The Association of Serum Bilirubin Levels With Cardiovascular Disease And Metabolic Syndrome In Palestinian Population

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Abstract

Introduction

Bilirubin is the end product of heme catabolism. At physiological levels, bilirubin is a potent antioxidant and anti-inflammatory molecule. Oxidative stress and inflammation are major events linked to cardiovascular disease (CVD) and metabolic syndrome (MS), in which CVD is considered as one of the leading cause of death. A Mildly increased circulating total serum bilirubin level (TSB) seems to be a promising target for decreasing the prevalence of CVD and MS. The aim of this study is to evaluate the association of TSB with CVD and MS among Palestinian-population.

Methods

A retrospective study was conducted in 2021 for 1446 participants and the medical records were collected from AL-Ahli hospital-Palestine. The examined samples were divided into four quartiles based on TSB (Q1= 0.1-0.4mg/dl, Q2 = 0.5-0.8mg/dl, Q3 = 0.9-1.2mg/dl, Q4 = >1.2 mg/dl). The prevalence of CVD, MS and the correspondence laboratory parameters (e.g. bilirubin, lipid profile, and HbA1c) were analyzed in relation to TSB.

Results

Total serum bilirubin and HDL were significantly lower in CVD patients compared to non-CVD persons. TSB, direct bilirubin and HDL were significantly higher in persons without MS compared to patients with MS. The prevalence of CVD in bilirubin level quartiles from 1-4 (95%CI in parentheses) was 33.7% (95%CI 28.5-42.1), 36% (95%CI 32.3-40.3), 7.7% (95% 3.4-11.8), 4.7% (95%CI 0.8-8.4) respectively (p-value <0.001). The prevalence of MS in bilirubin level quartiles from 1-4 (95%CI in parentheses) was 7.1% (95% 4.5-8.9), 5.7% (95%CI 3.2-7.1), 1.4% (95%CI 1.1-1.6), 0.8% (95%CI 0.6-1) respectively (p-value <0.05).

Conclusions

In Palestinian adults, TSB is inversely related to the prevalence of CVD and MS. We suggest that TSB should be routinely tested in patients with risk of CVD and MS.

Keywords

Anti-oxidants, CVD, LDL, Metabolic syndrome, Nrf2, Total serum bilirubin.

1. Introduction

Bilirubin is the yellow end product of heme catabolism. Most of bilirubin is generated by the destruction of red blood cells in spleen while the remaining (about 20%) is derived from heme-containing proteins. The Heme-oxygenase-1 enzyme (HO-1) converts heme into biliverdin which is then reduced to unconjugated bilirubin by biliverdin reductase (BVR) enzyme (Vítek & Ostrow, 2009). At physiological levels, bilirubin has a potential antioxidant effect both in vivo and in vitro. It has been shown that bilirubin prevents the oxidation of lipoproteins and lipids (Choi et al., 2013; J.-P. Lin et al., 2010). Bilirubin has an antioxidant role by lowering reactive oxygen species (ROS) (Jansen & Daiber, 2012). Our previous studies showed that bilirubin at higher levels induces Nrf2 (Qaisiya et al., 2014), a master cellular sensor of antioxidant response genes (Hayes & Dinkova-Kostova, 2014). Other studies demonstrated that bilirubin has an anti-inflammatory properties and inhibits pro-inflammatory genes such as: NFκB and TNFα (Bulmer et al., 2018; Yamashita et al., 2004).

Metabolic syndrome is a collection of complex metabolic abnormalities that constitute cardio-metabolic risk factors including hypertension, central obesity, insulin resistance, and dyslipidemia (Rochlani et al., 2017; Zhong et al., 2017). The pathogenesis of MS includes both genetic and acquired factors that altogether contribute to the final pathway of inflammation that leads to CVD (Rochlani et al., 2017). Cardiovascular disease is a cluster of diseases that affect the heart and the blood vessels (Flora & Nayak, 2019). Oxidative stress (OS) has also been involved in the pathogenesis mechanisms of CVD (Oda, 2017). Substantial evidence from experimental and clinical studies have indicated the role of OS and inflammation act as a central network in the progress of CVD and MS (García et al., 2017; Hutcheson & Rocic, 2012).

