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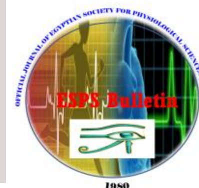

"COVID 19 m-RNA (Pfizer) vaccination impairs cardiac functions in adult male rats"

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- mRNA covid (Pfizer) vaccine
- Cardiac functions
- Inflammatory cytokines
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- Apoptosis.

Abstract

Safe and effective whole-population vaccination against severe acute respiratory syndrome coronavirus 2 is the long-term solution to the ongoing coronavirus disease 2019 pandemic. Administration of COVID-19 mRNA vaccine induced a rapid onset of multifocal myopericarditis predominantly after the second dose of mRNA COVID-19 vaccines (Pfizer-BioNTech). However, the underlying mechanisms for development of myocarditis are not clear. The current study aims to identify the effect of Pfizer vaccine on the cardiac function in male rats and to explore the underlying mechanisms for the disturbed cardiac function. 22 adult male Wistar rats were allocated to 2 groups. Control group were injected with normal saline, and Pfizer-injected group received 2 doses of Pfizer vaccine intravenous. Two weeks later, recording of ECG was done and rat hearts were isolated and perfused using Langendorff preparation, in addition, blood sampling was done. Significant bradycardia and significant decrease in the peak tension was demonstrated. In addition, significant prolongation of time to peak tension, half relaxation time and contraction time were reported. Biochemical findings revealed significant increase in cardiac tissue Interleukin-18, Interleukin-6, Tumor necrosis factor-alpha and cardiac malondialdehyde. This was accompanied by significant increase in plasma D-Dimer, cardiac troponin, C-reactive protein and cardiac catalase. Histological findings showed marked apoptotic cardiac muscle fibers. Therefore, Pfizer vaccine injection impaired the cardiac muscle function through myocardial inflammation, apoptosis and increasing myocardial oxidative stress.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a recently identified coronavirus that causes the infectious condition known as coronavirus disease-2019 (COVID-19)[1]. As there is presently no established curative therapy for COVID-19, prevention is the key management strategy for this pandemic. Safe and effective whole-population vaccination is the only long-term solution to the ongoing COVID-19 pandemic [2].

Vaccine hesitancy among the general public is a significant problem due to the potentially severe side effects of these rapidly developed novel vaccines. An example of such side effect is myopericarditis following mRNA COVID-19 vaccines [3]

Myocarditis is an inflammatory condition that affects the heart.It is associated with cardiac dysfunction, ventricular remodeling and of poor prognosis if complicated by left ventricular (LV) dysfunction, heart failure (HF) or arrhythmia. Furthermore, fulminant myocarditis, may take place and is considered to be one of the main causes of cardiogenic shock in young adults[4].

There have been over 1000 reports of myocarditis and pericarditis after COVID-19 vaccination particularly in adolescents and young adults [5,6,7],with highlights of occurrence predominantly after the second dose (mRNA-Pfizer-BioNTech).Nearly all reported cases of myocarditis occurred following vaccination with mRNA-based vaccines (Pfizer and Moderna) [8,9].A similar study reported post-vaccination myocarditis in 136 patients, 94.2% of which were after the Pfizer-BioNTech vaccine and 91.4% of which were after the second dose[10].

Despite well-described clinical manifestations, the immunological processes causing myocarditis following the COVID-19 mRNA vaccination are not entirely understood.

Aim of the work

The current study was designed to identify the effects of COVID-19 mRNA (Pfizer) vaccine on the cardiac functions in adult male rats and to explore the underlying mechanisms for the disturbed cardiac function resulting in such condition.

Materials& Methods

Ethics approval :

All animal protocols complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 2011) and were conducted with the approval of Faculty of Medicine, Ain Shams University Ethical Committee, the approval number is FMASU R 169/2022.

Animals

This study was performed on 22 adult male Wistar rats weighing between 150 and 200 g, purchased from the Holding Company for Biological Products and Vaccines (VACCERA), Helwan, Egypt. Animals were kept in plastic cages with enough ventilation, a temperature range of 22–25 °C, a 12-hour light/dark cycle, and free access to food and drink. To lessen the possibility of animal distress, the animals were allowed to adapt to the new surroundings for 7 days prior to experimental treatments. Animals were not subjected to unneeded suffering or stress, and all handling were done with the utmost care and cleanliness.

