxic environmental pollutants such as TCDD. The present finding revealed 100% of pregnancy in animals fed diet supplement with chitosan, these result may supported idea that infection with *B. melitensis* generated ROS that influence on reproductive activity of patients and chitosan act as antioxidant that protective the sperms and oocytes against oxidative stress ,these evidence was in consistent with [49] who demonstrated that increase in fertilization and pregnancy rate in mice post administration of melatonin which cause diminish in intra-follicular oxidative damage. Moreover, it was recorded low number pregnant females infected with Brucella as compared with other groups these result may indicated failure of implantation embryo ,these evidence was agreed with result of LH and FSH examination these result may indicated improper condition in the endometrium for implantation, LH cause ovulation and carpus luteum formation that produced progesterone which mediated preparation of the endometrium for implantation [50] , also the present result may indicated that during fertilization and pregnancy ,the gametes and embryos can exposed to oxidative stress [51] that associated with impairment of oocyte intracellular calcium ions homeostasis, oocytes maturation and fertilization [51], Oxidative damage of DNA were recorded by several authors[52; 53] these

changes cause poor fertilization, defect in embryo development, loss pregnancy and impairment birth ; in addition to offspring illness including autism and childhood cancer [54]. However, the low percentage of pregnancy in infected females as compared with vaccinated –chitosan group or chitosan group only after infection may supported idea that oxidative stress play important role in the pathogenesis of reproductive tract infection by *B.melitensis* which associated with impairment implantation embryo or dead of embryo at early stage of gestation due ,embryos is highly sensitive to ROS injury due to lack sufficient antioxidant system ,However, according to the present result with elevated of oxidative stress markers in the present study, it was suggested that ROS cause females infertility [55 ; 56] recorded that normal levels of ,ROS play role in normal function of the female reproductive tract. The current study explained increased in the levels of serum 8-OHdG in the animals infected with *B.melitensis* ,these result indicated that the brucellosis associated with production of oxidative stress that cause oxidative damage of guanine base of DNA at C8 site ,these evidence was in consistent with [57] ,who demonstrated that the DNA was highly susceptible to ROS due to their guanines base characterized by lowest ionization potential ,guanine can hydroxylation by ROS at C8 site that lead to generate8-hydroxy-2-deoxyguanosine (8-OHdG) which considered a main biomarkers of oxidative DNA base damage by ROS [58]. Also these products can used as a marker for mitochondrial dysfunction and defect in metabolism rate [59], however hydroxylated guanine can removed by body fluid with repair of DNA damage [60]. ROS also cause disrupted metabolic enzymes and cell me membrane transporters [61], the present result may indicated that ROS cause destruction of the mitochondrial DNA which is a target organs for oxidative damage ,these idea was agreement with [62] who suggested that mitochondrial DNA are more susceptible for oxidative damage due to DNA of mitochondria located closer to the site of ROS generation ,the high levels of 8-OHdG may indicated that Brucella melitensis may produce ROS that induced damage of DNA ,these idea was agreement with[43] in migraine patients who expressed bacterial infection induced damage of DNA and neurogenic inflammation[63]. The present study demonstrated that animals administrated with chitosan with or without vaccination expressed low levels of 8-DhG,these mean that chitosan can scavenger of ROS and help in repair damage DNA and act as antimutagenic agents ,these idea was in consistence with previous authors who demonstrated that chitosan act as antimutagenic [64] antimicrobial [65] and free radicals scavenger [66] .

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SOME ANATOMICAL AND HISTOLOGICAL STUDIES IN LIVER OF WHITE-EARED BULBUL (*Pycnonotus leucotis*)

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ABSTRACT

The objective of this investigation was to study the anatomical and histological structure of liver in Bulbul, the anatomical study showed that the liver in white-eared Bulbul located on each side of midline in the body in hepatoperitoneum region consist of undivided two lobes left lobe small in size, pyramid in shape and have three surfaces parietal, medial and visceral surface. The right lobe large in size, triangular in shape and extend more caudally than the left lobe and have two borders and two surfaces, medial and lateral border, parietal and visceral surface with the gallbladder. The histological study regarding the liver indicate enclose by serosa membrane with absence of interlobular septa and without boundaries between the liver lobules, the hepatocytes are dense, polygonal with granules in their cytoplasm and oval nucleus, the hepatocyte arranged as thick plates in 2-3 cells thickness, the sinusoids are very small in size and reduce in number.

Keywords: *White-eared Bulbul, Liver, Hepatocytes*

INTRODUCTION

The liver is the largest internal organ of the body and the largest gland tissue. Its functions include the production of bile, detoxification, and maintenance of the body metabolic homeostasis which includes the processing of carbohydrates, proteins, lipids and vitamins. The liver also plays a key role in the synthesis of plasma proteins, like albumin, fibrinogen, and complement factors (1). The avian liver is suspended by peritoneum that is connected with overlying air sac and surrounded by hepatic coelomic cavities (2, 3). The liver weight of the male turkey was (1.89 ± 0.112) % (4), in geese the liver weight (2.105 ± 0.071) % relation with the body weight (5). The liver differed in size, color, lobed shape and the presence of secondary lobes according to the species of birds (6). The avian liver is covered by a peritoneal layer of mesothelium (7). The principal cell of the avian liver is the hepatocyte, avian hepatocytes are polyhedral cells with a large rounded oval and centrally located nucleus, and the sheets of hepatocytes are separated by sinusoids (8, 9 and 10). The liver parenchyma of birds resemble the liver of mammalian but there is some difference in histological features such as the absence of lobules and interlobular trabeculae (9, 11, 12 and 13). The aim of the study: was to provide anatomical and histological information about the liver in white-eared Bulbul.

