Effect of pirfenidone on cardiac complications in a model of Kawasaki disease in female Balb/C Mice: Histological and Immunohistochemical study

Original Article

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ABSTRACT

Introduction: Kawasaki disease (KD) is an immune mediating vasculitis affecting many systems especially the cardiovascular system. Pirfenidone is known for its anti-fibrotic and anti-inflammatory effects.

Aim of work: to study the possible protective effect of pirfenidone on cardiac complications in a model of KD induced by Bacillus Calmette-Gue´rin (BCG) injection.

Materials and Methods: This study included 18 female Balb/C mice that were divided into three groups. Group I (control group), group II (KD group) that received single injection of BCG in tail vein, and group III (pirfenidone group) that received BCG as group II and daily oral pirfenidone till end of experiment. All mice were sacrificed 21 days after the injection, hearts were collected and examined.

Results: Group II revealed irregular separated cardiac myocytes and interstitial inflammation. Coronaries were seen with thin wall and irregular lumen. Localized intimal thickening and irregularly arranged smooth muscles were noticed in the media. Perivasular inflammation was also noticed. Mallory stained sections revealed interstitial, vascular and perivascular fibrosis. Orcein stained sections revealed disruption of internal and external elastic laminae. Positive immune reaction for TNF-α was also noticed in group of KD. Pirfenidone treatment minimized the histological changes induced in KD. Little mononuclear cellular infiltration was still noticed in the myocardium. Moderate collagen fibers were seen in the adventitia and intact elastic laminae.

Conclusions: Pirfenidone aborted the cardiovascular complications associated with KD.

Key Words: Coronary, heart, histology , Kawasaki disease, Pirefenidone, TNF-α

Revised: 19 May 2017, **Accepted:** 17 August 2017

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ISSN: 1110-0559, September Vol. 40, No. 3

INTRODUCTION

Kawasaki disease (KD) is an acute, self-limited vasculitis of unknown etiology[1,2]. It was first described by Tomisaku Kawasaki in Japan in 1967[3,4], and had replaced acute rheumatic fever as the leading cause of acquired heart disease among children in many parts of the world[5]. Kawasaki disease primarily affects infants and young children[6]. It is considered to be an immune-mediated systemic vasculitis and is also known as mucocutaneous lymph node syndrome. The disease affects the skin, lymph nodes, brain, eyes, joints, liver, and heart[3,6].

Incidence of KD has continued to rise around the world and took the top spot in pediatric acquired heart disease[3,6]. Cardiovascular sequel of untreated KD in young adults include myocardial ischemia, infarction, arrhythmia, congestive heart failure, sudden death[3,7,8] and also valvular lesions[9]. The most important and serious lesions in KD are coronary artery lesions[1,3,6,10,11,12] which can cause coronary artery aneurysms or stenosis[6,12]. Coronary artery aneurysm is a serious complication of KD and develop in 5% of treated and 30% of untreated patients[1,7,10]. Complications of coronary artery aneurysms associated with KD, include thrombosis, distal embolization, rupture, and vasospasm[5]. The inflammatory process in KD may also result in myocarditis[3,1012], pericarditis, and endocarditis[3].

The diagnosis of KD is made according to "Diagnostic Guidelines of KD" that describe the following six major findings. (1) Fever persisting for five days or more (inclusive of cases in which the fever has subsided before the 5th day in response to therapy). (2) Bilateral conjunctival congestion. (3) Changes of lips and oral cavity: as reddening of lips, strawberry tongue, and diffuse congesition of oral and pharyngeal mucosa. (4) Polymorphous exanthema. (5) Changes of peripheral extremities: in Acute phase: Redness of palms and soles. Convalescent phase: Membranous desquamation from fingertips. (6) Acute nonpurulent cervical lymphadenopathy[9,10]. Patients with at least five of the above six major findings are diagnosed as typical KD (described as "level A certainty"). A diagnosis of

 DOI: 10.21608/EJH.2017.4656

atypical KD ("level B certainty") is made for patients with four of the six major findings. Physicians should prescribe high-dose intravenous immunoglobulin (IVIG) therapy for patients with at least four major findings as those for patients with typical KD[9]. Treatment with IVIG should be started within the first 10 days of fever[4,13].

Administration of a single dose of IVIG in conjunction with aspirin within the first 10 days after the onset of fever reduces the incidence of coronary aneurysms from 25% to 3- 5%[1,5,11]. In Egypt, treatment of KD by IVIG is available only in certain governmental and university hospitals where its high cost is covered by the government and partly by insurance. However, many patients are not covered by insurance and the co-payment is prohibitive for many families. In Egypt, the incidence of KD is still unknown. But, it was found that 6.7% of young adults (40 years or younger) who undergo angiography to evaluate symptoms of suspected myocardial ischemia have coronary artery aneurysms that may be due to antecedent KD. This raises the possibility that KD is not uncommon in Egypt. Reports from around the world suggest that where there are children, there is KD[8].

Prevention and treatment of coronary artery disease is the most important target of KD treatment[6]. Recent studies also suggest that early and aggressive treatment of the blood vessels inflammation caused by KD may reduces the future risk of developing accelerated atherosclerosis[7].

Pirfenidone is an orally active small molecule consists of modified phenyl pyridone ring[14]. It exhibits antinflammatory and antifibrotic effects. Pirfenidone was also reported to reverse and prevent cardiac remodeling and fibrosis in diabetic hearts from streptozotocin-treated rats[15].

It was reported that flare and formation of crust at site of BCG inoculation is one of the typical symptoms of KD listed in the diagnostic criteria. It was suggested that a cross-reactive immune response to BCG and human antigens might cause immunopathogenesis that leads to systemic vasculitis characteristic of KD. Therefore, it was hypothesized that antigenic proteins of BCG might induce an immunopathologic reaction in the vascular wall[16].

The present study was designed to clarify the possible protective effects of pirfenidone on cardiac lesions in a model of KD induced by BCG injection in female Balb/C mice.

PATIENTS AND METHODS

Experimental Animals:

Eighteen female Balb/C mice 68- weeks old (1820-gram) were purchased from National Research Institute (Cairo, Egypt) and were housed in animal room in the Medical research center in Ain Shams University, with 12h light/dark cycle with free access to water. All animals' procedures were approved by the Institutional Animal Ethics Committee of Ain Shams University, Faculty of Medicine.

Experimental protocol:

After one week acclimatization period, mice were randomly divided into three groups, six animals each:

Group I (control group): each mouse received single injection of 0.2 ml Sauton growth medium in the tail vein at day zero. They also received 0.5ml saline by gastric gavage daily, and then they were sacrificed on day 21.