Experimental design

Animals were divided randomly into two groups:

1- Control group (10 rats): rats were injected with normal saline (0.25 µg per body weight) intravenous via rat tail veinon day 1 and another dose 14 days after[11].

2- COVID-19 mRNA vaccine (Pfizer) injected group (10 rats): Rats in this group received 2 doses of Pfizer vaccine (0.25 µg per body weight) on day 1 and another boosting dose 14 days after. The vaccine was injected intravenousvia rat tail vein to ensure reaching to the cardiac muscle to demonstrate its effects [11].The vaccine was purchased from the infection control unit, Faculty of Medicine, Ain shams University.

Experimental procedure:

On the day of sacrifice, overnight fasted rats were injected intraperitoneal with 5000 IU/kg heparin sodium and anaesthetized with intraperitoneal injection of Pentobarbitone (40 mg/kg body weight) with booster doses as needed. The dose was given according to the guideline of rodent anesthesia analgesia formulary-UBC animal care [12].

When the stage of surgical anesthesia (judged by loss of withdrawal reflexes) had been reached, the animal was placed on its back and fixed on the operating table where the following procedures were done:

1- Recording of Electrocardiogram (ECG): changes in cardiac electrical activity were recorded in lead II using Cardimax Fx-2111 (Fukuda Denshi Co., Ltd., Japan). The recorded ECG parameters were heart rate, R-wave voltage, durations of P-R interval and the Q-T interval.

2- Blood sample collection: Blood of heparinized tubes was centrifuged, and the separated plasma was used for determination of plasma cardiac troponin, D-dimer, and C-reactive protein (CRP).

3- In vitro study of isolated hearts: This was performed using a Langendorff's preparation to record intrinsic activity of the heart under basal condition. Myocardial perfusate collection was performed for determination of myocardial flow rate.

I-Preparation and perfusion technique of the isolated hearts

A V-shaped incision was performed between the upper abdomen and the base of the neck and the attachment of the diaphragm to the ribs was transected.

Langendorff preparation:

Hearts were removed quickly and immediately placed in ice-cold modified Krebs-Henseleit Bicarbonate (KHB) buffer solution for fast cardioplegia. This was done according to Langendorff technique [13] and modified by Ayobe and Tarazi [14].

The perfusion fluid (Krebs Henseleit solution) mimic the key ionic content of blood or plasma and have a pH of 7.4 at 37ºC, has the following composition (in mM/L): NaCl 118.5, $NaHCO_3$ 25, KCl 4.7, Na2 EDTA0.5mM, $MgSO_4$ 7 H_2O 1.2, KH_2PO_4 1.2, $CaCl_2$2.5and glucose 11mM was added as a substrate. The solution was supplied with oxygen and carbon dioxide (95%:5%) and temperature was adjusted at 37°C.

The aorta was then cannulated, and retrograde perfusion was started from a reservoir containing the perfusion fluid placed 75 cm above

the level of the heart from which the perfusion fluid passed through spiral glass tube immersed in a bath of warmed water to obtain an outflow temperature for the passing fluid of about 37°C. The heart was gently pressed once or twice to help removal of any air bubbles from its chambers and coronary vessels. The perfusion pressure was adjusted at 55 mmHg to shut the aortic valve and enforce the fluid to pass through the coronary arteries.

Recording of cardiac responses

Tension developed by the heart was measured by an isometric force transducer (ugobasile S.R.L., Model 7004-F, Serial N. 101014, Data EVO 14543, Italy). Isolated rat heart apex was attached by a clip to the transducer. The transducer is vertical to the heart with the distance adjusted to get the best recording of tension; the initial tension was 28 and then the transducer was kept in position without change during the whole period of recording.

The transducer was connected via a USB interface to the data capsule–Evo four channel digital recorder (ugobasile S.R.L. Biological Research Apparatus 21036, Model 17304, Serial N. O448A15, Italy) and to a computer provided with iWorx LabScribe2 TM Data Recording and Analysis Software. The heart was left to stabilize for 15 minutes, and then the baseline cardiac activities were recorded including heart rate (HR), peak developed tension (PT), time to peak tension (TPT) and half relaxation time (HRT). Myocardial flow rate (MFR) was determined by collecting the fluid passing out of the heart in glass beaker by the end of 3 minutes.

II-Biochemical Measurements

Following recording of cardiac responses, the hearts were stored at -80°C to be used for further biochemical analysis.