MATERIALS AND METHODS

The number of birds that used in this study five adult Bulbul for anatomical study and the same number for histological study were collected from Al-Hilla city markets, all birds were free from any diseases, the birds were deeply anesthetized by using intramuscular administration of a combination ketamine and diazepam at dose 25.5 mg/kg of body weight (14), after that open the chest to make bleeding by puncture the heart to occur full hemorrhage, then wash with tap water get rid of impurities that may be found during opening the chest. The liver separated for anatomical study, and fore histological study the liver were cut in different areas in size 1cm and fixed with formalin 10 concentrations, then dehydration, clearing, embedding and cutting in thickness 5 microns and mounted on glass slides then stained with hematoxylin and eosin, then used mounting medium and covered then examined under light microscope.

RESULTS AND DISCUSSION

Anatomically the liver of White-eared Bulbul located on each side of midline on the body in hepatoperitoneum region, red-brown to dark-brown in color (fig: 1) this is similar to results pointed by (4,5) who reported that the color of liver in male turkey (Meleagris gallopava) and adult male geese were red-brown to dark –brown respectively. The liver of white-eared Bulbul consists of two lobes, the left lobe smaller than right (fig: 1&2) this result agreed with (9, 12, 13, 16) whom reported the same result in liver of domestic fowl and also (15)in liver of local coot birds.

Left lobe of white-eared Bulbul are undivided this result similar with (17) who said no further lobular subdivisions of liver in Houbara Bustard and (6) who talk the left lobe undivided in liver of *(Agaporins fischeri)* and *(Larus canus)* but unlike with results of (9,12) whom mentioned the left lobe of domestic fowl subdivided into the dorsal and ventral parts and also differ with (18,19) whom indicate the left lobe in ostrich is subdivided into three parts, two parts small caudodorsal and intermediate ,and one part large caudoventral , the result of the current study showed the right lobe of white-eared Bulbul also undivided (fig:2) this agreed with results of (4,9) in male turkey and domestic fowl respectively and results of (6) in three species birds *(Agaporins fisheri)* , *(Numida meleagris)* and *(Larus canus)*. The left lobe pyramid in shape its base located cranially and apex toward caudally and have three surfaces , parietal surface slightly convex , this result similar with (6) who said the left lobe has concavity in its top in *(Numida meleagris)* , the parietal surface smooth in appearance and covered by peritoneum , the left side of the heart cupped by the medial surface of left lobe and engaged completely by its impression , the visceral surface is irregular and strongly attached with adjacent organs gizzard and intestine at the base while the apex free and also cover by peritoneum (fig:2).

The right lobe triangular in shape and extend more caudally than the left lobe, its cranial end wide while the caudal end pointed and bent cranially, the right lobe have two borders and two surfaces, the medial border, which contain impression of the right side of the heart in its cranial half while the caudal half of this border sharp, the lateral border also shop along its length, the parietal and visceral surfaces of right lobe are similar to the left lobe (fig: 1). Gall bladder is found in liver of white-eared Bulbul as small sac originated from visceral surface of right lobe and extend shortly between limbs of duodenum (fig:1b) that not similar with results of (6) about disappear of gallbladder in liver of *(Agaporins fisheri)* but like with him who said the gallbladder present and well developed with elongated shape and attached in dorsal side of right lobe and appears divided at its bottom in liver of *(Numida meleagris)* and also well developed in liver of *(Larus canus)* which appear long in shape ,looks like a grain of pumpkins and attached to the middle dorsal side of right lobe and also disagree with (19,20,21)

whom talk the gall bladder is exceptionally long and extend as far as cloaca in woodpeckers (Picidae), toucans (Ramphastidae) and barbets (Capitonidae). Histologically the parietal surface in liver of white eared Bulbul enclose by serosal membrane which exhibit thin layer of connective tissue (fig: 3b) that not similar with (23) who said the liver of ostrich enclose by thick connective tissue capsule, the capsule covered by simple squamous epithelial known mesothelioma (Glisson's capsule) that agree with (22) as the capsule in liver of chicken, the visceral surface attach to neighboring organs by connective tissue at base of liver while the apex was covered by mesothelioma. The interlobular septa are loss in the liver of white-eared Bulbul, there no distinct boundaries between the liver lobules, (24) notes the same result in most liver of mammals except camel and pig. There was a central vein in each hepatic lobule this result also seen by (25, 26) in chicken, (4) turkey and (7) psittacine. The liver parenchyma composed of dense polygonal hepatocyte with granular cytoplasm and oval nucleus ,the hepatocyte arranged as thick plates in 2-3 cells thickness in orientation forming hepatic cords ,between the hepatic cords there are very reduce sinusoids in size and numbers which open in the central vein (fig:3a) the results didn’t resemble with (27) in chicken and (15) in local coot birds whom talk the thickness of hepatic cords in this birds formed from 2 cells and also didn’t resemble with (6) how describe the hepatic cord or plates consisting 1-2 hepatocyte in three species birds *(Larus canus), (Agaporins fischeri)* and *(Numida meleagris).*

The central vein and sinusoid together lined with flat cells called endothelial cells, the Kupfer cells are less numerous and these cells characterized by a large nucleus with debris in their cytoplasm (figure 4) (28) showed the same result in ostrich. The portal area are identified by connective tissue with branch of portal vein, hepatic artery and two or three branch of bile duct ,the portal vein characterized by thin wall while the hepatic artery have thick wall, the portal vein and hepatic artery lined by endothelial cells, the bile duct lined by one layer of cuboidal cells underlying subendothelial connective tissue (fig:4) , our result are similar to the (3) in duck,(4) in Turkey and (15) in coot birds *(Fulica atra).*

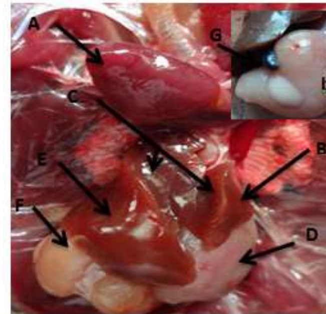

Figure 1. photograph position of liver A, heart - B, parietal surface of left lobe- C, medial surfaces &impressions of heart - D, gizzard - E, parietal surface of right lobe - F, small intestine- G, gallbladder