Group II (group of KD): mice received single injection of BCG in the tail vein (0.2 ml/mouse) at day zero. Mice were sacrificed on day 21[17,18]

Group III (Pirfenidone treated group): mice received single injection of BCG (0.2 ml/mouse) in the tail vein, and pirfenidone 500mg/kg/day by gastric gavage[19]; starting with BCG injection (day 0) till the end of experiment on day 21th.

Drugs:

BCG vaccine: is a culture preparation of BCG, an attenuated live strain freeze-dried form of BCG supplied as a white powder in a vial. It was provided with a Sauton growth medium for dissolving, consisting of sterile PBS containing 0.025% polysorbate 80. The concentration of the vaccine is 8 x 10^5 to 32 x 10^5 colony forming units per 0.1 mg BCG.

It was a gift from Veterinary Serum and Vaccine Research Institute, bacterial diagnostic product research department, Abbasia, Cairo, Egypt.

Pirfenidone tablets as Pirfenex Cipla (India) were dissolved in normal saline (0.9% NaCl).

Sample collection:

At the end of the experiment, all animals were sacrificed after ether inhalation anesthesia. Hearts were taken to examine the left ventricle and coronaries (especially the proximal parts). Specimens were fixed immediately in 10% formol saline solution and were processed to obtain paraffin sections of five μm thickness. Sections were then subjected to H&E stain, orcein and Mallory's triple stain[20].

Immunohistochemical study:

Paraffin sections were also stained with avidin-biotin peroxidase for demonstration of cells immunoreactive to tumor necrosis factor- alpha (TNF-α) and counterstained with Hx. Antibodies against TNF-α were purchased from R&D Systems (Minneapolis, Minnesota, USA). TNF-α

expression was cytoplasmic immunopositive reaction. According to some authors, to detect any unintended background staining, the regular immunohsitochemical staining protocol was processed on rat thoracic aorta without adding the primary antibody (negative control)[21].

Morphometric and statistical study:

Samples were analyzed by using Leica DM2500 microscope with built in camera (Wetzlar, Germany). All images were digitally acquired using an image analyzer Leica Q win V.3 program (Wetzlar, Germany) installed on a computer in the Histology & Cell Biology department faculty of Medicine, Ain Shams University. Five different non overlapping fields from five different sections of different mice were examined in each group for measuring the mean area percentage of collagen fibers between cardiac myocytes and the mean area percentage of positive immune reaction for TNF-α.

All measurements were taken at magnification of ×200. All data were collected, revised, and subjected to statistical analysis using one-way analysis of variance (ANOVA) performed using SPSS.21 program (IBM Inc., Chicago, Illinois, USA). The significance of data was determined by P values. P values greater than 0.05 were considered non significant (NS) and P values less than 0.05 were considered significant.

RESULTS

In mouse model of KD, examination of the myocardium in H&E stained sections revealed irregularly arranged distorted separated cardiac muscle fibers, with deep acidophilic cytoplasm, loss of striations, rarified myofibriilar content, and degenerative changes in the nuclei as pyknosis and Karyorrhexis. Inflammatory cells were also seen in the interstitium between cardiac muscle fibers (Fig. 2). Extravasation of blood was seen between the degenerated cardiac myocytes. Adipocytes were also seen between cardiac muscle fibers (Fig. 3). Coronaries were frequently seen with irregular lumen and localized areas of intimal thickening. Focal areas of denuded endothelium were also seen. The media of some coronaries showed focal degeneration (Fig. 4). The media also appeared with disoriented irregularly arranged smooth muscle cells (Fig. 4 and 5) which were seen sometimes vacuolated. Shrunken deeply stained pyknotic nuclei, were also seen in some smooth muscles of media (Fig. 6). Perivascular inflammatory infiltration was also frequently noticed in most of the coronary vessels (Figs. 2, 4 and 5).

Pirfenidone treated mice showed greatly minimized histolopathological findings, compared to those of group II. Cardiac muscles were seen with acidophilic cytoplasm, vesicular nuclei, and distinct striations (Fig. 7). Cellular infiltration was infrequently seen in-between cardiac myocytes (Fig. 8). Most of the coronaries were seen with normal architecture (Fig. 7).

In a mouse model of KD, Mallory stained sections showed increased collagen fibers between the cardiac myocytes (Fig. 10), and in the adventitia of coronary vessels (Fig. 11 and 12). Increased collagen content was also noticed extending in the media of some coronary vessels (Fig. 12). While in pirfenidone treated mice, minimal amounts of collagen fibers was noticed in-between cardiac myocytes and an apparent minimal increase was also noticed in the adventitia of some coronary vessels compared to the control group (Fig. 13).

In orcein stained sections, coronaries of KD model showed disruption of internal elastic lamina (IEL) and sever distortion of external elastic lamina (EEL) (Fig. 15) with fragmentation of elastic fibers in the media. While in pirfenidone treated mice, intact wavy IEL and EEL were seen in most of the coronaries (Fig. 16).

In mouse model of KD, intense positive immune reaction for TNF-α was frequently seen (Fig. :18), while in pirfenidone treated mice, weak immune reaction for TNF-α was infrequently seen (Fig. 19).

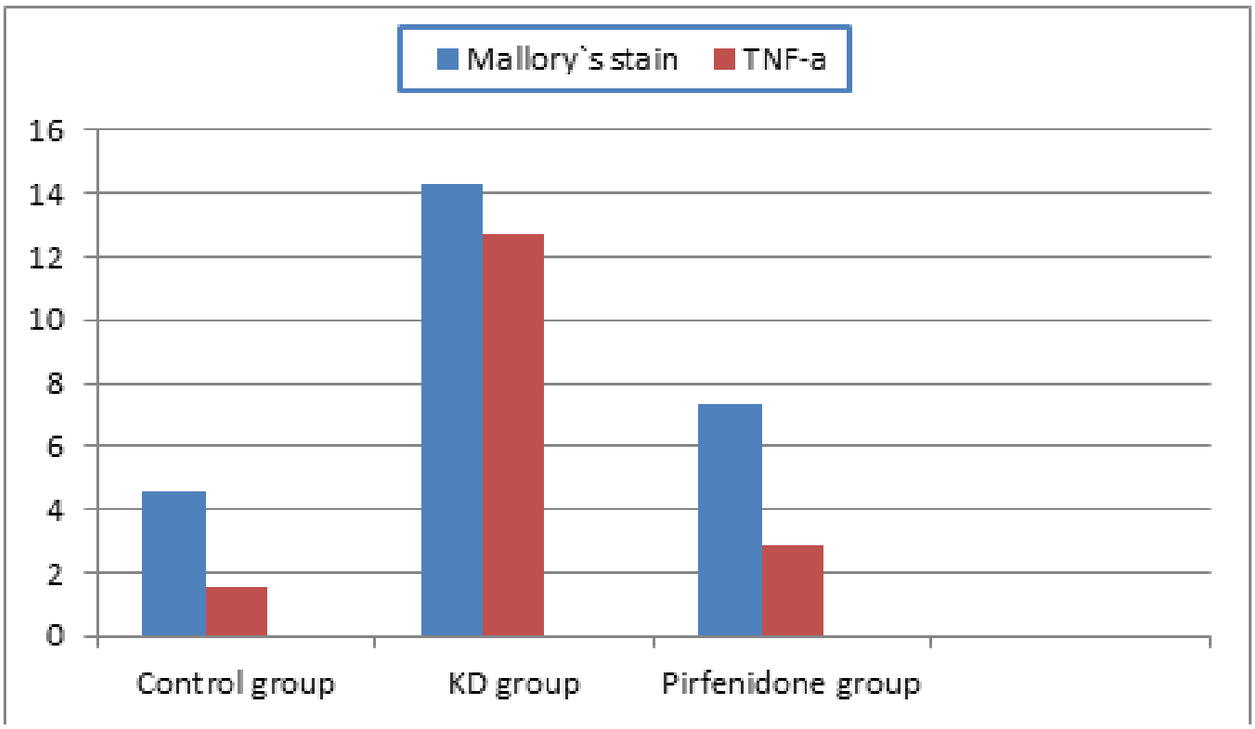
Table 1: showing the mean ± SD of area percentage of collagen fibers between cardiac myocytes and positive TNF-α immune reaction in different groups