The epidemic of CVD is due to the sedentary lifestyle and to significant changes of dietary habits that increased rates of risk factors including diabetes, hypertension, hyperlipidemia and obesity (Traina et al., 2017). The cyto-protective role of bilirubin against MS and CVD was well established in several studies (Guzek et al., 2012; J.-P. Lin et al., 2010). A Mildly increased circulating bilirubin level seems to be a promising target for prevention and reduction of the prevalence of MS and its related diseases including CVD and diabetes (Vítek, 2012). An inverse correlation between the presence of CVD and bilirubin in circulation was reported in several independent studies (Lan et al., 2019; Stojanov et al., 2013). A recent study in Koreans showed that bilirubin levels are negatively correlated with the incidence of CVD (Suh et al., 2018). A representative study conducted on sample of adults from Poland showed that total TSB level is inversely related to the prevalence of MS and insulin resistance (Guzek et al., 2012). Furthermore, bilirubin is suggested as a potential biological marker in the risk prognosis of chronic inflammatory disorders, in which CVD is considered as one of the leading cause of mortality worldwide (Suh et al., 2018).

The prevalence of CVD and associated risk factors is high in most Asian and Middle Eastern countries, and considered the highest in the world (Bhagavathula et al., 2021). There is limited information on the prevalence of CVD and its related risk factors among adults in the Gulf area and the United Arab Emirates (Aljefree & Ahmed, 2015; Razzak et al., 2018).

To our knowledge, no such study has been performed in the adult population of Palestinians. The aim of the present study is to evaluate the association (if any) of serum bilirubin level with the prevalence of CVD and MS among the adult population in Palestine.

2. Methods

2.1 Study design and population

This representative retrospective study was launched with the ethical approval between Hebron University and Al-Ahli Hospital. We have reviewed and evaluated 3381 files for persons (aged between 18 to 97 years) who admitted to cardiology department in 2021 at Al-Ahli Hospital. All records with incomplete laboratory data were excluded from our analysis. People who have anemia or being cigarette smokers or have viral hepatitis markers were excluded from our analysis because these factor rises TSB levels. Subjects with higher bilirubin level (>3 mg/dl) and transaminase activities were excluded from the study to rule out patients with impaired liver function. The final number of analyzed records was 1446 (858 males and 588 females).

From the data records, different parameters were collected including: Age [years], gender, BMI kg/m2], waist circumference

[cm], hypertension [mmHg], total serum bilirubin (TSB) [mg/dl], direct bilirubin (DBil) [mg/dl], triglycerides (TG) [mg/dl], total cholesterol (TC) [mg/dl], high-density lipoprotein cholesterol (HDL) [mg/dl], low-density lipoproten cholesterol (LDL) [mg/dl], and HbA1c measurements [%]. Data were divided into four groups from (Q1- Q4) according to particular TSB level quartiles; Q1= 0.1-0.4 mg/dl, Q2 = 0.5-0.8 mg/dl, Q3 = 0.9-1.2 mg/dl, Q4 = >1.2 mg/dl. The baseline characteristics of the study population were compared within the quartiles.

2.2 Determination of CVD

CVD was defined as the occurrence of coronary heart disease and/or stroke based on the electrocardiogram, enzyme assay and coronary angiography confirmation. Data was obtained from medical records for persons found during routine follow-up examinations. People diagnosed with CVD were 1188 patients, while 258 were considered non-CVD.

2.3 Definition of the metabolic syndrome (MS)

The define metabolic syndromes is based on having three or more of the following traits: insulin resistance, fasting blood glucose level (≥ 100mg/dl), high triglycerides (≥150mg/dl), reduce HDL-cholesterol (<40mg/dl in male, <50mg/dl), hypertension (SBP ≥130mg/dl and DBP ≥ 85mg/dl), and obesity (waist circumference ≥ 90cm for male and ≥85cm for female) (Choi et al., 2013; Huang, 2009). In this study subjects were diagnosed with the MS according to the following criteria: (1) triglyceride levels higher than 150 mg/dl, (2) high-density lipoprotein cholesterol (HDL) levels of less than 40 mg/dl in males or less than 50 mg/dl in females, (3) HbA1c greater than 6% and obesity. Subjects who classified as MS patients were 217 and those without were 1229.