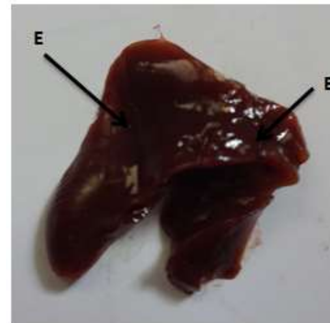

Figure 2. photograph visceral surface of liver B, left lobe - E, right lobe

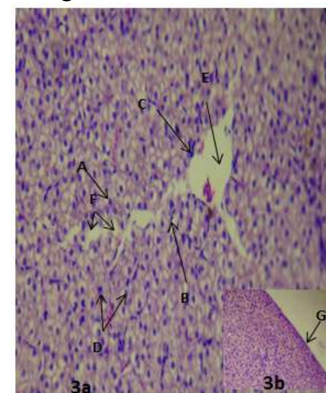

Figure 3a and b. Histological cross- section of liver in Bulbul show (a) A, hepatocyte – B, endothelial cells – C, Kupffers cells- D, hepatic cord – E, central vein – F, sinusoids .H&E (40X) - (b) liver capsule- G, mesothelium. H&E (10X)

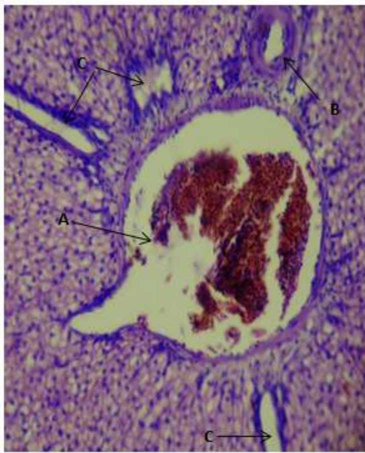

Figure 4. portal area of liver in Bulbul show A, hepatic vein – B-hepatic artery – C, bile ducts H&E (40X)

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ASSESSMENT OF SOME IMMUNOLOGICAL AND BIOCHEMICAL CHANGES WITH HEPATITIS B IN BABYLON PROVINCE

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ABSTRACT

This study was done to illustrate some immunological and biochemical changes in patients infected with hepatitis B virus in Babylon province. Thirty serum samples were collected from patients with hepatitis B (HBs) who were admitted to Teaching Hospital in Babylon province aged from 20-60 years during the period from October 2015 to March 2016, also serum samples were collected from fifteen healthy aged from 18-52 years as a control group. In this study, the serum levels of complement types C3, C4 measured by Radial immunodiffusion plate kit but liver enzymes as Glutamic-Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT) and Total protein were measured by biochemical kits. These parameters measures in two groups. The results review that serum complement components (C3 and C4) were different in HB patients in comparison to control group. It was found that there was a significant decrease in the levels of C3 and C4 patients group compared to control group. The results of this study indicate that serum GOT and GPT concentration was a significant increase in the levels of these enzymes patients group compared to control group. While results of the total protein recorded a significant decrease in the levels of patients group compared to control group.

Keywords: *C3, C4, GOT. GPT. Total protein*

INTRODUCTION

Hepatitis is a Latin word describes the inflammations that occur in the liver [1]. Hepatitis may start and get better quickly (acute hepatitis) or cause long-term disease (chronic hepatitis). In some instances, it may lead to liver damage, liver failure, or even liver cancer and how severe hepatitis depends on many factors including the cause of the liver damage and any illnesses in the body [2]. Viruses, bacteria, parasites and helminths are important infectious etiologic factors of hepatitis [3]. Hepatitis B is a viral disease with a high incidence and prevalence worldwide and it can cause acute and chronic liver disease , Approximately (8%) of the world's population has been infected with HBV, and about (350 million, 5–6%) are persistent carriers of HBV [4] The clinical presentation ranges from subclinical to symptomatic and, in rare instances, fulminate hepatitis [5] . Prenatal or childhood infection is associated with few or no symptoms, but it has a high risk of becoming

chronic. There are a limited number of medications that can be used to effectively treat chronic hepatitis B and effective vaccine is available to prevent hepatitis B infection [6]. The aim of study assessment of C_3, C_4, GOT, GPT and total protein in patients with Hepatitis B in Babylon province compared with healthy.

MATERIALS AND METHODS

A- Patients:
Thirty patients were included in this study. Those patients were attended to the teaching hospital and diagnosed by specialist doctors as clinical and lab. test to hepatitis B infection by rapid supply from biotech. co.

B- Controls:
Fifteen apparently healthy subjects (clinically assessed by specialist doctors) were included as controls in this study, which consist of (10 males) and (5 females). The range age of those subjects was (20-40 years). Those subjects were selected randomly from the population.
Measurement of C3 and C4 proteins: The concentration of, complement component C3, C4 were measured by a radial immune diffusion (RID) method in which equal volumes of reference sera and test samples were added to wells in agarose containing monospecific antisera. The sample diffuses radially through this gel and the substance being assayed from a precipitin ring with the monospecific antisera. Ring diameters were measured and a reference curve is constructed on graph paper. These methods include follwoing steps: 1-Five µl of each serum sample was dispensed by a micropipette into one well of each plate (containing 15 wells for C3 and 15 wells for C4. The plate was left open for (10–20) minutes, then covered and left at room temperature (20–25) °C for (3–4) days for precipitin ring to be forms. 3- The diameter at each immune precipitating using formed around each well was measured in mm by the immune viewer and the concentration of complement level was calculated from a standard curve.
Measurement of GOT, GPT: The concentration of GOT, GPT in patients infected with HBs was done according to kits supplied from Randox Company, U. K.

RESULTS

Clinic-pathological characteristics of patients with HBs included in this study are classified according to the age and sex as showing in table (1).
The humeral immunity is provoked by specific antibodies that distinguish and react to a challenge, for that reason the humeral immunity or antibodies – mediated arm of the immune system as well as the humeral part mediated by complement components, as a result the serialized assessment of serum complement may make available marker as useful for disease progression and therapeutic monitoring [7] . Concerning the serum complement components (C3 and C4) were evaluated in HBs patients in comparison to control group. It was found that there was a significant decrease ($p \leq 0.01$) in the mean value of C4 (9.83 ± 5.34) and C3 (35.22± 20.47)among patients group compared to C4 control group (32.54 ±7.67) and C3 control group (117.52 ± 17.89) as shown in (table 1,2).