	Area % of fibrosis (Mallory's stain)	Area % of positive TNF-α immune reaction
Control group	4.6± 0.3	1.5±0.6
KD group	14.3± 1.2*	12.7±1.1*
Pirfenidone group	7.3±-0.9▲	2.9±1.0▲

SD= Standard deviation

*Significant increase compared to other groups
▲ Significant decrease compared to group II

Histomorphometric results confirmed the previous histological findings. A significant increase in both the mean area percentage of collagen fibers between cardiac myocytes, and the mean area percentage of TNF-α immune reaction was noticed in KD group compared to the control group. While pirfendione treated mice showed significant decrease in both parameters compared to KD group (Table 1, Histogram1).



Histogram 1: showing the mean area % of collagen fibers and positive TNF-α immune reaction in the different groups

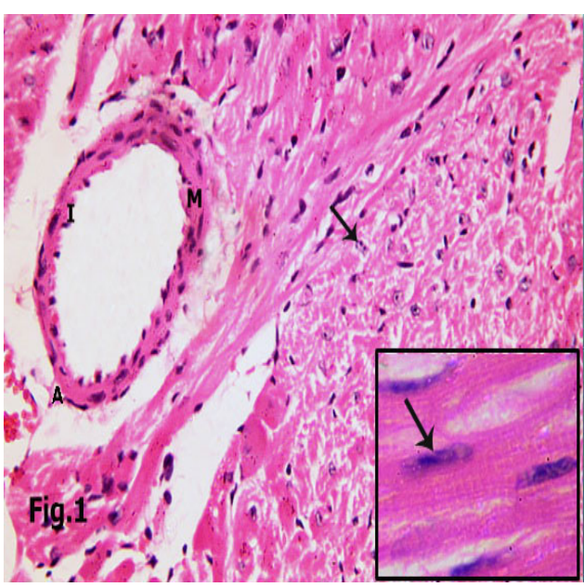


Fig. 1: showing acidophilic cardiac myocytes running in different directions and containing central oval vesicular nuclei (↑). Coronary artery is seen with intima (I), media (M), and adventitia (A). Inset showing distinct striation in the cardiac myocytes.

Group I (H&EX400) Inset X 1000

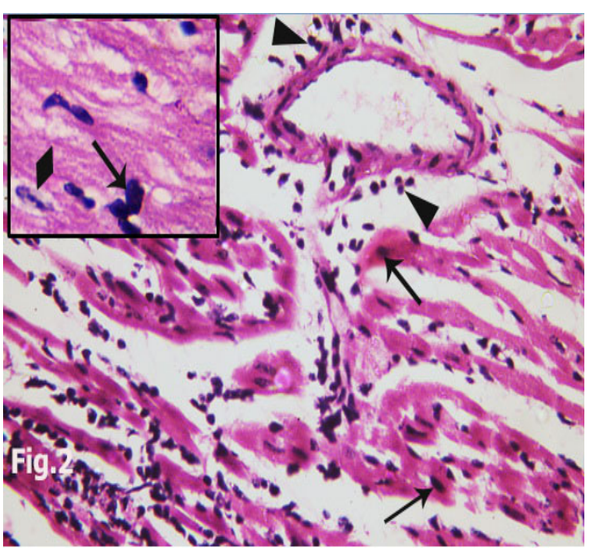


Fig. 2: showing distorted widely separated cardiac myocytes with deep acidophilic cytoplasm and dense nuclei (↑). Inflammatory cellular infiltration can be seen in between myocytes with perivascular (▲) inflteration can also be seen. Inset showing loss of striation of cardiac myocytes with rarified myofibrillar contents. Nuclei show degenerative changes in the form of pyknosis (↑) and karyohexis (♦).

Group II (H&EX400) Inset X 1000

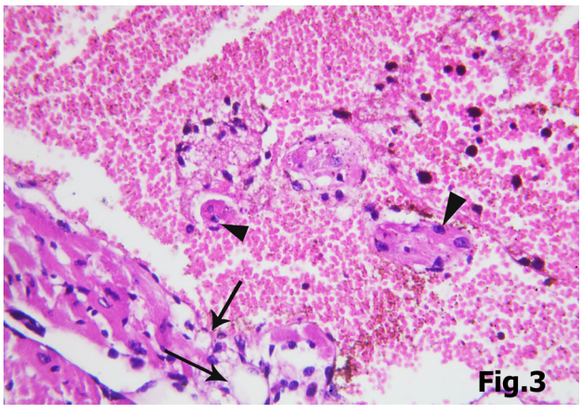


Fig. 3: showing extarvasation of blood between the degenerated cardiac myocytes with pyknotic nuclei (▲). Adipocytes can also be seen between cardiac muscle fibers (↑).

