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ORIGINAL ARTICLE

# Evaluation of some oxidative markers in diabetes and diabetic retinopathy

Hadeel Ahmed Shawki[1,3] · Rasha Elzehery[2] · Maha Shahin[3] · Ekbal M. Abo-hashem[2] · Magdy M. Youssef[1]

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## Abstract

**Aims** Diabetes mellitus and diabetic retinopathy (DR) are major public health concerns globally. Oxidative stress plays a central role in the pathogenesis of diabetes and DR. The aim of this study was to investigate the association of malondialdehyde, uric acid and bilirubin with diabetes and diabetic retinopathy development.

**Methods** This study was conducted on 110 diabetics (with and without retinopathy). Beside 40 healthy individuals as a control group. The level of three markers (malondialdehyde, uric acid and bilirubin) was estimated in the studied groups. Receiver operating characteristic analysis and a logistic regression model was performed.

**Results** The present study revealed significantly higher uric acid and malondialdehyde levels, while bilirubin showed significantly lower levels in diabetics compared to control and similarly in diabetic retinopathy compared to those without DR. Furthermore, combination of the three markers increased the accuracy and effect size for differentiation between diabetes with and without DR. In addition, higher levels of uric acid and malondialdehyde were associated with risk of diabetes and DR development.

**Conclusion** This study concluded that higher levels of uric acid and malondialdehyde were associated with increase in the risk of diabetes and DR development, while bilirubin wasn't associated with decreasing the risk of diabetes or DR. However, the combination of malondialdehyde, uric acid and bilirubin may be a valuable addition to the current options for the prognosis of DR. In addition, malondialdehyde may be independent predictor of diabetes and DR as well as uric acid may be used as independent biomarker to predict the risk of DR.

**Keywords** Uric acid · Malondialdehyde · Bilirubin · Diabetes · Diabetic retinopathy

## Introduction

Diabetes mellitus is a major public health problem worldwide. It is a chronic disease characterized by chronic hyperglycemia due to inability of the body to adequately produce or use insulin effectively [1]. Hyperglycemia causes oxidative stress which is associated with the pathophysiology of DM. Severe oxidative stress may cause cell damage leading to microvascular complications that include diabetic retinopathy, neuropathy, and nephropathy. In addition, macrovascular complications such as stroke, coronary artery disease, and peripheral arterial disease [2]. Microvascular and macrovascular disorders are responsible for morbidity and mortality in diabetic patients [3].

DR is one of the most severe diabetic microvascular complications that affect the blood vessels in the retina due to prolonged hyperglycemia. It is the fifth most common cause of blindness that contributes to 4.8% of vision loss worldwide [4]. Early detection of diabetic retinopathy can reduce the risk of blindness by 50% [5].

Oxidative stress plays a central role in the pathogenesis of diabetic retinopathy. It produces mitochondrial dysfunction, inflammation and cell death by apoptosis and neurodegeneration that leads to vascular, neural and retinal tissue damage [6, 7]. Malondialdehyde (MDA) is a highly reactive three-carbon dialdehyde, produced as an end product of lipid peroxidation. It is a highly toxic and reactive bifunctional

✉ Hadeel Ahmed Shawki
biolab_msd@yahoo.com

1 Biochemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt

2 Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

3 Mansoura Ophthalmic Center, Mansoura University, Mansoura, Egypt

molecule that reacts with DNA and forms adducts with deoxyguanosine and deoxyadenosine. It can also modify RNA, proteins and other biomolecules [8]. Beside, uric acid (UA), a metabolic end-product of purine degradation, possesses pro-oxidant property in human and is also a promoter of oxidative and inflammatory processes [9]. Hyperuricemia has a vital role in the development of metabolic syndrome and increases the risk of microvascular complications. Elevated serum uric acid is a feature of hyperinsulinemia or insulin resistance [10].

Conversely, bilirubin is mainly generated from heme degradation and has strong antioxidant and anti-inflammatory effects on the microvasculature. It not only scavenges superoxide and peroxide radical but also prevents oxidation modifications of low-density lipoprotein and lipid oxidation [11]. Elevated serum total bilirubin (TB) levels protect against peripheral arterial disease, stroke and cardiovascular disease [12]. Hence, for the early detection of DM and DR, it is recommended to estimate the oxidative stress markers which might be helpful in assessing the risk of DM and its related complications. Thus, the present study aimed to investigate the relation between serum levels of oxidative stress markers (MDA and UA) and antioxidant marker (TB) with the development of diabetes and diabetic retinopathy.