Table 1. Clinic-pathological characteristics of patients with HBs included in this study

Clinic-pathological variables	NO.	**%**
Total no. of patients	30	100%
- **Age**		
- <30	12	36%
- ≥30	18	64%
- **Sex**		
- Male	16	48%
- Female	14	52%

Table 2. Mean values of C4 protein (mg/dl) for patients infected with Hepatitis B virus compared with control group.

Complement (C4)		Mean ± SD	No.
Groups	Patients	9.83 ± 5.34	30
	Control	32.54 ±7.67	15
Total		p value =(p≤ 0.01)	45

Table 3. Mean values of C3 protein (mg/dl) for patients infected with Hepatitis B virus compared with control group.

Complement (C3)		Mean ± SD (mg/dl)	No.
Groups	Patients	35.22 ± 20.47	30
	Control	117.52± 17.89	15
Total		p value =(p≤ 0.01)	45

Results of serum GOT and GPT indicated to evaluated in HBs patients in comparison to control group . These results recorded in figures (1,2) that show a significant increase in the levels of GOT (41.13±16.70) and GPT concentration in patients infected (38.43± 17.34) with HBs compared with healthy group or control group that mentioned concentration of GOT in healthy group is (13.80±2.45)while the concentration of GPT in healthy group is (10.26± 1.944)

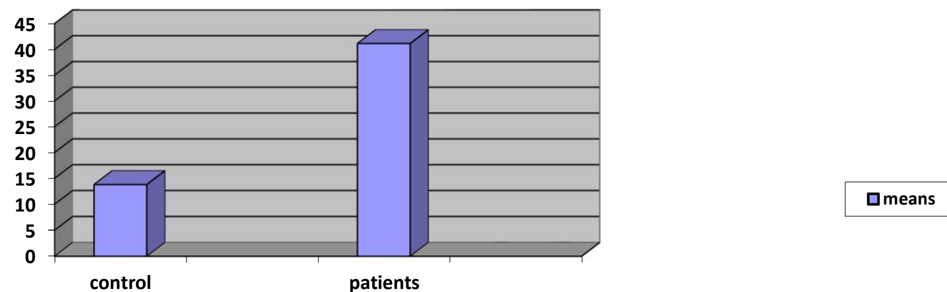

Figure 1. The Mean value of GOT (UI/ L) in patients with Hepatitis B (HBs) group compared with control group

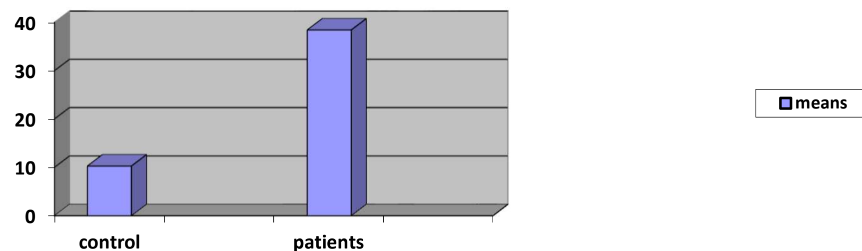

Figure 2. The Mean value of GPT (UI/ L) in patients with Hepatitis B (HBs) group compared with control group

The present study suggested positive correlation between ages and C4 levels(R2=0.242, P-value > 0.05), as showing in figure (3).

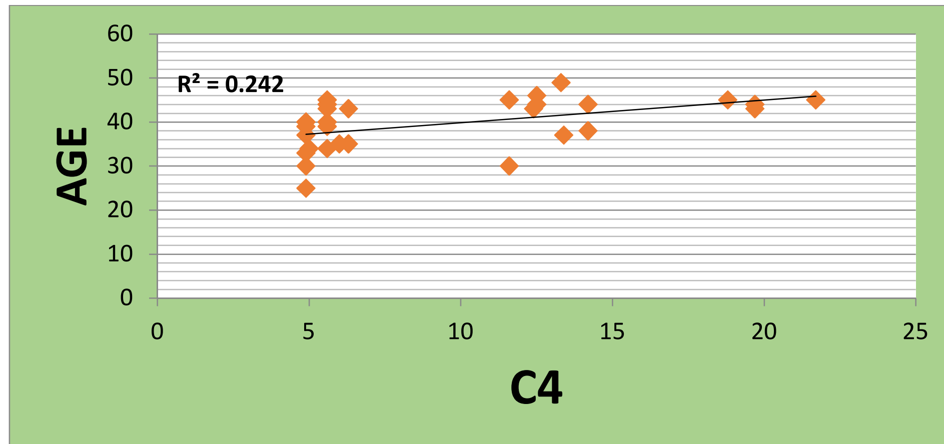

Figure 3. correlation between ages and C4 levels of patients with HBs.

Lastly, the results of total protein in patients infected with HBs proved finding a significant decrease in the levels of total protein in patients (4.38 ± 1.9) compared with control group (6.65 ± 1.3). These results showed in figure (4).