Group II (H&EX400)

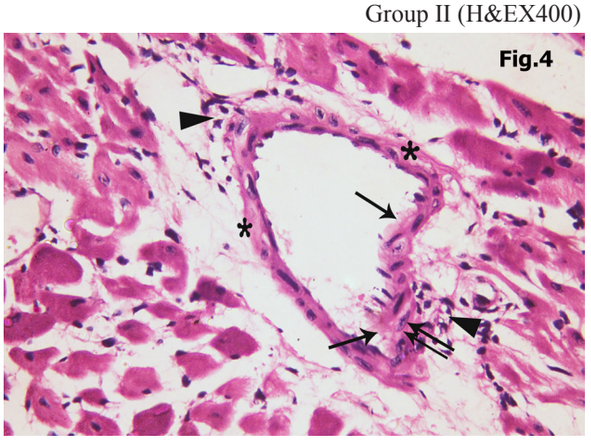


Fig. 4: showing irregularity in the lumen of coronary artery. The endothelium is seen denuded in focal area (↑). Disorientation of smooth muscle cells (↑↑) is seen in the media under the denuded endothelium. Area of focal degeneration is seen in the media (*) Perivascular inflammatory cells (▲) can also be seen.

Group II (H&EX400)

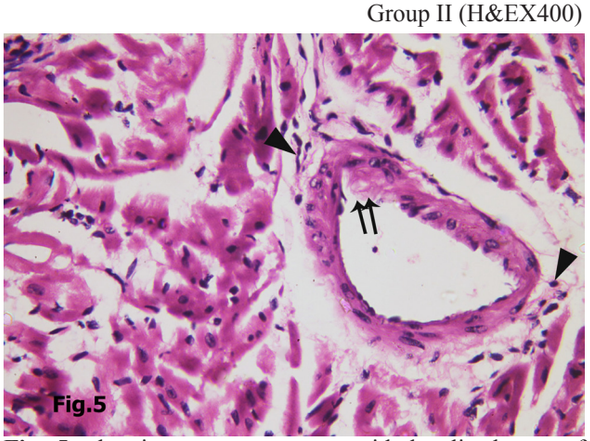


Fig. 5: showing coronary artery with localized area of irregular intimal thickening and loss of endothelial lining (↑). Perivascular infiltration (▲) can also be detected. Notice the distorted widely separated cardiac myocytes with deep acidophilic cytoplasm and pyknotic nuclei.

Group II (H&EX400)

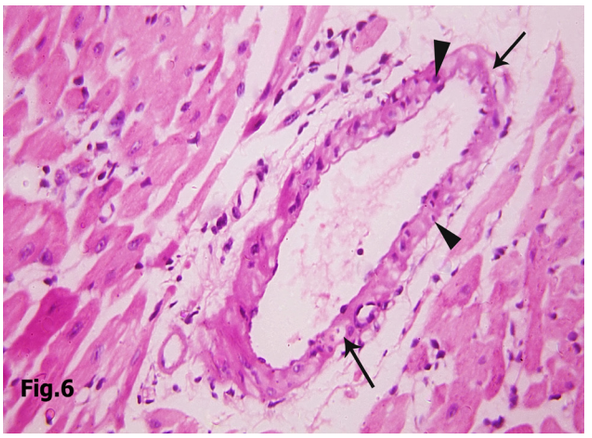


Fig. 6: showing smooth muscle cells (↑) in the media of coronary artery with vacuolated cytoplasm. Shrunken deeply stained pyknotic nuclei (▲), are also seen in smooth muscles of media.

Group II (H&EX400)

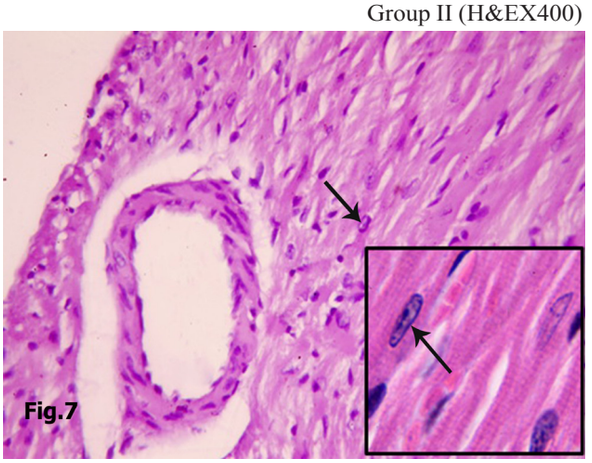


Fig. 7: showing acidophilic cardiac muscle fibers with vesicular central nuclei (↑). Notice coronary artery with intima, media and adventitia. Inset showing distinct cross striations.

Group III (H&EX400) Inset X1000

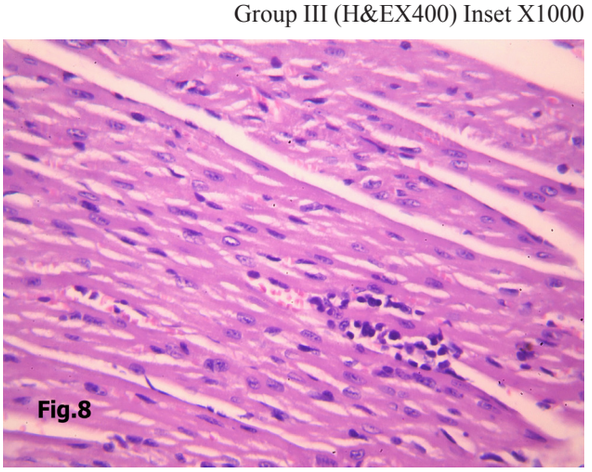


Fig. 8: showing cellular infiltration in the myocardium. Closely packed cardiac myocytes with acidophilic cytoplasm and central vesicular nuclei are seen.

Group III (H&EX400)

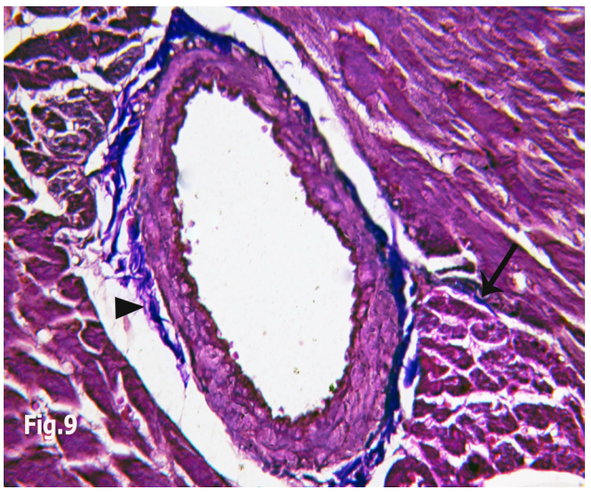


Fig. 9: showing minimal amounts of collagen fibers in-between cardiac myocytes (↑) and in the adventitia of the coronary artery (▲)

Group I (Mallory's trichrome stain X 400)

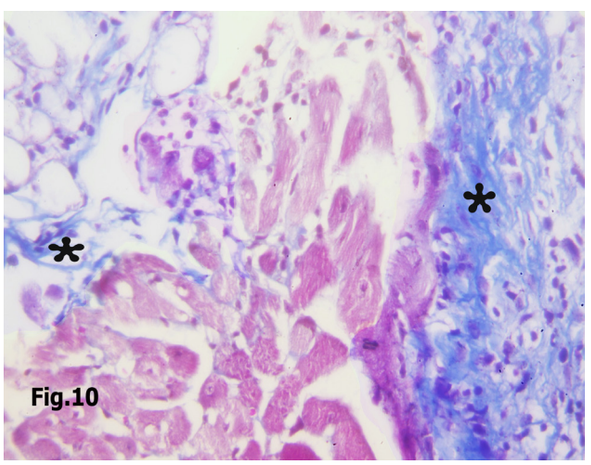


Fig. 10: showing increased amount of collagenous fibers (*) between cardiac muscle fibers.