2.4 Statistical analysis

All statistical analyses were performed with use of SPSS, v.27 software (SPSS Inc., Chicago, Illinois). The results were expressed as mean ± standard deviation to compare the differences between groups. Normality testing for non-normal distribution of the parameters (triglycerides, DBil and TSB) was performed. Skewness, kurtosis, Shapiro-wilk and Intequartile range (IQR) values were obtained. Pearson's chi-square test was used to analyze statistical differences in the characteristics of the study participants among quartiles. Trends between categorical variables were tested for statistical significance using chi-square tests for linear-by-linear association. Multivariate linear regression analysis was done to identify the major predictors of the TSB. Logistic regression analyses were used to estimate the odds ratios for CVD in the high bilirubin quartiles (Q3 and Q4). A probability value of $p < 0.05$ was considered significant.

3. Results

3.1 Differences of the general parameters between genders

General characteristics of the study population based on the gender are presented in (Table 1). TSB, triglyceride and LDL were significantly higher in males, while total cholesterol and HDL were significantly higher in females. Certain data including TG, TB, and DB, appear to be non-normally distributed.

3.2 Distribution of the study sample based on TSB levels

Subjects were categorized into four quartiles based on TSB levels. The baseline characteristics of the study population according to these quartiles are presented in (Table 2). Negative relationships were observed between the bilirubin quartiles and systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, triglyceride, LDL and HbA1c. Positive relationships were observed between the bilirubin quartiles and HDL. No clinical significance was observed between bilirubin quartiles and age, BMI and waist circumferences.

3.3 High TSB is inversely associated with the prevalence of CVD and MS

According to the distribution of bilirubin level in quartiles, the prevalence of CVD and MS relatively decreased as the bilirubin level quartiles increased. The prevalence was 33.7% (95%CI 28.5-42.1), 36% (95%CI 32.3-40.3), 7.7% (95% 3.4-11.8), 4.7% (95%CI 0.8-8.4) respectively for CVD patients (Figure 1). It was 7.1% (95% 4.5-8.9), 5.7% (95%CI 3.2-7.1), 1.4% (95%CI 1.1-1.6), 0.8% (95%CI 0.6-1) respectively for MS patients (Figure 2).

Table 1. General characteristics of the study population based on the gender.

Variable	Male (N= 858)	Median Male score [IQR]	Female (N= 588)	Median Female score [IQR]	p-value
Age [years]	54.79±15.75		57.72±15		0.001*
TSB [mg/dl]	1.90±0.85	1.0 [1.00-2.00]	1.77±0.88	1.0 [1.00-2.00]	0.005*
DBil [mg/dl]		0.2 [0.100-0.300]		0.2 [0.100-0.300]	0.530
TC [mg/dl]	168.28±46.50		176.40±78.07		0.022*
TG [mg/dl]		114 [104.0-217.0]		115 [105.0-220.0]	< 0.001*
HDL [md/dl]	36.92±11.73		44.47±12.24		< 0.001*
LDL [mg/dl]	102.74±42.42		97.21±41		0.023*
HbA1c [%]	5.8±0.6		5.3±0.4		0.297

Table 2. Baseline characteristics of the study population based on bilirubin quartile.

Variable	Q1 (0.1-0.4mg/dl)	Q2, (0.5-0.8mg/dl)	Q3 (0.9-1.2mg/dl)	Q4 (>1.2mg/dl)	P-value
Age [years]	55±16	56±15	59±16	58±18	
BMI [kg/m2]	23.4±4.2	22.7±3.5	24.5±5.1	23.8±3.2	0.253
Waist (cm)	82.31±8.13	83.52±7.42	82.34±4.33	83.63±7.04	0.126
SBP (mmHg)	119.12±13.53	118.41±17.00	115.41±18.57	114.41±15.3*	0.014*
DBP (mmHg)	77.43±11.3	74.86±12.45	75.67±10.87	74.32±16.21*	0.038*
TSB [mg/dl]	0.32±0.07	0.60±0.10	1.02±0.11	1.74±0.48*	<0.001*
DBil [mg/dl]	0.2±0.1	0.2±0.1	0.3±0.1	0.4±0.3	0.128
TC [mg/dl]	181±82	169±46	158±36	154±38*	<0.001*
TG [mg/dl]	207±186	169±135	150±90	146±113*	<0.001*
HDL[my/dl]	40±13	39±12	41±13	44±14*	<0.001*
LDL [mg/dl]	105±42	102±44	90±36	86±35*	<0.001*
HbA1c (%)	5.8 ± 0.3	5.6 ± 0.2	5.9 ± 0.5	5.1± 0.2*	<0.001*

Data are expressed as mean ± SD

*Significantly differences is related to group Q1 (control)

TSB: total serum bilirubin; DBil: direct bilirubin; TC: total cholesterol; TG: tri-
glyceride; HDL: high-density lipoprotein cholesterol; LDL: low-density lipo-
protein cholesterol.