## Materials and methods

This study was conducted on 110 diabetic patients classified into 70 diabetics with diabetic retinopathy (40 females and 30 males); their age ranges from 20 to 70 years with mean ± SD (43.0 ± 10.7); 40 diabetic patients without any complications (26 females and 14 males); their age ranges from 18 to 68 years with mean ± SD (45.4 ± 15.1). Besides, 40 healthy subjects (based on clinical examination and laboratory investigations) (28 females and 12 males); their age ranges from 25 to 60 years with mean ± SD (42.6 ± 9.4). Patients were selected from Clinics diabetes and endocrinology in Specialized Medical Hospital, Mansoura University. Ocular examinations were performed in the Ophthalmology Centre, Mansoura University. This study was performed in Clinical Pathology Department, Mansoura University. Patients were excluded if they had any evidence of other endocrinal diseases, liver or pancreatic diseases, and previous kidney diseases of any aetiology, severe infections or cancer.

### Blood sampling

Six ml venous blood was withdrawn from all subjects after fasting for 10–12 h, the blood was divided into two aliquots: two ml was collected into a tube containing Ethylenediaminetetraacetic acid (EDTA) for hemoglobin A1c (HbA1c) assay. Four ml was collected into a plain tube, left to clot and then centrifuged; the separated serum was used for some biochemical parameters investigations (blood glucose level, serum creatinine, and lipid profile) and measurement of three markers (MDA, uric acid and total bilirubin).

### Investigations

Fasting, post-prandial blood glucose, creatinine levels and lipid profile (triglyceride, total cholesterol, HDL-c) were done on automated device HITACHI 902, automatic analyzer (serial no.1928013). HbA1c was performed using Stanbio kit for quantitative colorimetric determination [13]. eGFR (estimated glomerular filtration rate) was estimated from the simplified equation developed using data from the Modification of Diet in Renal Disease (MDRD) Study as follows [14]:

$$\text{MDRD equation}: \ \text{eGFR} = 186.3 \times (\text{Serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times 0.742[\text{if female}] \times 1.212[\text{if Black}].$$

Assessment of lipid peroxidation by measuring serum malondialdehyde as a marker of oxidative stress by colorimetric method (Sigma chemical co). In this method, malondialdehyde (MDA) reacts with thiobarbituric acid in which coupling of MDA with thiobarbituric acid in 2 molar sodium sulfate solutions produce color that is proportional to MDA concentration and is measured at wave length of 534 nm [15, 16].

Assessment of pro-oxidative stress by measuring serum uric acid as a pro-oxidative marker by colorimetric method (Spinreact): uric acid is oxidized by uricase to allantoine and hydrogen peroxide (2H2O2), which under the influence of POD, 4-aminophenazone (4-AP) and 2-4 Dichlorophenolsulfonate (DCPS) forms a red quinoneimine compound [17]. The intensity of the red color formed is proportional to the uric acid concentration in the sample that is measured at 520 nm.

Measurement of serum total bilirubin concentration as antioxidant by colorimetric method (Randox). Total bilirubin is determined in the presence of caffeine, which releases albumin-bound bilirubin, by the reaction with diazotized sulphanilic acid produced color [18]. The absorbance measured at 578 nm against sample blank is proportional to the amount of total bilirubin concentration in specimen.

### Statistic analysis

All statistical calculations were done using computer program Statistical Package for Social Science (IBM Corp. Released in 2011. IBM SPSS Statistics for Windows, Version 20.0. (Armonk, NY: IBM Corp.). Data were presented

and suitable analysis was done according to the type of data obtained for each parameter. Mean ± standard deviation (± SD); median (interquartile range) were used for the description of numerical data. Receiver operating characteristic (ROC) analysis was performed to determine the optimal cut-off point of serum MDA, uric acid and total bilirubin levels for the prognosis of diabetes and diabetic retinopathy. AUC was transformed into Cohen's d for effect size [19]. The effect size was categorized into small (0.2–0.5), medium (0.5–0.8) and large (more than 0.8). Large effect size indicates better discriminating ability [20]. Sensitivity, specificity positive predictive value (PPV) and negative predictive value (NPP) were all calculated. Logistic regression was used for prediction of diabetes, dependent variables were control or DM, independent variables were age, BMI (body mass index), creatinine, TG, TC/HDL, bilirubin, UA, MDA. Logistic regression analysis was performed for the prediction of risk factors of diabetic retinopathy, dependent variables were DM with and without retinopathy, independent variables were age, BMI, HBA1C, TC/HDL, bilirubin, creatinine, duration of diabetics, UA, MDA. $P < 0.05$ were considered statistically significant.