Group II (Mallory's trichrome stain X 400)

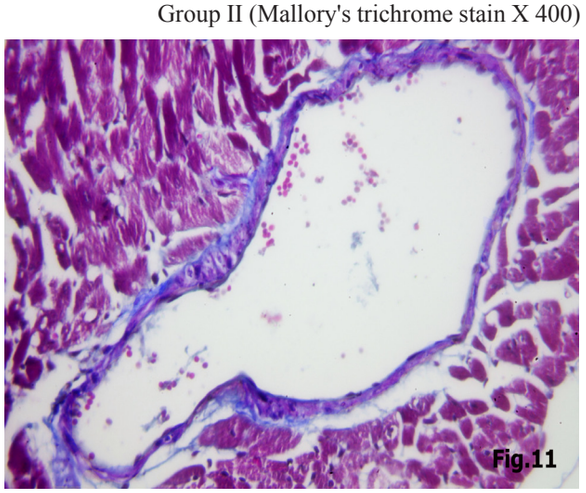


Fig. 11: showing increased collagenous fibers in the adventitia of a thin wall dilated irregular coronary vessel.

Group II (Mallory's trichrome stain X 400)

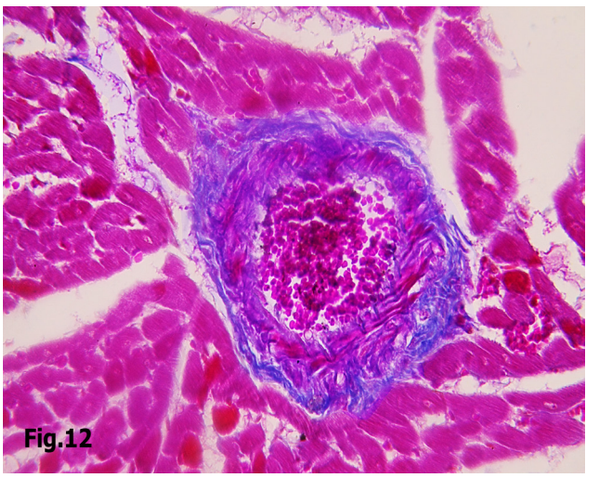


Fig. 12: showing increased collagenous fibers in the media and adventitia of a coronary artery.

Group II (Mallory's trichrome stain X 400)

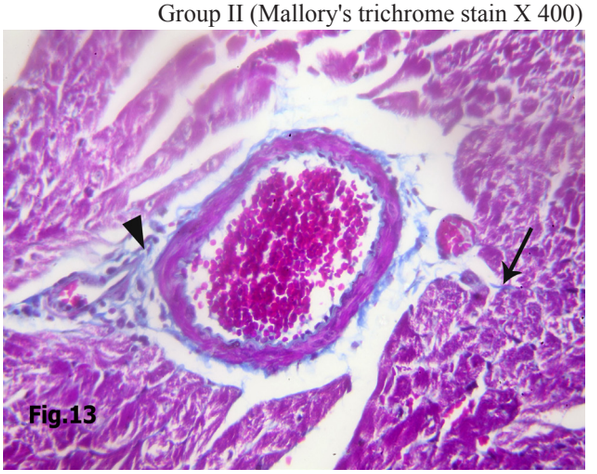


Fig. 13: showing minimal amounts of collagen fibers in-between cardiac muscle fibers (↑) and an apparent minimal increase in collagen fiber content in the adventitia of a coronary artery (▲).

Group III (Mallory's trichrome stain X 400)

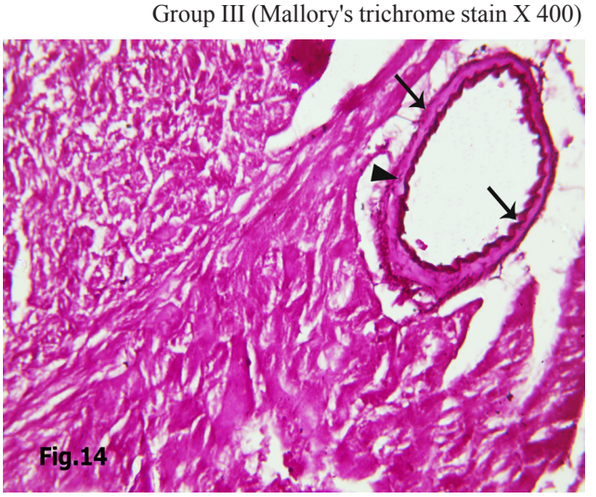


Fig. 14: showing an intact wavy IEL and EEL (↑). Thin elastic fibers (▲) are seen in the media of a coronary artery.

Group I (Orcein stain X 400

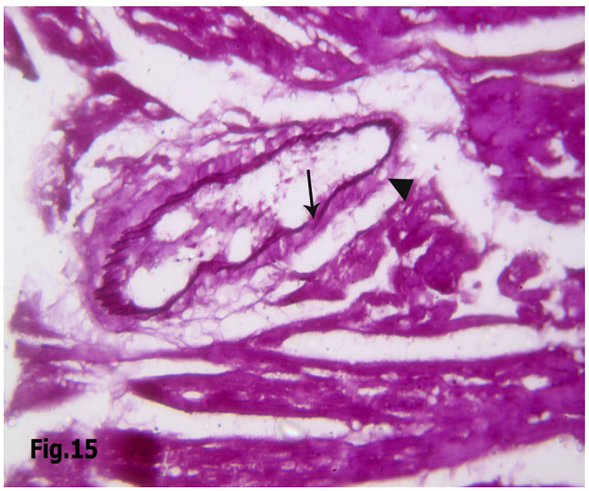


Fig. 15: showing localized area of loss of continuity of IEL (↑) and sever distortion of EEL (▲) in a coronary artery.
Group II (Orcein stain X 400)