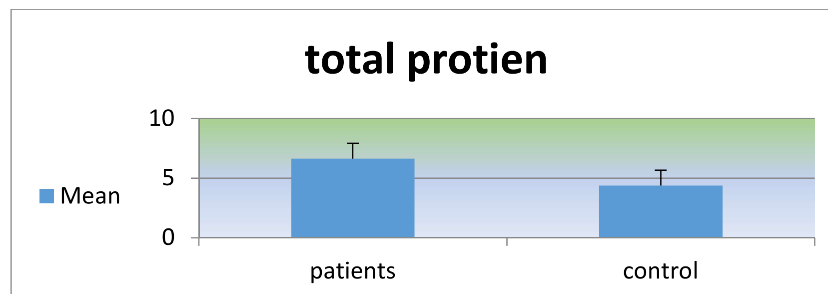

Figure 4. The mean value of total protein (g/dl) in patients with Hepatitis B (HBs) group compared with control group

DISCUSSION

Hepatitis B virus (HBV) infection is a major public health problem and its outcome depends on the kinetics of the virus-host interaction and in particular on the strength of the innate and adaptive humoral and cellular immune response [8]. The results of the present study revealed to a significant reduction in serum C3 and C4 levels in patients with viral hepatitis compared with control group. These results resembled those reported by other investigators [9] so complement activation is one of the earliest responses to infection including viral hepatitis and its level has been shown to be reduced in those diseases [7]. Other studies were reported a significant reduction in serum C3 and C4 levels in some patients with viral hepatitis during different phases of the disease [10] and the decreasing of complement levels in liver diseases has been assumed to be as the result of the failure of components synthesis in the liver [11].

The results of the present study indicated that the aminotransferase GOT and GPT enzymes will elevate in HBV infection and this finding is consisted with other study done by and it accept with other study that mentioned the mean value of GOT and GPT for HBs patients was higher than of controls [12]. Aminotransferase are normally intracellular enzymes (mainly hepatic cells), and low levels found in the plasma represent the release of cellular contents during normal cells turnover. Due to the elevation of plasma aminotransferase level indicates damage to cells rich in these enzymes, such as viral hepatitis [13]. Also, the results of this study indicate that the total serum protein decreases significantly ($p<0.01$) in patient groups that means impaired hepatic function. Because of the liver reserve capacity and the relatively long half-life of these proteins, the measuring of total protein is, for example, refer to hepatitis [14]. The decreasing in complement C4 was more than the falling in the levels of C3 and these results may be due to effecting of bacterial infections. The present study suggested that the elevation in aminotransferase enzymes and falls in the levels of immune proteins in combination may be a useful indicator to diagnosis and monitoring of patients with hepatitis B. Further studies are needed to understand the reduction in C3 and C4 levels and its correlation with other HBs parameters.

Conflicts of interest

The authors have no conflicts of interest to declare in relation to this paper.

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RESEARCH PAPER Journal of Al-Qasim Green University (JQGU)

INVESTIGATION OF HBV AMONG HOSPITAL PATEINTS IN BABYLON PROVINCE: A COMPARITIVE ASSESSMENT STUDY

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ABSTRACT

The prevalence of viral hepatitis B in general population in Babylon Governorate were covered. A total of (354) general population sample (age range <15->45 years with mean age 38.64 years) were collected for research investigation on random basis then tested for anti-HBsAg. The results showed that prevalence of HBV in general population was (3.95%) since that there are (14) samples had positive result for Anti-HBc IgM and patients with age group (>45) had the higher rate of infection (5.6%). According to the sex; male was more infected than female (10:4) respectively with prevalence rate (71.:4:28.6) respectively. A total of (14) positive anti-HBsAg (9) of them were in rural regions while the rest (5) were in urban with rate of infection≈ (2:1) respectively. The diagnostic marker anti-HBc IgM were more compatible with viral load /Ml of HBV by RT-PCR than anti-HBsAg which obtained by ELISA test.

Keywords: HBsAg , HBcIgM, ELISA, Minividas ,RT-PCR

INTRODUCTION

Hepatitis is a Latin word describes inflammation of the liver [1]**.**Hepatitis may start and get better quickly (acute hepatitis) or cause long-term disease (chronic hepatitis). In some instances, it may lead to liver damage, liver failure, or even liver cancer and how severe hepatitis depends on many factors including the cause of the liver damage and any illnesses in the body [2]. Two billion people worldwide have been infected with the virus and about 600 000 people die every year due to the consequences of hepatitis B [3]. The prevalence of anti-HBsAg varies among countries of the region: 4%–5% in Iraq, 3%–11% in Egypt, 2.6%–10% in Jordan, 2%–6% in the Libyan Arab Jamahiriya, 2.3%–10% in Oman, 5%–6% in Palestine,7.4%–17%in Saudi Arabia, 16%–20% in Sudan,6.5% in Tunisia, 2%–5% in United Arab Emirates and 12.7%–18.5% in Yemen [4] It has a circular form of partially double-stranded DNA and is approximately 3200 nucleotides in length [5]. A 42-45 nm long HBV spherical form named"Dane particle" is the full virion with infectivity that can be visualized by electron microscopy [6].The virus has two- layered shells. The outer shell is the envelope protein referred to as HBs protein which is further divided into small, middle, and large HBs proteins (SHBs, MHBs and LHBs proteins, respectively) and the inner shell is a core protein referred to as the HBc protein in which viral polymerase and HBV genome is enclosed [7] . The Aims of this study is to assessment different technique (ELISA ,Minividas and Real Time for detection HBV, and to investigate HBV

prevalence in Babylon province.
.

MATERIALS AND METHODS

Distribution of samples:
A total of (354) general population sample (age range 6-64 years with mean age 38.64 years) collected from the central public health laboratory, blood bank, Hilla teaching hospital and Marjan teaching hospital in Babylon province.

Blood collection
Three to five milliliters of venous blood was collected from each enrolled people at 9-12 PM in public health laboratory. One ml was mixed with anticoagulant (EDTA) for plasma collection. The rest were used for serum collection which is separated by centrifuge at 1000 rpm for 5-10 minute and then refrigerated or frozen at -20 until used for the required test. For determined anti-HBsAg we used a third-generation enzyme immunoassay kits (EIA-3) was used Acon–USA).The anti-HBsAg reactivity was confirmed by enzyme –linked fluorescent immunoassay (ELFA) that is performed in the automated VIDAS system (Minividas Kit/HbsAg, Biomerieux-france). Then the viral load was measured by RT-PCR using Exiprep^{TM} viral DNA/RNA kit (Bioneer-Koria) for extraction of the DNA of the viruses and RT-PCR Amplification kit, (Sacace-Italia) was used to DNA amplification of this virus.