## Results

No significant differences in age and gender between control group and different studied groups ($P > 0.05$). The studied groups are matched as regard age and gender. DM with DR showed significantly longer disease duration when compared to those without DR ($P < 0.001$). Higher fasting blood glucose (FBG), 2-h post-prandial glucose (2HPPG), HbA1c levels were significantly associated with diabetic patient when compared to control group ($P < 0.001$ for each). There were no significant differences in creatinine level eGFR and BMI between all studied groups ($P > 0.05$). HDL concentration was significantly lower in diabetics compared to control group ($P < 0.001$). Otherwise, no significant differences were found in lipid profile concentration between all studied groups ($P > 0.05$ for each).

UA and MDA showed significantly higher levels, while bilirubin showed significantly lower levels in DM compared to control group and similarly in diabetic patients with DR compared to those without DR as shown in Table 1.

ROC curve of TB, UA and MDA levels was conducted to estimate the predictive value of discrimination between DM and control groups. TB, UA and MDA showed the area under the curve (AUC) of (0.590, 0.661 and 0.994; respectively) and effect size (0.321, 0.587 and 3.554; respectively). Combinations of the three markers (TB, UA and MDA) for discrimination between DM and controls had nearly the

**Table 1** The clinical characteristics of participants

| Parameter | | Control $N=40$ | DM $N=110$ | $p$ | DM without DR $N=40$ | DM without D $N=70$ | $p$ |
|---|---|---|---|---|---|---|---|
| Age (years) | | 42.6 ± 9.4 | 43.8 ± 12.5 | 0.649 | 45.4 ± 15.1 | 43.0 ± 10.7 | 0. 340 |
| Duration | | – | 18.3 ± 11 | – | 8.7 ± 2.3 | 21.3 ± 10.9 | < 0.001 |
| Gender | Male | 12 (30%) | 44 (40%) | 0.266 | 14 (35%) | 30 (43%) | 0.423 |
| | Female | 28 (70%) | 66 (60%) | | 26 (65%) | 40 (57%) | |
| HbA1C (%) | | 5.3 ± 0.7 | 8.4 ± 2.0 | < 0.001 | 8.0 ± 2.2 | 8.7 ± 1.9 | 0.095 |
| FBG (mg/dL) | | 89 (77–100) | 255 (97–654) | < 0.001 | 232 (97–513) | 269 (111–654) | 0.092 |
| PPBG (mg/dL) | | 128 (94–194) | 351 (118–781) | < 0.001 | 308 (111–667) | 354 (128–781) | 0.094 |
| Creatinine (mg/dL) | | 0.85 ± 0.16 | 0.98 ± 0.26 | 0.054 | 0.90 ± 0.18 | 1.0 ± 0.29 | 0.053 |
| TC (mg/dL) | | 197 (102–260) | 192 (111–300) | 0.196 | 184 (116–298) | 193 (111–300) | 0.759 |
| TG (mg/dL) | | 150.2 ± 51.2 | 149.8 ± 64.37 | 0.404 | 153.9 ± 54.7 | 147.5 ± 69.5 | 0.322 |
| HDL (mg/dL) | | 54.8 ± 9.9 | 47.8 ± 10.7 | < 0.001 | 45.9 ± 10.6 | 48.9 ± 10.8 | 0.552 |
| LDL (mg/dL) | | 114.9 ± 42.4 | 114.0 ± 40.0 | 0.854 | 112.2 ± 41.4 | 115.1 ± 39.5 | 0.322 |
| TC_HDL | | 3.9 ± 1.5 | 4.3 ± 1.6 | 0.949 | 4.3 ± 1.4 | 4.3 ± 1.8 | 0.256 |
| LDL_HDL | | 2.3 ± 1.2 | 2.5 ± 1.3 | 0.580 | 2.6 ± 1.2 | 2.5 ± 1.4 | 0.732 |
| Bilirubin (mg/dL) | | 0.786 ± 0.17 | 0.672 ± .14 | 0.048 | 0.715 ± 0.163 | 0.649 ± 0.137 | 0.034 |
| Uric acid (mg/dL) | | 5.43 ± 2.04 | 6.6 ± 2.08 | 0.003 | 3.4 ± 1.8 | 8.5 ± 2.1 | < 0.001 |
| MDA (nmole/mL) | | 5.47 ± 1.6 | 16.43 ± 4.2 | < 0.001 | 12.0 ± 4.99 | 18.6 ± 3.67 | < 0.001 |
| eGFR ml/min/1.73m$^2$ | | 89.0 ± 17.6 | 88.5 ± 19.1 | 0.885 | 86.5 ± 19.5 | 86.3 ± 19.1 | 0.958 |
| BMI (kg/m$^2$) | | 30.8 ± 5.9 | 32.1 ± 6.8 | 0.285 | 31.7 ± 6.7 | 32.4 ± 6.9 | 0.634 |