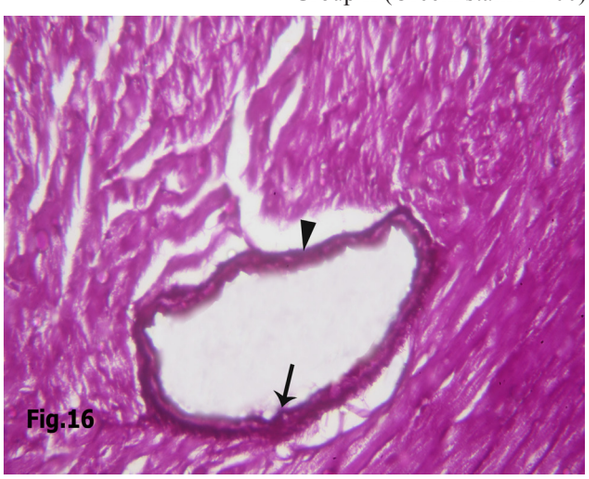


Fig. 16: showing an intact wavy IEL (↑) and EEL (▲) in a coronary artery.
Group III (Orcein stain X 400)

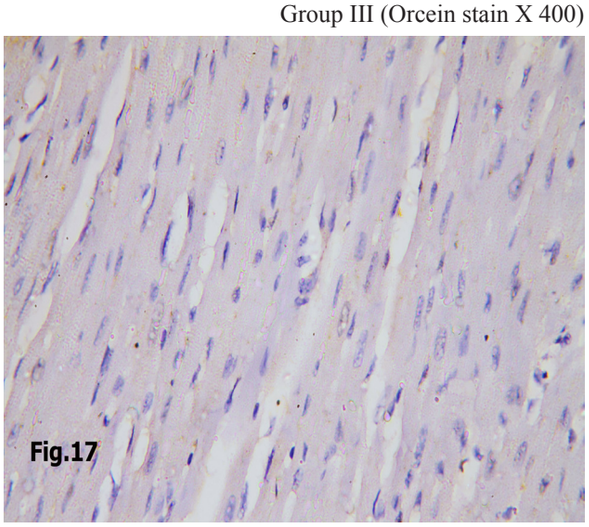


Fig. 17: showing negative reaction for TNF- α between cardiac muscle fibers.
Group I (Avidin–biotin peroxidase for TNF-α × 400)

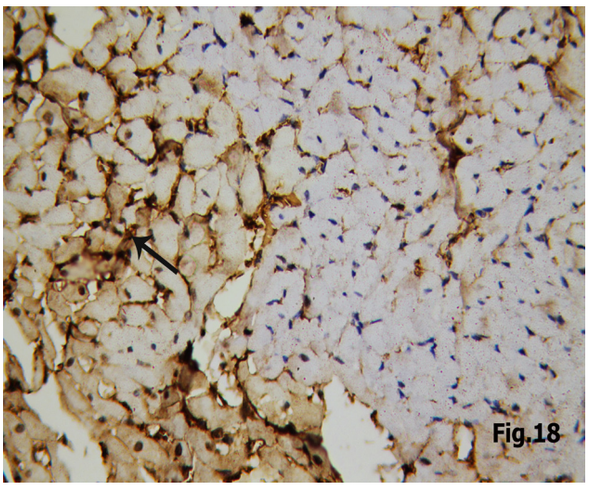


Fig. 18: showing frequent intense positive immune reaction for TNF-α (↑).
Group II (Avidin–biotin peroxidase for TNF-α × 400)

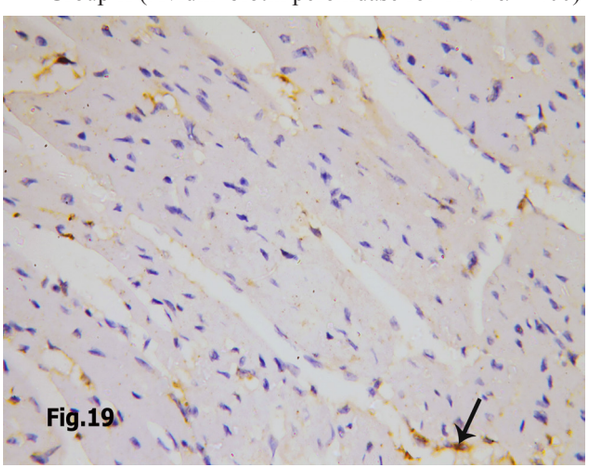


Fig. 19: showing infrequent weak immune reaction for TNF-α (↑).
Group III (Avidin–biotin peroxidase for TNF-α × 400)

DISCUSSION

Kawasaki disease is a childhood vasculitis of blood vessels predominately affects medium sized arteries with a striking predilection for the coronaries[5,10]. Myocardial ischemia remains an important concern. Some authors reported that patients with KD might be at risk of developing ischemic cardiomyopathy due to abnormal myocardial perfusion and reduced coronary flow[12].

The clinical course of the disease has been described in three phases: the acute phase begins with an abrupt onset of fever, followed by conjunctivitis, rash, involvement of the oral mucosa, redness, swelling of hands and feet, and enlargement of cervical lymph nodes[3,4]. It lasts 12- weeks and myocarditis is the main feature. The subacute phase lasts till the 4th week with development of coronary artery aneurysms[10].

During this phase desquamation of skin of fingers and toe tips occurs. The convalescence stage usually lasts for eight weeks. About 15%-25% of cases result in coronary artery aneurysms or ectasia[3,4]. If the diagnosis is not established and the treatment is not instituted, some patients may suffer from sudden death secondary to myocardial infarction, or coronary artery rupture, or may develop serious cardiac disease that is unrecognized till symptoms of myocardial ischemia and heart failure develop later in life. Occasional patients have refractory KD that doesn't respond to IVIG infusion[10].

The etiology and pathogenesis of the disease is unknown, but it is thought to be of immunologic origin. Increased activation of helper T cells and increased levels of immune mediators and antibodies that destroy endothelial cells have been detected during the acute phase of the disease. It was hypothesized that some unknown antigen triggers the immune response in a genetically predisposed children[3,6]. Also, circulating immune complexes; triggered by infectious agents; have been detected in the early phase of KD patients[2]. Therefore, increased blood levels of cytokines and fragments of endothelial cell adhesion molecules, indicate widespread immune activation. The absence of KD in neonates and its rarity in adults, suggests that protection may occur through maternal antibodies and through acquired immunity respectively[10,13].

As flare and crust formation at the site of BCG vaccination are observed in patients with KD[16], BCG inoculation could induce the production of inflammatory cytokines, active free radicals, and nitric oxide with subsequent activation of macrophages and T lymphocytes to release several proinflammatory cytokines, which have been proven to be a kind of cell-mediated immune response[22].