RESULTS AND DISCUSSION

Infection by HBV begins when the immune response that normally clears the infection fails to take place or is too weak to be effective; thus infections are more common in low immunity subjects as a result of poverty [8]. The current study indicated that epidemiology of anti-HBsAg in general population was (3.95%) and the age group (<45) had the higher prevalence of infection (5.6%) then the age group (25-44) with prevalence (5.5%) as reveals in table (1). This may back that these age groups are more exposure to the risk factor of infection may be during the work, sexual activity or travel, however; the statistical analysis indicated there is relation between age and age of infected persons (p>0.05)in these age group compare to the ages enrolled in this study.

Table 1. Tests result of anti-HBsAg in general population by ELISA test

Age groups	No. test	Anti-HBs Ag+	Anti-HBc IgM+		ELISA		
			NO	Index	Mean of the O.D(650nm) of negative control	Mean of the O.D(650nm) of positive control	Mean of the O.D(650nm) of patient samples
>15	95	2	1	0.67	0.011	0.496	1.64
15-24	98	4	3	0.89	0.034	0.360	1.42
25-44	72	3	2	0.83	0.03	0.322	1.86
45<	89	5	1	0.49	0.06	0.400	2.00

The statistical analysis relieved significant difference between the mean of optical density of patients samples and negative control in all age groups ($LSD_{(0.05)}$1.783).

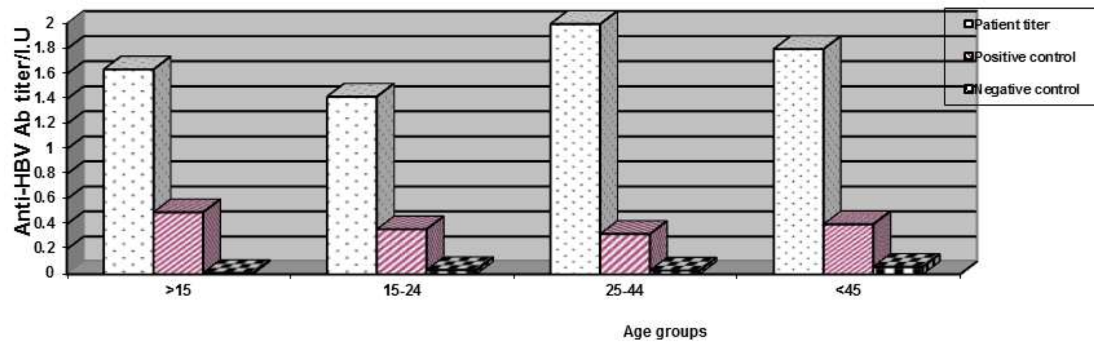

Figure 1. The status of anti-HBs Ag in different age groups of general population by ELISA test

Table (1) indicated the incidence of anti-HBsAg in general population was (3.95%) since that a total of 14 positive samples , there were 7 samples with positive anti-HBcIgM which is a marker for acute HBV infection or recent hepatitis B virus infection[9]. This result was also documented by [10] who explain that; the age of infection is mainly at birth time by the vertical transmission (from mother to baby) but the time of presentation between 25 to 35 years old is because the patients are asymptomatic and discovered accidentally by routine investigation during blood donation, mar ried or employment and all of them in this age group. The prevalence of infection in the older age groups explained on the basis of multiple risk exposures of aging groups including injections with syringes, blood transfusions and invasive procedures. Several results reported by [11], [12] and [13] confirm the result of the present study. According to the sex; the male was more infected with the disease than female (10:4) with rate (71.4: 28.6) as relive in the figure (2):

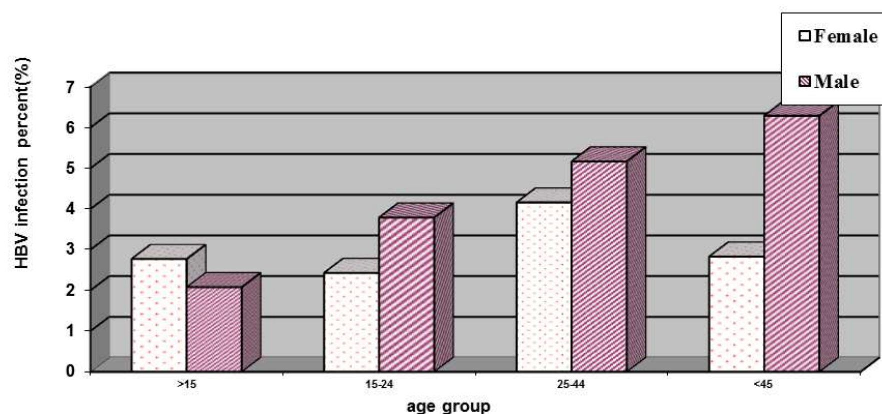

Figure 2. The relation between age groups and HBV infection in general population according to age and sex.

This result was confirmed by other studies, like [14] also this fact is well documented by [15] that higher anti-HBsAg seroprevalence has been reported in male than in female for populations in any Asia country as well as [16] and [17] found that men predominate women in all populations of anti-HBsAg carriers. The sex is well established, but poorly understood as well as a determinant of chronicity; however, women are more likely than men to clear anti-HBsAg [18]. These results confirm the previous studies in this field in Iraq [19]) and [20]. A total of 14

positive anti-HBsAg (8) of them was in urban regions, while the rest (6) were in rural with rate of infection (2:1). The distributions of hepatitis B viruses according to residence in this study indicated the prevalence of them in urban areas than those in rural areas with a significant differences ($p < 0.05$). This finding is may be due to the health education in the urban is better than in rural areas which leads to early discovery of this disease .the high prevalence in urban areas might be described to the crowdness in urban areas which may facilitate the transmission of HBV and HCV. These results are compatible with other studies in Iraq by [19] and [10]. According to the economic status the distributions of hepatitis B virus in this study indicated the high prevalence of them in low economic stats, then the medium and the lower prevalence were in high economic status with a significant difference ($p < 0.05$).The ratio of good, medium and low economic status in HBV was about (1:1:2) respectively. The present study agrees with other studies done by [21] who found a lower socio- economical level and less hygienic living conditions are more susceptible HBV than the others. It is similar to results of other studies were done in Japan by [16] who showed that the prevalence of HBV is higher in the lower socioeconomic state .Other results reported by [22] and [23] were confirmed these results. According to the educational level the distributions of hepatitis B virus in this study indicated the high prevalence of them in patients low educational level (primary and secondary school education) compared to those with high educational level (graduate and post graduate education) with a significant differences ($p < 0.05$).This study found that the subjects with low educational level are more affected than those with higher educational level with HBV at ratio of (6:1) respectively.