***Preparation of tissue homogenate**

After the animals were sacrificed, tissues were washed thoroughly and rinsed with ice. They were gently blotted between the folds of a filter paper and weighed in an analytical balance. 10% of homogenate was prepared in 0.05 M phosphate buffer (pH 7) using a polytron homogenizer at 4°C. The homogenate was centrifuged at 10,000 rpm for 20 minutes for removing the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant (cytoplasmic extract) was used according to manual instructions.

1-Assessment of the antioxidant activity in cardiac tissue

***Catalase (CAT) assessment**

Catalase activity was measured using an enzymatic colorimetric technique [15]. Using kits supplied by BioDiagnosticlab.

***Malondialdehyde (MDA) assessment**

This was carried out using an enzymatic colorimetric technique according to [16]. Using kits supplied by bio-diagnostic lab.

2-Measurements of proteins by enzyme-linked immunosorbent assay (ELISA) in rat hearts

***Determination of tissue protein**

Protein content in the tissue was determined using Genei, Bangalore, protein estimation kit**[17].** Color absorbance was read at OD range 490 to 630 nm using an ELISA plate reader (Stat Fax 2200, Awareness Technologies, Florida, USA).

Interleukin-18 was measured using IL-18 ELISA kit (Elabscience). Interleukin-6 was measured using (IL-6) ELISA kit (BioVision). Tumor

Necrosis Factor Alpha was measured using (TNF-α) ELISA kit (Cloud-Clone Corp.).

3-Measurement of Plasma D-Dimer, C-reactive protein and Cardiac troponin I

Plasma D-Dimer was measured using D-Dimer ELISA kit (Fine Biotech Co.). Plasma high sensitivity cardiac troponin I (cTnI) using cTnI ELISA kit (Life Diagnostics). Plasma C-reactive protein (CRP) was measured using Latex (Spectrum).

III-Histological examination

The rats' hearts from different groups were isolated, washed with cold saline, and fixed for 24 h in 10% formalin. Tissue sections from the myocardia of different groups were processed and embedded in paraffin, stained with Hematoxylin and Eosin (H&E), and examined by light microscopy for histopathological studies**[18].**

Statistical analysis

All results in this study were expressed as mean ± SEM. Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) program, version 20.0 was used to compare significance between each two groups. One Way ANOVA (Analysis Of Variance) for difference between means of different groups was performed on the results obtained in the present study. Differences were considered significant when P is ≤ 0.05.

Results

(I) Biochemical Results (Tables1& 2)

***Cardiac tissue Interleukin-18(IL-18) , Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Malondialdehyde (MDA) levels and Catalase (CAT) activity:**

Pfizer injected group showed significant increase in the cardiac tissue IL-18, IL-6 andTNF-αcompared to the control group. The cardiac tissue MDA level was significantly increased in the Pfizer injected group in relation to the control one. On the contrary, the cardiac tissue catalase enzyme activity was significantly decreased in the Pfizer injected group compared to their matching controls. Significant increase in the plasma levels of D-Dimer, cardiac troponin I and CRPwas present in the in the Pfizer injected group compared to the control group.

Table (1) Mean values ±SEM of IL-18, IL-6, TNF-α, MDA and catalase activity in the cardiac tissues of studied groups

	Control group (10)	Pfizer injected group (10)
IL-18 (pg/g tissue)	226.4±5.38	273.8±6.16*
IL-6 (pg/g tissue)	270±30.9	547±68.7*
TNF-α (pg/g tissue)	489.2±29.6	618.6±15.6*
MDA (nmol/g tissue)	4.2 ±0.17	7.49 ± 0.5*
Catalase (U/g tissue)	3 ±0.06	2 ± 0.13*

In parenthesis is the number of observations

P: calculated by Student's t-test for unpaired data compared to control rats (P<0.001)

IL-18: Interlukein-18, IL-6: Interleukin-6, TNF-α: Tumor Necrosis Factor-alpha, MDA: Malondialdehyde.

Table (2) Mean values±SEM of plasma levels of D-Dimer), Cardiac Troponin I and C-reactive protein levels in the studied groups

	Control group (10)	Pfizer injected group (10)
D-Dimer (ng/ml)	2.62±0.05	3± 0.06*
Cardiac Troponin I (ng/ml)	2.18±0.18	2.5± 0.08*
C-reactive protein (mg/L)	12.4±1	29.4±2.74*

In parenthesis is the number of observations

P*: calculated by Student's t-test for unpaired data compared to control rats (P*<0.001)

(II) Electrocardiographic changes (table 3)

Significant bradycardia was observed in the Pfizer injected rats compared to the control rats. In addition, significant decrease in the R-wave voltage and significant prolongation of the Q-T interval in the Pfizer injected rats compared to the control rats. No significant difference was observed in the P-R interval between the studied groups.