It was reported that pirfenidone exerted anti-inflammatory, anti-oxidant, and anti-fibrotic effects on the heart[23]. The present study was designed to investigate the potential protective effect of oral pirfenidone (500mg/kg/day) on the cardiac complications in a model of BCG-induced KD (immune mediated).

Female Balb/c mice were used in this study as the experimental and clinical data indicated that they have a naturally occurring sexual dimorphism in the normal immune response[24]. Female Balb/c mice produce a more vigorous cellular and humoral immune response, have a more developed thymus, and have higher immunoglobulin concentration[25].

Young mice were used in this study, their average weight ranged from 1820-gm, which were equivalent to human age from four months to one year as it was reported that more than 80% of patients with KD are infants and children aged < 5 years[10,13].

It was reported that, in immune complex diseases, antigen antibody complex is formed in the circulation leading to complement activation and deposition of immune complex in the tissue, with initiation of inflammatory reaction in various sites. It was also reported that, when massive immune complexes bind to inflammatory cells via Fc and complement receptor, they trigger release of proteases; vasoactive mediator; that increase vascular permeability. When the complexes deposit in tissue, inflammatory reaction occurs. Attempts of phagocytosis of immune complex result in release of inflammatory mediators and lysosomal enzyme that digest basement membrane, collagen, and elastin. Immune complexes can also lead to platelet aggregation that can lead to formation of microthrombi producing local ischemia[26].

In the current study, administration of BCG vaccine resulted in a picture similar to KD as reported by other authors. Irregularly distorted widely separated cardiac muscles[11,27] with loss of cross striation[27] and degeneration of myocytes with deep acidophilic cytoplasm[11,27] were noticed. Inflammatory cells were also observed between cardiac myocytes[2,11] that was suggestive of myocarditis which is considered as a feature of KD[9.11,13,27,28]. Myocardial fibrosis was also observed in this study in Mallory stained sections[9,11,12]. This fibrosis was explained by authors who reported that it might be due to previous ischemia in the area perfused by the affected coronary artery[9,11]. Others reported that increased collagen content is a common response to myocarditis. Their results suggested that cellular infiltration in the ventricular myocardium develops in the acute phase of KD and persists for a long time. They also noticed absence of myocardial fibrosis in patients of KD with normal coronary arteries[11,12]. It was also reported that endothelial abnormalities and intimal proliferation may lead to cardiac ischemia with subsequent myocardial infarction[13].

Fibrosis is a slowly-evolving process which leads to chronic impairment of cardiac functions[29]. Areas of fibrotic tissue compromise cardiac function and interfere with coordinated electrical conduction and increase vulnerability to arrhythmias[30]. Cardiac fibroblasts are the primary cell responsible for cardiac fibrosis. Several studies illustrated that pirfenidone attenuates cardiac fibrosis in different animal models[31]. Transforming growth factor- β (TGF-β) pathway is important for endothelial/epithelial-to-mesenchymal transition and the creation of myofibroblasts that influence the generation of regulatory T-cells[32,33]. Myofibroblasts mediate damage to the arterial wall through recruitment of pro-inflammatory cells. Myofibroblasts can be generated from many areas as endothelial cells, resident fibroblasts

in the adventitia, smooth muscle cells,[32] or recruitment of pericyte-like progenitor cells. The mechanism of myofibroblast differentiation involves several cytokines as TGF-β, endothelin-1, angiotensin II, and platelet-derived growth factor. These cytokines work together to induce activation of the resident interstitial fibroblasts and induce the expression of extracellular matrix components, including collagen type I[34]. Prevention of myofibroblasts differentiation might therefore represent a potential target for therapies aimed at limiting fibrosis in the heart[31].

In the current work, pirfenidone exerted anti-fibrotic effects as were evident in Mallory stained sections. This was explained by authors who reported that pirfenidone might inhibit cardiac fibrosis by suppressing cardiac fibroblast activation, collagen synthesis, and inhibiting the over-expression of fibrosis-related genes[23]. Others reported that, pirfenidone reduced the expression of profibrotic cytokines as TGF-β[14,30,31,35,36]. The best known inducer of fibrosis is TGF- β, and is known to induce the expression of fibrogenic mediators and facilitate myofibroblasts differentiation[30]. Pirfenidone also increases collagen breakdown by reducing the TGF-β induced inhibition of the degrading enzymes; the matrix metalloproteinases[15]. Pirfenidone also effectively inhibits the proliferation and differentiation of myofibroblasts, collagen formation, and migration of cardiac fibroblasts[29].

The clinical significance of KD stems from the involvement of coronary arteries. Pathologic changes outside cardiovascular system are of rare significance[26]. Up to 25% of untreated children will develop permanent damage of the coronary arteries[11]. In the current study, coronary arteries showed various changes similar to those of KD as reported by other authors. Coronaries were frequently noticed with irregular lumens and thin walls[11]. Discrete regions of intimal proliferation[10,11,13,1,28,], vacuolation and disorientation of smooth muscle cells in the media[28], perivascular infiltration[2,28], pyknotic nuclei in the smooth muscles of the media[37], disruption of the internal[1,2,10,11] and external elastic laminae,[1,11] and deposition of collagen fibers in the wall[11,28] were seen.

It was noticed that apoptosis of vascular smooth muscle cell that occurred in many arterial diseases; including aneurysm and atherosclerosis[37] was probably due to nitric oxide which was known for its capability of inducing apoptosis[38].

It was reported that, the coronary vessels in KD lose their structural integrity resulting in focal weakness, dilatation, and aneurysmal formation. In the healing phase, the vascular wall could become progressively fibrotic with intimal proliferation which may lead to stenotic occlusion of the vessel overtime[10].

Changes that occurred in the coronary vessels might lead to thinning of the wall. Subsequent rupture of the wall might occur with extravastion of blood between cardiac myocytes. As a result of theses coronary changes, ischemia of cardiac muscles could occur, leading to necrosis of muscle fibers that appeared with deep acidophilic cytoplasm and pyknotic nuclei. Some cardiac myocytes were also seen replaced by fat cells.

Vascular inflammation observed in this study was explained by some authors who reported that when cytokines secreted by activated macrophages, stimulate the hepatocytes to produce C- reactive protein which combine with platelet-activating factor, inducing platelet aggregation, granulocyte and monocyte activation, smooth muscle contraction, increased vascular permeability with neutrophil margination[6]. It was also reported that, in KD vasculitis is characterized by T cell and macrophage activation in response to unknown antigen. This leads to formation of antibody against endothelial cells and smooth muscle cells, which precipitates an acute vasculitis[26].