Table 2. Distribution of patients by (residency, economical status and educational level

The parameter	Hepatitis B		
	No	(%)	
Residency	Urban	125	(8\125)= 6.4
	Rural	229	(6\299=2.6)
Economic Status	Good	59	(2\59=3)
	Medium	118	6\118=5)
	Low	177	(6\177=3)
Educational level	High	118	(2\118=1.69)
	Low	236	(12\236= 5.1)

Table 3. Tests results of anti-HBsAg in general population by ELISA, Minividas and RT-PCR.

Age groups	Anti-HBs Ag+(ELISA)		Minividas IU/Ml	value	RT-PCR	
	NO.	Index	No.	Value	No.	Viral load IU/Ml
>15	2	20.5	2	15.14	2	$13.07*10^7$
15-24	4	14.2	3	13.90	3	$85.4*10^5$
25-44	3	20.0	8	17.80	8	$11.06*10^6$
<45	5	15.72	13	17.52	13	$1.668*10^7$

Table (3) indicates that the index means of anti-HBsAg obtained by ELISA technique doesn't completely related with viral load of the virus, but the higher mean titer is the same in both techniques (ELISA and RT-PCR) (2.00 and $1.668*10^7$) respectively. This also obtained in the lower mean titer as relieved in the table (3). The same anti-HBsAg prevalence obtained by Minividas technique, but it was more specific than ELISA technique and this back to that all results obtained automatically by the apparatus which measure the result value depending on Calibrator 1 (represent positive control) and calibrator 2 (represent negative control). RT-PCR is an advanced molecular technique by which viral load of the positive specimens was measured. In this study manual and automated procedure was done and the result was the same in all tested blood samples. The results shown in figure3 in general population group refer to positive correlations with the (IgM immunoglobulin) as a part of the adaptive immune response and viral load of RT-PCR test since the used kit of (IgM-HBc (was based on competitive combination principle. The result is compatible with the study done by [24] who found that combination of IgM anti-HBc and HBV DNA viral load has positive colorations and improve diagnostic power. The high viral load titer in infected children in the age group is (≤10). This can be explained by the fact that they were born with the unvaccinated or infected mother or by the contaminated device or the source may be inside of the hospital (nosocomial infection).The higher viremia notice in the age group (31-40) and this means there are newly infected persons in this age group since most of them with positive anti-HBc IgM test results. The graduated decrease is seen in viral load of higher age. This may be because these groups were exposure to the infectious agent before the diagnosis of it occurred and since they had an older age they have more chance to medical procedures as well as the blood transfusion. In conclusion, the prevalence of HBs Ag among general population in Babylon province 3.96%, which is much higher than HBsAg prevalence among healthy blood donors (0.76%). The HBsAg infection was significantly associated with male sex, urban communities, low economic status and low educational level. The determination of viral load of HBV infection by RT-PCR is a forecasting technique to state of prevalence status of Hepatitis B disease.

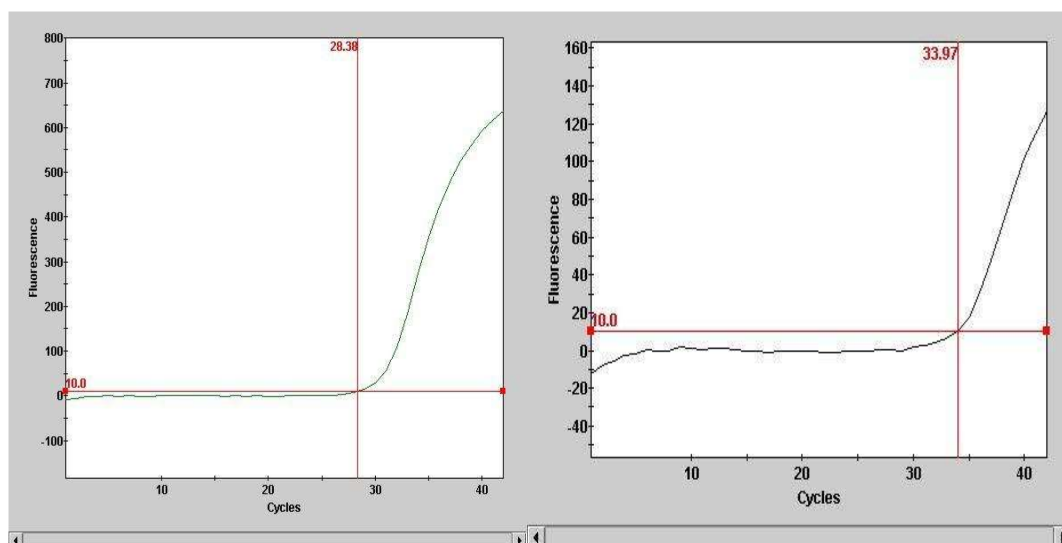

Figure 3. Relative fluorescence vs – cycle number – amplification plot showing two positive cases with two different (Ct)s (28.3 and 33.9 which indicates different levels of viral genome .