$P > 0.05$ is considered non-significant, $P < 0.05$ is considered significant. Data are expressed as mean (SD) or median (interquartile range)

*DM* Diabetes mellitus, *DR* Diabetic retinopathy, *FBG* Fasting blood glucose, *PPBG* Postprandial blood glucose *TG* Triglyceride, *TC* Total cholesterol, HbA1c Hemoglobin A1c; *HDL* High-density lipoprotein; *LDL* Low-density lipoprotein, *MDA* Malondialdehyde, *eGFR* estimated glomerular filtration rate, *BMI* Body mass index, *N* number

same AUC (0.996), performance criteria of MDA and effect size (3.751). Performance criteria are shown in Table 2.

Furthermore, to explore the predictive value for diabetic retinopathy, ROC of TB, UA and MDA levels was analyzed. TB, UA and MDA showed AUC of (0.622, 0.762 and 0.986; respectively), and effect size (0.439, 1.008 and 3.108; respectively). Combinations of the three markers had perfect AUC) 1.0) and increased both the performance criteria and effect size (4.372) for discrimination between DR and non-DR group. Performance criteria are shown in Table 2.

All possible correlations between MDA, uric acid, bilirubin and other studied parameters in diabetic patients were estimated and summarized in Table 3. MDA showed significant positive correlations with FBG, PPBG and BMI. Uric acid showed significant positive correlations with BMI. Bilirubin showed significant negative correlations with FBG and PPBG. MDA showed significant negative correlation with bilirubin. Otherwise, no significant correlations were found regarding MDA, uric acid, bilirubin with other studied parameters in diabetics.

The correlation between three markers (MDA, UA and TB) and HbA1c in all studied groups was estimated and summarized in Table 4. MDA and uric acid showed significant positive correlations with HbA1c in diabetic and diabetic retinopathy patients. While bilirubin showed significant negative correlations with HbA1c in diabetic and diabetic retinopathy patients. No significant correlations were found between MDA and uric acid with HbA1c in healthy individuals. While bilirubin showed marginal significant negative correlations with HbA1c in healthy individuals.

Regression analysis was conducted for the prediction of DM development in healthy individuals. Higher UA and MDA were associated with increase in the risk of DM development in univariable analysis. However, conducting multivariable analysis revealed that higher MDA was suggested to be independent predictor of DM development (Table 5).

Regression analysis was conducted for the prediction of DR development in diabetics. Duration of diabetes is associated with increase in the risk of DR development in univariable analysis. Higher UA and MDA were suggested to be independent predictors of DR development in uni- and multivariable analyses (Table 6).

## Discussion

Diabetes mellitus and diabetic retinopathy are one of the fastest growing diseases and a leading cause of vision loss in the world [21]. Hyperglycemia plays an important role in the pathogenesis of retinal microvascular damage [22]. It promotes changes in vascular and neuronal structures through ischemic or hyperosmotic damage. The retina is a high energy-demanding organ, which makes it susceptible