Q1 as an internal control

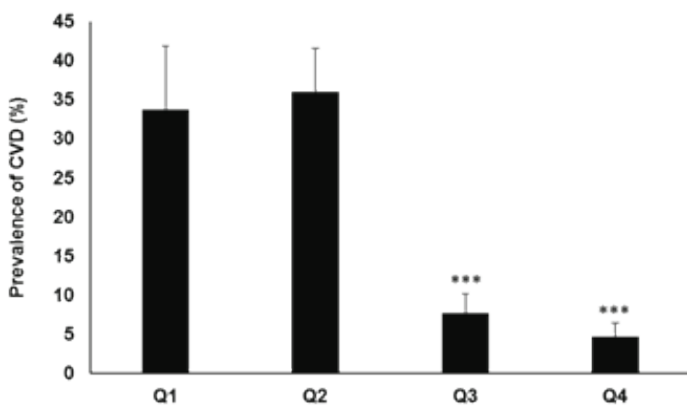p < 0.05 is considered significant (*)

Figure 1: Prevalence of CVD in relation to total serum bilirubin con-
centration (adjusted for gender). Q1= 0.1-0.4mg/dl, Q2 = 0.5-0.8mg/dl,
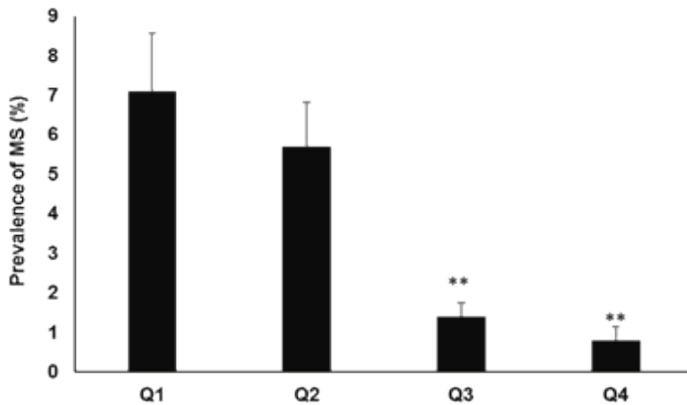Q3 = 0.9-1.2mg/dl, Q4 = >1.2mg/dl. p-value <0.001

Figure 2: Prevalence of MS in relation to total serum bilirubin concen-
tration (adjusted for gender). Q1= 0.1-0.4mg/dl, Q2 = 0.5-0.8mg/dl, Q3
= 0.9-1.2mg/dl, Q4 = >1.2mg/dl. p-value <0.01

3.4 Low TSB in CVD associated with high triglycerides, cholesterol and LDL

Comparison of clinical differences between patients
with CVD and those without CVD are presented in (Table 3).
TSB and HDL were significantly higher in non-CVD persons
compared to CVD patients. Total cholesterol, triglyceride, and
LDL were significantly higher in CVD patients compared to
non-CVD persons.

Table 3. Comparison of clinical differences between persons with CVD
and those without.

Variable	Non - CVD (N= 258)	CVD (N= 1188)	P-value
	Mean ±SD	Mean ±SD	
Age [years]	55.09±16.14	56.18±15.77	
TSB [mg/dl]	2.10±0.99	1.80±0.83	< 0.001*
DBil [mg/dl]	0.21±0.09	0.22±0.14	0.215
TC [mg/dl]	150.91±18.15	177.03±67.50	< 0.001*
TG [mg/dl]	117.88±26.36	197.03±168.73	< 0.001*
HDL [mg/dl]	52.73±9.78	36.69±10.91	< 0.001*
LDL [mg/dl]	63.47±14.26	110.15±41.76	< 0.001*

Data are expressed as mean ± SD

CVD: cardiovascular disease; TSB: total serum bilirubin; DBil: direct bilirubin;
TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein cholester-
ol; LDL: low-density lipoprotein cholesterol.

p < 0.05 is considered significant (*)

3.5 Low TSB in MS associated with high triglycerides, cholesterol, LDL and HbA1c

The results of the relationship between clinical parameters
and MS are presented in (Table 3). TSB, direct bilirubin, and
HDL were significantly higher in persons without MS compared
to patients with MS. Total cholesterol, triglyceride, LDL, and
HbA1c were significantly higher in patients with MS compared
with those without.