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DIAGNOSTIC STUDY OF BOVINE BROCELLOSIS

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ABSTRACT

This study was conducted to investigate the percentage of infected cows with *Brocella abortus* in Babylon city. Seventy three samples were collected from several rural and farm areas of Babylon city. The total samples were divided into aborted (34 samples) and non-aborted (39 samples) depending on the case history, then examined by using Rose Bengal screening test and confirmatory serological indirect (i) ELISA test. The results of Rose Bengal test appear as in aborted animals 13 samples (38.23%) were positive and 8 samples (20.51%) in non-aborted animals, while the result of indirect ELISA test in aborted animals is 0.887-2.543 1.003 ±0.44 and in non-aborted animals is 0.991-2.901 1.231 ±0.55.

Keywords: *Brocella abortus*, detection, cattle, Babylon city.

INTRODUCTION

Brucellosis is the most world wide spread zoonotic disease and is of major health and economic significance [1]. The disease is caused by *Brucella* spp. which can infect several important livestock species, including cattle, water buffaloes, sheep, goat and pigs [2]. The main pathogenic species distributed worldwide are *B. abortus*, which responsible for bovine brucellosis, *B. melitensis*, the main etiological agent of ovine and caprine, and *B. suis*, which responsible for swine brocllosis [3, 4, 5]. The principal symptoms of the infection in all animal species are aborted or premature expulsion of the fetus [6]. The available strategies to control brocellosis are based on very strict management procedures, including the slaughter of all seropositive animals and were allowed vaccination [7]. The traditional and well documented technique for serological diagnosis include Rose Bengal test (RBT), serum agglutination test (SAT) complement fixation test (CFT) and more recently, enzyme linked immunosorbent assay ELISA being put into more regular use [8, 9, 10, 11]. Depending on the sensitivity and specificity tests can be used to screen for, or confirm, disease. Traditionally screening tests are inexpensive, fast and highly sensitive but not necessarily highly specific [12]. The enzyme linked immunosorbent assay is an appropriate screening test since its highly sensitive and specific making it an ideal test for used in international trade [13]. Rose Bengal plate test (RBPT) is considered the more important screening test for detection the early infection than tube agglutination test, also its result in the diagnosis of chronic cases is equivalent to the result of complement fixation test (CFT) [14, 15]. CFT used to detect the infection in vaccinated animal and it is considered more efficacy than tube agglutination test, and complement fixation test becomes negative in

vaccinated animals after 6 months while still positive for a long period in infected animals [16, 17]. This study aimed to diagnose *Brocella abortus* by using serological tests ELISA and Rose Bengal test.

MATERIALS AND METHODS

Animals

A total number of 73 cows from a governmental farm and rural located in Babylon city were used in the present study, The animal history showed abortion of some animals, anther showed reproductive disorder as metritis, endometritis, retained placenta and decrease milk, but some animals showed no signs, all animals of experiment weren't vaccinated against Brucellosis and were examined for brucellosis by Rose Bengal test and ELISA.

Blood samples

A total number of 73 blood samples were collected from the jugular vein of animals, the blood samples were put in evacuated tubes to complete clotting and taken to laboratory for separation of serum by using centrifuge at 300r/min and then the sera were preserved at -20 º C. until used. These serum samples were used for serological examination, information about animals such as case history, health state, age of animals were recorded and the serological tests that used in this study were done according to manufacturer's instructions. ELISA was performed using commercial brucellosis serum ELISA (France) whereas RBT were done according to official instruction and protocol [18-22].

RESULTS AND DISCUSSION

Quick and accurate diagnosis of brucellosis is very important for a positive outcome of eradication and monitoring programs**.** By Rose Bengal plate test (RBT) a total of 73 samples collected from aborted and non-aborted cows were examined by RBPT for Brucellosis. 13, 38.23% of 34 samples of aborted cows showed positive results, the remaining 21 samples considered negative, while out of 39 non-aborted cows 8, 20.51% were positive, the remaining 31 samples considered negative. However, the total percentage of positive results (aborted and non-aborted) was 28.76% 21:73 (table 1); while by using iELISA test the results appeared as a total of 73 samples collected from aborted and non-aborted cows were examined by iELISA test 11 samples 32.35% of 34 samples of aborted cows showed positive results, the remaining 23 samples considered negative, while the non-aborted cows showed 5 samples 12.82% of 39 samples and the remaining 34 samples considered negative. However, the total percentage of positive results (aborted and non-aborted) was 21.91% 16:73 positive samples (table 2). These results were agreed with [23].

Table 1. The percentage of the infection by RBPR of aborted and non-aborted cows.

State of cows	RBPT	+Ve	%
Aborted	34	13	38.23
Non- aborted	39	8	20.51
Total	73	21	28.76

Table 2. The percentage of the infection by iELISA of aborted and non-aborted cows. Mean ±SE 1.003 ±0.44 1.231±0.55

State of cows	*OD range	Mean ±SE
Aborted	0.887-2.543	1.003 ±0.44
Non- aborted	0.991-2.901	1.231 ±0.55

*OD: optical density.

The present study showed that the commonly used conventional serodiagnostic tests for Brucellosis, RBPT may not be absolutely reliable [24]. Though this test is considered one of important screening and rapid test to investigate Brucellosis in animals, there are some false positive results that may be due to the presence of antibodies originated from vaccination or may be due to infection with other organisms, such as *Yersinia enterocolitica* [25], and the RBPT give a false negative results in early stage of infection, or immediately after abortion [26]. Many samples give a rapid agglutination, the agglutination appears after (30) seconds to (2) minute, that referred to the high titer of antibodies in serum [27]. The iELISA test is rapid, easy to perform and cane be automated [28]. The test is considered as one of the most important confirmatory serological tests in the diagnosis of *Brucella* in farm animals because it had 100% sensitivity and 99.7% specificity [29]. Moreover, ELISA is a valuable and reliable addition of brucellosis sero-tests [30]. In conclusion, this study has confirmed the validity of the indirect ELISA is a good confirmatory test to practically being applied in the diagnosis of brucellosis in farm animals. Eventually, RBT is an important screening test in diagnosis of brucellosis infection in cattle. Nevertheless, I recommended making a study involving all Iraq governorates including all animals' types in addition to human at the same time and isolate of all abortion causes in farm animals.

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