Table-3: Mean±SEM of changes in the Electrocardiographic parameters in the different studied groups

	Heart rate (bpm)	R-wave voltage (µv)	P-R interval (msec)	Q-T interval (msec)
Control rats (10)	510±10	310±10	42±2	66±3
Pfizer injected rats (10)	458±23.8	270±15.2	44±2.66	76±2.7
P*	**<0.05**	**<0.05**	**NS**	**<0.05**

In parenthesis is the number of observations

P*: calculated by Student's t-test for unpaired data compared to control rats.

NS: Non-significant.

(III)In vitro study of isolated perfused heart (Langendorff preparation)

Responses during the 30 minutes reperfusion following 30 minutes of global ischemia:

1- Heart rate responses (HR,bpm "table 4"):

Baseline preischemic values of the heart rate of hearts isolated from control as well as Pfizer injected groups was insignificantly different. After 30-minute ischemia, significant bradycardia was observed in Pfizer injected rats compared to the control rats which occurred from the start to the end of the 30-minute reperfusion. For the Pfizer injected group, significant bradycardia was observed after 15 and 30 minutes of the reperfusion compared to the preischemic baseline values.

Table-4: Mean±SEM of heart rate (HR, bpm) changes in response to 30 minutes ischemia-reperfusion of hearts isolated from the different studied groups

HR	Preischemic baseline value (bpm)	Reperfusion		
		5 min. (bpm)	15 min. (bpm)	30 min. (bpm)
Control **Mean** **±SEM** **P**	(10) 139 5.35	(10) 154 13.9 **NS**	(10) 147.2 10.7 **NS**	(10) 133 14.9 **NS**
Pfizer **Mean** **± SEM** **P**	(10) 137 6.57	(10) 106.8 16.7 **NS**	(10) 103.4 12.4 **<0.05**	(10) 87.9 10.7 **<0.01**
P*	**NS**	**<0.05**	**<0.05**	**<0.05**

In parenthesis is the number of observations NS: Not significant.

P: calculated by Student's t-test for paired data compared to preischemic values.

P*: calculated by Student's t-test for unpaired data compared to control rats.

2- **Peak developed tension (PT,g "table 5")**

Significant decrease in the baseline preischemic PT was observed in the Pfizer injected group compared to the control group. After 30-minute ischemia, significant decrease in the PT in Pfizer injected rats compared to the control rats which occurred from the start to the end of the 30-minute reperfusion. Meanwhile, on comparing the post ischemic responses of PT to baseline values, no significant differences were observed in both groups.

Table-5: Mean± SEM of Peak developed tension (PT, g) changes in response to 30 minutes ischemia-reperfusion of hearts isolated from the different studied groups

Peak developed tension (g)				
PT	**Preischemic baseline value (g)**	**Reperfusion**		
		5 min. (g)	**15 min. (g)**	**30 min. (g)**
Control **Mean** **±SEM** **P**	(10) 5.79 0.36	(10) 5.31 0.44 NS	(10) 5.58 0.56 NS	(10) 5.72 0.44 NS
Pfizer **Mean** **± SEM** **P**	(10) 4.34 0.11 NS	(10) 4.1 0.19 NS	(10) 4 0.26 NS	(10) 4 0.24 NS
P*	**<0.001**	**<0.05**	**<0.05**	**<0.01**

Inparenthesis is the number of observations NS: Not significant.

P: calculated by Student's t-test for paired data compared to preischemic values.

P*: calculated by Student's t-test for unpaired data compared to control rats.

4- Myocardial flow rate (MFR, ml/min, table 6)

Significant decrease in the baseline preischemic MFR was observed in the Pfizer injected group compared to the control group. After 30-minute ischemia, significant decrease in the MFR in Pfizer injected rats compared to the control rats which occurred from the start to the end of the 30-minute reperfusion.Moreover, both groups showed significant decrease in the post-ischemic MFR on comparing to their baseline values.