3.6 Risk of cardiovascular disease factors based on TSB quartiles

The results of multivariate analysis of the relationship
between bilirubin and traditional CVD disease risk factors are
presented in (Table 5). The strongest negative correlation with
bilirubin level were obtained for LDL-C and TGs with odd ratio
0.53 (0.37-0.73) and 0.35 (0.26-0.52), respectively, between the
Q4 vs. Q1. An inverse association of bilirubin level with HbA1c
was observed in Q4 vs. Q1 with odd ration level (OR=0.60
(0.37-0.73). No association was found for blood pressure and
direct bilirubin.

Table 4. Comparison of Clinical differences between persons with MS and those without.

Variable	Without MS (N=1229)	With MS (N=217)	P-value
	Mean ±SD	Mean ±SD	
Age [years]	55.21±16.39	60.33±11.30	
TSB [mg/dl]	1.87±0.87	1.72±0.83	0.016*
DBil [mg/dl]	0.22±0.13	0.19±0.13	0.019*
TC [mg/dl]	168.41±63.15	187.42±50.38	< 0.001*
TG [mg/dl]	151.29±113.45	312.03±227.13	< 0.001*
HDL [mg/dl]	41.62±12.75	32.26±7.35	< 0.001*
LDL [mg/dl]	98.49±41.73	110.44±43.35	< 0.001*
HbA1c [%]	5.6±0.2	7.2±0.6	< 0.001*

Data are expressed as mean ± SD

MS: metabolic syndrome; TSB: total serum bilirubin; DBil: direct bilirubin; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; HbA1c: glycated hemoglobin.

$p < 0.05$ is considered significant (*)

Table 5. The risk of CVD based on the four quartile. Adjusted odds ratios of particular CVD or risk factors in relation to bilirubin level values (distributed in quartiles) for the whole study population

Group	Q2 Vs Q1	Q3 Vs Q1	Q4 vs Q1	P-value
CVD 1	0.972 (0.819–1.211)	0.803 (0.712-0.953)*	0.70 (0.50-0.97)*	<0.004
LDL-c 2	0.74 (0.53-1.01)	0.68 (0.57-0.97)	0.53 (0.37-0.73)*	<0.003
TGs 2	0.65 (0.45-0.90)	0.48 (0.33-0.67)*	0.35 (0.26-0.52)*	<0.001
HbA1c 2	1.05 (0.86-1.49)	0.91 (0.50-0.93)	0.60 (0.46-0.85)*	<0.001
Blood pressure 2	0.87 (0.65-1.29)	0.85 (0.59-1.37	0.98 (0.79-1.24	0.72
DBili 2	1.03 (0.72-1.47)	0.93 (0.76-1.34)	1.07 (0.79-1.50)	0.396

Data are expressed as mean ± SD

*Significantly differences are related to group Q1 (control) which is considered as 1

1 adjusted to age, BMI, sex, waist circumference and DBil, LDL-cholesterol and HbA1c

2 adjusted to age, sex and other CVD-risk factors

$p < 0.05$ is considered significant (*)

4. Discussion

Bilirubin is an endogenous antioxidant and anti-inflammatory molecule. At mild levels, bilirubin directly scavenge reactive specieses. Studies performed on endothelial cells demonstrated that bilirubin able to scavenges NADPH oxidase-derived ROS and reduce cell toxicity (Maruhashi et al., 2019). Wegiel et al. have shown that the knockout of BVR enzyme in vitro leads to an increase in cell death in response to low level of bilirubin and high hydrogen peroxide (B & Le, 2012). Furthermore, A large number of therapeutic drugs including non-steroidal anti-inflammatory drugs (e.g., coxibs) and hypolipidemic (e.g., statins) have been reported to induce HO-1 that increases bilirubin level (Vitek et al., 2019). Other studies showed that bilirubin indirectly activates the Nrf2 antioxidant genes (Qaisiya et al., 2014), that represent a promesing target to reduce the incidence of CVD and MS (da Costa et al., 2019). These benificial effects of bilirubin is supported by several studies showing that TSB level is inversely related to MS, CVD and diabetes mellitus (Franchini et al., 2010; Guzek et al., 2012; Kim et al., 2014; Suh et al., 2018). In this study, we evaluated the possible relatioship between TSB and the prevelance of CVD and MS amonge Palestinian patients.