**Table 2** Validity of TB, UA and MDA for discrimination between studied groups

| Groups | Parameters | AUC | Effect size | 95% CI | Cut off | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Diabetics and control group | Total bilirubin | 0.590 | 0.321 | 0.434–0.653 | 0.66 | 46.4 | 70 | 81.0 | 32.2 | 52.7 |
| | Uric acid | 0.661 | 0.587 | 0.573–0.750 | 5.6 | 64.5 | 65 | 83.5 | 40.0 | 64.6 |
| | MDA | 0.994 | 3.554 | 0.987–1 | 8.9 | 94.5 | 100 | 100 | 86.9 | 96.0 |
| | TB + UA + MDA | 0.996 | 3.751 | 0.990–1 | – | 97.3 | 95 | 98.2 | 92.8 | 96.7 |
| DR and non DR group | Total bilirubin | 0.622 | 0.439 | 0.511–0.733 | 0.67 | 54.3 | 75 | 79.2 | 48.4 | 61.8 |
| | Uric acid | 0.762 | 1.008 | 0.664–0.861 | 4.6 | 88.6 | 60 | 79.5 | 75 | 78.2 |
| | MDA | 0.986 | 3.108 | 0.972–1 | 16.7 | – | 100 | 100 | 86.9 | 94.5 |
| | TB + UA + MDA | 1 | 4.372 | 1–1 | 91.4 | 100 | 100 | 100 | 100 | 100 |

*DR* diabetic retinopathy, *AUC* area under curve, *PPV* positive predictive value, *NPV* negative predictive value, *MDA* malondialdehyde, *UA* uric acid, *TB* total bilirubin

**Table 3** Correlations of MDA, uric acid, bilirubin with other studied parameters in DM cases

| Parameters | MDA | | Uric acid | | Bilirubin | |
|---|---|---|---|---|---|---|
| | *r* | *p* | *r* | *p* | *r* | *p* |
| Age | − 0.129 | 0.180 | 0.073 | 0.447 | 0.125 | 0.196 |
| Duration | 0.187 | 0.160 | 0.034 | 0.803 | − 0.193 | 0.147 |
| Creatinine | 0.157 | 0.252 | 0.073 | 0.448 | − 0.045 | 0.663 |
| FBG | 0.553 | < 0.001 | 0.031 | 0.749 | − 0.528 | < 0.001 |
| PPBG | 0.998 | < 0.001 | 0.045 | 0.644 | − 0.974 | < 0.001 |
| BMI | 0.490 | 0.013 | 0.423 | 0.020 | − 0.048 | 0.821 |
| TC | 0.109 | 0.129 | − 0.057 | 0.552 | − 0.186 | 0.053 |
| TG | 0.185 | 0.053 | − 0.022 | 0.818 | − 0.163 | 0.091 |
| HDL | − 0.048 | 0.617 | − 0.042 | 0.666 | 0.026 | 0.788 |
| LDL | 0.142 | 0.138 | 0.002 | 0.985 | − 0.114 | 0.239 |
| MDA | – | – | –0.054 | 0.574 | − 0.974 | < 0.001 |
| UA | – | – | – | – | 0.091 | 0.349 |

$P < 0.05$ is considered significant

*FBG* fasting blood glucose, *PPBG* postprandial blood glucose, *TG* triglyceride, *TC* total cholesterol, *HbA1c* hemoglobin A1c, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *MDA* malondialdehyde, *UA* uric acid, *BMI* body mass index

**Table 4** Correlations of MDA, uric acid, bilirubin with HbA1c in all studied groups

| Parameters | HbA1c | | | | | |
|---|---|---|---|---|---|---|
| | MDA | | Uric acid | | Bilirubin | |
| | *r* | *p* | *r* | *p* | *r* | *p* |
| Healthy control | 0.110 | 0.498 | 0.087 | 0.593 | − 0.311 | 0.051 |
| DM cases | 0.971 | < 0.001 | 0.163 | 0.046 | − 0.899 | < 0.001 |
| DR patients | 0.951 | < 0.001 | 0.319 | 0.018 | − 0.902 | < 0.001 |

*DM* diabetes mellitus, *DR* diabetic retinopathy, *MDA* malondialdehyde, *HbA1c* hemoglobin A1c

**Table 5** Regression analysis for prediction of DM cases

| Factors | Univariable | | | | Multivariable | | | |
|---|---|---|---|---|---|---|---|---|
| | *p* | OR | 95% CI | | *p* | OR | | 95% CI |
| Age | 0.549 | 1.006 | 0.987 | 1.025 | | | | |
| BMI | 0.341 | 1.365 | 0.792 | 1.928 | | | | |
| Creatinine | 0.105 | 1.724 | 0.494 | 3.283 | | | | |
| TG | 0.974 | 1.003 | 0.996 | 1.014 | | | | |
| TC/HDL | 0.112 | 1.123 | 0.973 | 1.295 | | | | |
| Bilirubin | 0.857 | 1.152 | 0.246 | 5.384 | | | | |
| Uric acid | 0.004 | 1.143 | 1.044 | 1.251 | 0.208 | 0.862 | 0.684 | 1.086 |
| MDA | < 0.001 | 1.961 | 1.436 | 2.677 | < 0.001 | 2.088 | 1.458 | 2.991 |