Table-6: Mean±SEM of myocardial flow rate (MFR, ml/min) changes in response to 30 minutes ischemia-reperfusion of hearts isolated from the different studied groups

Myocardial flow rate (ml/min.)				
MFR	**Preischemic baseline value (ml/min)**	**Reperfusion**		
		5 min. (ml/min.)	**15 min. (ml/min.)**	**30 min. (ml/min.)**
Control	(10)	(10)	(10)	(10)
Mean	7.49	5.78	5.51	4.98
±SEM	0.57	0.55	0.57	0.44
P		**<0.01**	**<0.01**	**<0.001**
Pfizer	(10)	(10)	(10)	(10)
Mean	5.15	3.69	3.32	3.13
± SEM	0.59	0.62	0.52	0.54
P		**<0.05**	**<0.01**	**<0.001**
P*	**<0.05**	**<0.05**	**<0.01**	**<0.05**

In parenthesis is the number of observations

P: calculated by Student’s t-test for paired data compared to preischemic values.

P*: calculated by Student’s t-test for unpaired data compared to control rats

5-Time relations:Time to peak tension (TPT, msec,), Half relaxation time (HRT, msec,), Contraction time (CT,msec,) (Table 7):

Significant prolongation in the baseline pre-ischemic TPT, HRT, CT in the Pfizer injected group compared to the control group. After 30-minute ischemia, both groups showed significant prolongation in the post-ischemic TPT, HRT, CT compared to their preischemic values. However, TPT, HRT, CT was significantly prolonged in the in the Pfizer injected group compared to the control group from the start to the end of the 30-minute reperfusion.

Table-7: Mean±SEM of Time to peak tension (TPT, msec), Half relaxation time (HRT, msec) and Contraction time (CT, msec) changes in response to 30 minutes ischemia-reperfusion of hearts isolated from the different studied groups

TPT	Preischemic baseline value	Reperfusion		
		5 min.	**15 min.**	**30 min.**
Control **Mean** **±SEM** **P**	(10) 72 4.42	(10) 96 4.98 **<0.01**	(10) 98 3.59 **<0.01**	(10) 98 2.5 **<0.001**
Pfizer **Mean** **± SEM** **P**	(10) 112 5.33	(10) 150 6.83 **<0.01**	(10) 124 4.98 **NS**	(10) 144 10.6 **<0.05**
P*	**<0.001**	**<0.001**	**<0.01**	**<0.001**
HRT	**Preischemic baseline value**	**Reperfusion**		
		5 min.	**15 min.**	**30 min.**
Control **Mean** **±SEM** **P**	(10) 86 3	(10) 92 5.33 **NS**	(10) 96 4 NS	(10) 96 4 NS
Pfizer **Mean** **± SEM** **P**	(10) 112 8	(10) 154 11.9 **<0.05**	(10) 136.6 8.4 **NS**	(10) 156 11.2 **<0.05**
P*	**<0.01**	**<0.001**	**<0.001**	**<0.001**
CT	**Preischemic baseline value**	**Reperfusion**		
		5 min.	**15 min.**	**30 min.**
Control **Mean** **±SEM** **P**	(10) 158 5.53	(10) 188 14.3 **NS**	(10) 202 8.13 **<0.01**	(10) 206 8.45 **<0.001**
Pfizer **Mean** **± SEM** **P**	(10) 218 10.9	(10) 276 21.4 **<0.05**	(10) 252 18.6 **NS**	(10) 286 23.4 **<0.05**
P*	**<0.001**	**<0.01**	**<0.01**	**<0.01**

(IV) Correlation Analysis Results:

***Correlation study between cardiac tissue IL-18 and different biochemical variables (cardiac IL-6 , TNF-α, MDA , CAT andplasma D-Dimer, Troponin I, CRP)**

A significant positive correlation exists between cardiac tissue IL-18 and cardiac tissue IL-6 (r=0.8, P <0.001), cardiac tissue TNF-α (r= 0.75, P <0.001), cardiac tissue MDA (r=0.9, P <0.001), while a significant negative correlation between cardiac tissue IL-18 and cardiac tissue catalase activity (r=-0.9, P <0.001). A significant positive correlation exists between cardiac tissue IL-18and plasma levels of D-Dimer (r=0.5, P< 0.05), Cardiac Troponin I(r=0.4, P < 0.05) and CRP (r=0.6, P < 0.01).