Our retrospective study has indicated a significant inverse relationship between TSB and the prevalence of CVD and its related diseases such as MS (Fig. 1 and Fig. 2). These results are compatible with a study that supports the concept that bilirubin via its antioxidant potential has a protective effect against CVD in young males and females in Serbia (Stojanov et al., 2013), and with the results that founds a close association between low serum bilirubin concentration and increased CVD risk in Chinese population (Ko et al., 1996). Our results are also consistent with a study conducted by Guzek et al, showing the inverse association of TSB with the prevalence of MS in Polish adults (Guzek et al., 2012). Similarly, a study in Egypt demonstrated a strong inverse association between the reduced severity of coronary artery disease and increased total and indirect bilirubin levels (Adel et al., 2019).

The most important elements characterizing the CVD and increase the risk of its development are oxidative stress and inflammation. In CVD patients an elevation of oxidized LDL (oxLDL) concentration was reported. The ox-LDL can cause lipid accumulation in macrophages and foam cell formation as well as being cytotoxic to many cell types probably through inactivation of endothelial cell-derived relaxing factors (Yoshida & Kisugi, 2010). Research has provided strong evidence that LDL oxidation plays an important role in the pathogenesis of CVD and MS (Yla-Herttuala, 1999). In CVD there is an imbalance between the free radicals and the antioxidant defense system of the body which leads to the damage of nucleic acids, proteins, and lipids. On the other hand, It has been reported that mild hyperbilirubinemia inhibits oxidative stress which acts as ROS scavenger (Maruhashi et al., 2019). This is consistence to our results as shown in (Table 2 and Table 3), that revealed a significant decrease in LDL, TC, TG and HbA1c as TSB increases, while HDL is significantly increased. These findings suggest that elevation of TSB within the normal range are favorable condition from a CVD standpoint (Suh et al., 2018). This is supported by the results of the adjusted odd ration in (Table 5) showing that sample of high TSB in Q4 reduces the risk factors of CVD and inversely associated with LDL-c and TGs levels.

A previous study performed by Lin et al, showing that children and adolescent revealed a negative association between TSB and insulin resistance (L.-Y. Lin et al., 2009). From this study, we can speculate an inverse relationship between TSB and glucose level. No enough data was obtained concerning insulin resistance although the percentage of HbA1c was significantly elevated in MS patients compared to Non-MS (Table 5). Therefore, we still need more specific tests to measure insulin resistance (e.g., HOMA-IR). The use of TSB in this study makes it limited to state whether indirect, direct bilirubin or the free bilirubin has the strongest relationship with CVD and MS. Another limitation to this study is the marked discrepancy in the number of patients with CVD (1188) and non-CVD controls (258), implying some selection bias that undoubtedly affects the reliability of the results.

5. Conclusion

There is an inverse association between circulating TSB and the prevalence of CVD in Palestinian adults. The mechanism behind this relationship may be related to the beneficial effect of mild increase of TSB as antioxidant and anti-inflammatory molecule. Further investigations are needed to prove the inverse correlation of TSB with the risk factors of CVD and MS including insulin resistance, diabetes mellitus type 2, hypertension and obesity among Palestinian population.

Conflict of interests

The authors report no conflicts of interest. This research has not been previously published in any way, whether written, read, published, visual or audio.

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Contribution of authors:

Dr. Mohammad Qaisiya: Conceptualization, project administration, supervision, formal analysis, writing original draft and approving the final draft

Dr. Haneen Nur: Validation, data curation, formal analysis and approving the final draft

Sarah Yaghi: Investigation, writing original draft, methodology, formal analysis and approving the final draft

Haneen Aqel: Investigation, methodology, formal analysis and approving the final draft

Aya Najjar: Investigation, methodology, formal analysis and approving the final draft

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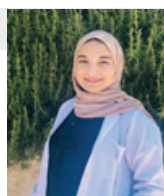
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