In the current study, pirfendione was found to abort perivascular and myocardial inflammatory cells infiltration as it might inhibits the vascular hyperpermeability[23]

Moreover, Pirfenidone has immune modulating activities. It inhibits expression of fibrogenic cytokines, as TGF-β, interleukin (IL)-4, IL-13, and inhibits expression of TNF-α. It also has anti-inflammatory effects that result from the inhibition of pro-inflammatory cytokines. Pirfenidone inhibits T cells proliferation in vitro and in vivo. Pirfenidone also has an inhibitory effect on T cell activation and production of multiple cytokines. The later inhibitory effects are believed to help resolving inflammation. TGF-β has an early pro-inflammatory role in tissue, which can recruit monocytes and granulocytes to the injured areas by acting as a chemoattractant[36]. Several in vivo studies described the ability of pirfenidone to regulate the production of cytokines and growth factors or reduce oxidative stress[23,30]. Pirfenidone inhibits alpha-smooth muscle actin (α-SMA) expression in the cultured cardiac fibroblasts. Alpha-SMA is involved in morphologic transformation of fibroblasts into myofibroblasts. Pirfenidone inhibits the perivascular and interstitial tissue fibrosis, and reduces TGF-β1 mRNA gene expression in mice cardiac tissue[35].

In the current study, immunohistochemical staining for TNF- α was done. A significant increase in the mean area percentage of TNF-α positive immune reaction were noticed in KD group compared to control group, while significant decreases were noticed in pirfenidone treated mice compared to KD group. It was reported that pirfenidone reduces TNF-α expression[39]. TNF-α plays a vital role in various immune and inflammatory processes,

including cellular activation, survival, proliferation, as well as cell death by necrosis and apoptosis. TNF-α is produced primarily by cells of hematopoietic origin as monocytes and macrophages, when stimulated by innate sensors, which are involved in initial step of host defense against microorganisms, and are widely expressed on immune cells[40]. It was reported that anti-TNF-α, could have a role in KD patients who are IVIG-resistant. Serum TNF-α is elevated in KD patients, and higher levels correlate with the development of coronary artery aneurysms[33]. TNF α largely contributes to the progress of inflammation in KD in the coronary arteries. This was demonstrated in a TNF α knockout KD rat model which was resistant to coronary arteries vasculitis[39]. Pirfenidone also inhibits the expression of the pro-inflammatory cytokine TNF-α, and is thus considered a potent anti-inflammatory agent. Potent pirfenidone anti-inflammatory effects could also be due to suppressing nitric oxide synthase expression and acting as a scavenger of reactive oxygen species[14].

Experimental evidence demonstrated that presence of TNF-α in tissue could induce inflammatory reactions, hemorrhagic necrosis, fibroblasts proliferation, and collagen deposition as well time and dose dependant cell killing[41].

To the best of our knowledge, this is the first study to address possible plausible coronary and myocardial anti-inflammatory, anti-fibrotic effects of pirfenidone on a model of BCG-induced KD in female Balb/C mice. Such potential effects may have future therapeutic implications in such grave and life threatening disease. Further researches should be carried on to consolidate the findings of the current study and to establish possible immunological mechanisms of favorable pirfenidone effects.

Cardiologists should be familiar and aware of the signs and symptoms of acute KD to allow questioning of the patient or parents about an antecedent KD. Features of the illness that are frequently recalled by patients and parents are the prolonged fever, rash, "bloodshot" eyes in the acute phase, and peeling of the skin of fingers and toes in the convalescent phase. Common misdiagnoses for KD include viral syndrome, measles, scarlet fever, allergic reaction to antibiotics, and Stevens Johnson syndrome[11].

CONCLUSION

Already known anti-fibrotic, anti-inflammatory effect of pirfenidone may be extended to include those of BCG induced KD in female Balb/C mice, an effect which could have potential therapeutic implications.

CONFLICT OF INTEREST

There are no conflicts of interest

ACKNOWLEDGMENTS

Many thanks are due to Prof. Doctor Adel Zohdy and Prof. Doctor Amany Elshawarby for reviewing this paper.

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الملخص العربى

تأثير عقار البيرفينيدون على مضاعفات القلب في نموذج لمرض كاواساكي في إناث فئران البالب/سي. دراسة هستولوجية وهستوكيميائية مناعية.

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المقدمة: يعد مرض كاواساكي مرضا مناعيا في الأوعية يؤثر على العديد من أجهزة الجسم خاصة جهاز القلب والأوعية الدموية. يعرف البيرفينيدون بتأثيره المضاد للتليف والإلتهاب.

الهدف من البحث: دراسة التأثير الوقائي المحتمل للبيرفينيدون علي مضاعفات القلب في نموذج مرض كاوساكي الناجم عن حقن لقاح البي سي جي

المواد وطرق البحث: شملت هذه الدراسة 18 من إناث فئران البالب/سي. تم تقسيم الفئران إلى ثلاث مجموعات. المجموعة الأولى (المجموعة الضابطة)،المجموعة الثانية (مجموعة مرض كاواساكي): تلقت حقنه واحدة من لقاح البي سي جي في الوريد الذيلي، والمجموعة الثالثة (مجموعة البيرفينيدون) التي تلقت لقاح البي سي جي كما في المجموعة الثانية، بالاضافة الى عقار البيرفينيدون عن طريق الفم يوميا حتى نهاية التجربة. تمت التضحية بجميع الفئران في اليوم الحادي والعشرين بعد الحقن. تم تجميع قلوب الفئران و فحصها.

النتائج: كشفت نتائج المجموعة الثانية عن عدم انتظام وتباعد بين خلايا عضلة القلب مع وجود التهاب خلالي. وشوهدت الشرايين التاجية رقيقة الجدار مع عدم انتظام لمعتها. كما لوحظ عدم انتظام سماكة الباطنة, وظهرت العضلات الملساء غير مرتبة في الطبقة الوسطى. لوحظ ايضا التهابات حول الاوعية الدموية. كشف الفحص بصبغة المالوري عن وجود تليف خلالي وتليف في الاوعية الدموية وماحولها. أظهر فحص شرائح صبغة الأورسين انقطاع في الصفيحة المطاطية الداخلية والخارجية. لوحظ أيضا رد فعل مناعي إيجابي لعامل نخر الورم ألفا في مجموعة مرض الكاواساكي. أظهر العلاج بالبيرفينيدون قلة في التغييرات النسيجية المستحثة في الكاواساكي. لوحظ القليل من الارتشاح الخلوي لخلايا وحيدات الأنوية في عضلة القلب. وشوهد القليل من ألياف الكولاجين في الغلالة و سلامة الصفائح المطاطية.

الاستنتاج: أحبط عقار البيرفينيدون مضاعفات القلب والأوعية الدموية المرتبطة بمرض كاواساكي