***Correlation study between cardiac tissue IL-18 and HR, PT, TPT, HRT and CT changes in response to 30 minutes ischemia-reperfusion of hearts isolated from the different studied groups**

A negative correlation exists between cardiac tissue IL-18 and HR (basal and 30 min ischemia-reperfusion period), being only significant during 10- and 15-min. reperfusion (r=0.55, $P < 0.05$). As regards peak tension, a significant negative correlation exists between cardiac tissue IL-18 and basal PT (r= 0.5, $P < 0.05$). Moreover, significant negative correlation exists between cardiac tissue IL-18 during 10- and 15-min reperfusion (r= 0.5, $P < 0.05$).A significant positive correlation exists between cardiac tissue IL-18 and basal TPT and basal CT (r=0.7, $P < 0.001$, r=0.5, $P < 0.05$) respectively. As regards during reperfusion period, a significant positive correlation between cardiac tissue IL-18 and TPT, CT and HRT during 5, 10 and 15 min (range of r= 0.5-0.8, range of P < 0.05-0.001).

***Correlation study between plasma D-Dimer and MFR changes in response to 30 minutes ischemia-reperfusion of hearts isolated from the different studied groups**

A significant negative correlation exists between plasma D-Dimer and MFR whether basal or during 5, 10, 15 min reperfusion period (r= -0.7, $P < 0.001$).

***Correlation study between MDA and HR changes**

A significant negative correlation exists between MDA and HR whether in vivo(ECG,r=-0.6, $P < 0.05$) or in vitro study being significant during 5, 10, 15 min reperfusion period (r=-0.7, $P < 0.001$).

(V) Cardiac histological examination (Fig.1a-b)

Control group (Fig1a): showed viable cardiac muscle fibers with distinct cell borders (black arrow) and central oval\elongated nuclei (blue arrow), and average intervening blood capillaries (red arrow) (H&E X 400)

Pfizer injected group (Fig 1b): showed markedly apoptotic cardiac muscle fibers (black arrow) and others with small pyknotic nuclei (red arrow) (H&E X 400)

Cardiac histological examination(Figures 1a-b)

Fig 1a

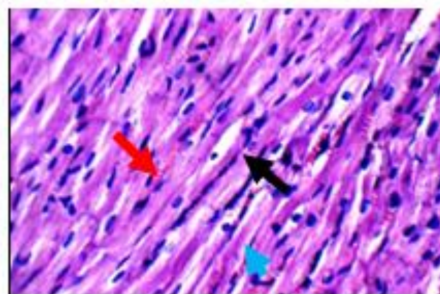
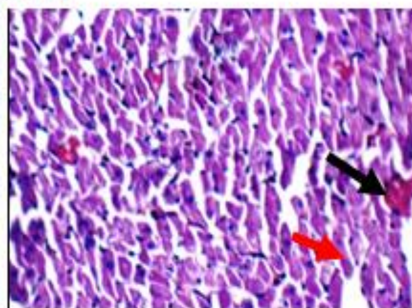


Fig 1b



Discussion

As the COVID-19 pandemic continues, international efforts to promote immunization are essential to restore health and economic and social recovery [19]. Different COVID-19 vaccines have been created [20].

Mechanisms of an unspecific inflammatory response secondary to vaccination and cross-reactivity of antibodies due to the molecular mimicry have been hypothesized [20].Another proposed mechanism is that through dendritic cells and toll-like receptors of the innate immune system, the mRNA may cause an overactive immunological response that results in proinflammatory immune cascades and cytokine activation [21]. Moreover, various new hypotheses have been made such as hyperviscosity-induced heart problems and strenuous exercise-induced secretion of the pro-inflammatory IL-6 [22].A similar hypothesis was proposed hypothesized that high secretion of IL-6 in both COVID-19 infection and vaccination could lead to myocarditis and thrombosis[23].

It is more plausible that the side effects of mRNA immunization that are immune-mediated due to the activation of already dysregulated pathways in certain persons who are prone to them compared to the vaccine's innate immunogenicity [21]. IL-6 polymorphisms have been indicated as a crucial genetic factor for defining the dysregulation of autoimmunity may result from contact with SARS-CoV-2, although further research is required to clarify these possibilities [24]. In addition, IL-18 has been proven to play a role in the pathogenesis of cardiovascular diseases. Circulating IL-18 levels are considered as a prognostic marker associated with congestive heart failure, myocardial infarction, and cardiovascular death in patients with coronary heart disease [25,26].

In our study, Pfizer injection induced immune and inflammatory responses in the cardiac muscle. This was confirmed by the significant increase of cardiac IL-18, IL-6, and TNF-α.

Approving our findings, a study performed on a mouse model to test the effect of intravenous injection of COVID-19 mRNA vaccine on the heart reported that cardiac tissue mRNA expression of IL-1β, interferon (IFN)-β, IL-6, and TNF-α increased significantly with the presence of myopericarditis [11]. Furthermore, a recent study explored the involvement of immune system activation in patients with myopericarditis after COVID-19 mRNA vaccination and their study showed elevated plasma IL-18 in myopericarditis after COVID-19 vaccine. They also showed that the patient with COVID-19 mRNA vaccine-associated myopericarditis presented increased levels of pro-inflammatory cytokines (IL-6 and TNF- α)[26].

In the current study, Pfizer vaccine injection in rats resulted in a state of hypercoagulability in blood and blood clotting disorder demonstrated by the significant increase in the plasma D-Dimer level. This was associated with significant increase in cardiac troponin I and CRP reflecting increased inflammatory condition that resulted in cardiac damage

Supporting our findings, multiple studies reported elevation of D-Dimer in 25-50% of cases of myocarditis following mRNA COVID-19 vaccine occurred [27,28,29].

Our study showed significant increase in troponin level of vaccinated rats as compared to

the control group indicating cardiac cell damage resulting in leakage of troponin. This came in agreement with *Dionne et al.* [30]who reported that troponin levels were elevated in all patients with myocarditis after receiving the BNT162b2 (Pfizer) vaccine as a result of immunogenic or inflammatory response to the vaccine. Also, further studies reported that elevated cardiac troponin levels were seen in all cases, and these ranged from 10-fold to 400-fold increases in a case series of 23 patients after mRNA vaccination[8].

As for CRP, the current study showed higher CRP levels in vaccinated rats and this finding is supported by *Truong et al.* [10]who reported mild elevation of CRP in post-vaccination myocarditis patients. Moreover, several case reports of post-COVID-19 vaccine myocarditis documented elevation of CRP [11, 28].Another systemic review revealed marked elevations in cardiac troponins and CRP in COVID-19 patients with myocarditis [31].

Assessment of the oxidative stress in our study showed significant increase in MDA and decrease in catalase in cardiac tissue indicating increased oxidative stress and diminished antioxidant capacity. There have been strong indications that oxidative stress and the defense against reactive oxygen species (ROS) are crucial in COVID-19 pathogenesis on various mechanistic levels [32].

In support of our findings, increased inflammasome complex formation in monocytes and increased plasma levels of IL-18 was observed in COVID-19 patients with more severe symptoms [33]. In addition, peripheral blood mononuclear cells (PBMCs) from COVID-19 patients released IL-1 β, IL-6, and TNF- α ex vivo. Cytokine levels were positively correlated with oxidative stress markers in COVID-19 patients [34]. This was also in accordance with the results of another study that reported vaccine-induced myopericarditis cases are associated with oxidative stress test abnormality [17].

Studying the cardiac functions revealed electrocardiographic changes (ECG) so that Pfizer-injected group showed significant bradycardia and significant decrease in the R-wave voltage when compared to their controls. These findings reflect the state of myocardial injury that occurred after Pfizer vaccine injection (2nd dose) that affected both the chronotropic and the inotropic activity of the cardiac muscle.

These findings go with *Kaur etal.*[35] who reported abnormal ECG findings following Pfizer vaccine injection in the form of arrythmia (30% bradycardia and 60% tachycardia), decreased R wave voltage and ST elevation [10].

Prolonged QT duration was also observed which indicates prolonged cardiac action potential that leads to variable forms of arrythmia. The prolonged QT is considered as a good predictor of myocarditis and is useful for early recognition of the fulminant one [36]. This finding is consistent with a recent study that documented many cases of long QT syndrome following injection of Pfizer COVID vaccine [37].

The previous findings indicate that cardiac cells may be a target for inflammatory cytokines released due to vaccine injection and increased oxidative stress state. This resulted in a change in heart rate dynamics as well as cardiac contraction force.

As for the cardiac intrinsic properties, chronotropic, inotropic ,and myocardial flow rate

changes were observed. The significant bradycardia which was observed in the Pfizer injected group when compared to the control group, and to its pre-ischemic value indicate inefficient chronotropic recovery in Pfizer injected rats following 30-minute ischemia. This could be attributed to the increase in the oxidative stress in hearts isolated from these rats (significant increase in MDA "oxidative stress marker" which has a negative correlation with heart rate ($r=-0.6$, $P < 0.05$).

Oxidative stress resulted in sinoatrial node dysfunction demonstrated by decreased heart rate both in vivo and in vitro. Reactive oxygen species negatively affect myocardial calcium handling, causing arrhythmias, and contributing to cardiac remodeling by inducing hypertrophic signaling, apoptosis, and necrosis[38].

Moreover, isolated hearts from Pfizer injected group demonstrated disturbed myocardial mechanics including systolic dysfunction manifested by the significant decrease in the peak tension when compared to the control group following 30 minutes ischemia.

Peak developed tension describes the maximum force generated by the heart, therefore, hearts isolated from Pfizer injected group were not protected from the inotropic insult induced by ischemia reperfusion and deterioration in the cardiac contractility occurred. This could be attributed to the disturbance of the heart muscle energy balance that could limit the cardiac function recovery in I/R. ATP depletion and/or cytoplasmic calcium-overload, might be the cause of the fall of peak tension in hearts isolated from Pfizer injected group[39,40].

Moreover, time to peak tension, half relaxation time, contraction time were significantly prolonged in the isolated hearts from Pfizer injected rats compared to their controls. These findings further confirm the state of systolic and diastolic dysfunctions which could be due to calcium overload and impaired calcium homeostasis. In addition, the myocardial ischemia in this group can be a possible explanation for the contractile dysfunction as well as abolished calcium uptake by sarcoplasmic reticulum and hence lusitropic dysfunction (prolonged HRT).

The systolic and diastolic dysfunction could be attributed to the significant increase in IL-18 level which has a positive correlation with Time to peak tension, Half relaxation time, Contraction time.IL-18 reduces the responsiveness of the myofilaments to $Ca2+$, induces myocardial hypertrophy and remodeling [41]. Moreover, IL-18 induces myocardial fibrosis, and dysfunction resulting in increased ventricular stiffness and decreasing cardiac contractility [42]

Thus, elevated IL-18 directly cause cardiomyocyte injury in the patient with myopericarditis after COVID-19 vaccination, which was proven by high cardiac troponin levels and decreased ventricular and diastolic systolic functions [26].

The significant decrease in the myocardial flow of hearts isolated from Pfizer injected group rate further augmented the ischemic insult for these hearts. This significant decrease is attributed to the significant increase in the D-Dimer level reflecting a state of hypercagulability (significant negative correlation exists between plasma D-Dimer and myocardial flow rate,$r= -0.7$, $P < 0.001$).

Carli et al.[43] reported thrombotic events two weeks after a booster dose of the Pfizer-COVID-19 mRNA injection which could be attributed to vaccine induced thrombocytopenic purpura through activating antibodies towards platelet factor 4. This is a fatal condition that is characterized by mild to severe thrombocytopenia and a striking hypercoagulable state, it leads to widespread venous or arterial thrombosis.

Interestingly, the proportion of arterial thrombotic events was higher in the Pfizer (mRNA) vaccine group than the adenovirus group, supporting the hypothesis that the mechanism of vaccine induced thrombosis is likely different between adenovirus and mRNA platform vaccine [44].The significant increase in plasma D-Dimer could be attributed to the significant increase in IL-18 (significant positive correlation between IL-18 and plasma D-Dimer r=0.5, $P < 0.05$).

In the current study, Pfizer vaccine resulted in major histological changes in the form of marked apoptotic cardiac muscle fibers and other with small pyknotic cells that reflects necrosis of cardiac muscle. This finding goes in agreement with *Li et al.* [11] who reported widely distributed apoptotic cardiac cells both sporadically and in the form of multiple large foci.

Conclusion:

Interestingly in the current study, administration of COVID-19 mRNA vaccine has been shown to induce a rapid onset myocarditis that impaired the cardiac function in rats. A possible explanation for the cardiac damage that occurred after COVID-19 mRNA vaccine could be mediated by vaccination-triggered excessive IL-18 production which further increased the immune (IL-16, TNF- α), increased inflammatory (cardiac troponin-I and CRP) and oxidative stress(MDA) in the in the hearts of injected rats. Moreover, the vaccine resulted in a hypercoagulable state (increased D-Dimer) that led to decreased cardiac muscle perfusion which further increased damage of cardiac muscle fibers and accelerated the rate of programmed cell death i.e., apoptosis.

Recommendations

Although COVID-19 vaccination is important in the disease control, our study recommends accurate cardiac investigation before vaccination in all age groups especially those with cardiac risk factors or past medical history of cardiac diseases.

Ethics Declaration

Conflict of interest: The authors declare that there are no conflict of interest.

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