$P < 0.05$ is considered significant. OR, odds ratio; OR > 1 indicates increased occurrence of event. OR < 1 indicates decreased occurrence of event (protective exposure)

*TG* triglyceride, *TC* total cholesterol, *HDL* high-density lipoprotein, *MDA* malondialdehyde, *BMI* body mass index, *CI* confidence interval

to high levels of free radicals or reactive oxygen species (ROS) [7]. The excessive production of ROS contributes to the development of many signs of diabetic retinopathy ranging from vascular leakage and vascular dysfunction to pathological angiogenesis [23]. The increased oxidative stress and decreased antioxidant status may predict diabetes mellitus and its related microvascular complications. Therefore, the present study aimed to investigate the

**Table 6** Regression analysis for prediction of DR

| Factors | Univariable | | | | Multivariable | | | |
|---|---|---|---|---|---|---|---|---|
| | *p* | OR | 95% CI | | *p* | OR | 95% CI | |
| Age | 0.346 | 0.991 | 0.972 | 1.010 | | | | |
| BMI | 0.630 | 1.009 | 0.973 | 1.045 | | | | |
| HA1C | 0.098 | 1.105 | 0.982 | 1.245 | | | | |
| TC/HDL | 0.860 | 0.987 | 0.853 | 1.142 | | | | |
| Bilirubin | 0.082 | 5.434 | 0.808 | 6.541 | | | | |
| Creatinine | 0.209 | 1.182 | 0.911 | 1.534 | | | | |
| Duration of diabetes | 0.013 | 1.432 | 1.195 | 1.931 | 0.135 | 1.634 | 1.182 | 1. 985 |
| Uric acid | < 0.001 | 1.248 | 1.129 | 1.378 | 0.023 | 1.613 | 1.125 | 1.978 |
| MDA | < 0.001 | 2.188 | 1.598 | 2.995 | < 0.001 | 1.142 | 1.061 | 1.301 |

$P < 0.05$ is considered significant. OR, odds ratio; OR > 1 indicates increased occurrence of event. OR < 1 indicates decreased occurrence of event (protective exposure)

*HbA1c* HemoglobinA1c, *TC* total cholesterol, *HDL* high-density lipoprotein, *BMI* body mass index, *MDA* malondialdehyde, *CI* confidence interval

association of MDA, uric acid and bilirubin with DM and DR development.

The present study showed that diabetic patients with DR had significantly longer disease duration compared to those without DR. Similar findings were observed by [24, 25]. Longer duration may represent a longer period of retinal toxicity induced by hyperglycemia that is associated with neural and vascular death in the retina [26]. The current study indicated that higher FBG, 2HPPG and HBA1C concentrations were significantly associated with diabetics when compared to control group. While there was no significant differences in creatinine level eGFR and BMI between all studied groups. HDL concentration was significantly lower in diabetics compared to control group. Similarly, in Egyptian population, type 2 diabetic patients had a significant increase in FBS, PPBS and HbA1C ($P = 0.001$) compared to control group. In addition, the duration of DM was significant increase in diabetics with DR when compared to diabetics without DR ($P = 0.001$) [27]. There was a significant increase in serum creatinine, FBG, 2HPPG and HbA1c levels in diabetics patients when compared to the healthy control subjects [28]. In addition, blood glucose and LDL levels were significantly higher in the DR patients than in the non-DR patients. However, no significant difference was found in the level of cholesterol [26].

An Egyptian study carried on type 2 diabetics found that 45.7% of the patients have diabetic retinopathy. Regarding GFR stages, 25.8% of the patients are of G1 (GFR 90 mL/min/1.73 m2), 31.8% G2 (GFR, 60 to 89 mL/min/1.73 m2) [29]. In Thailand type 2 diabetic patients, the prevalence of DR was 9.5%, 11.0%, 14.1%, and 20.9% in patients with GFR of ≥ 90, 60–89, 30–59 and < 30 mL/min/1.73 $m^2$, respectively ($P < 0.001$) [30]. In Japan population, type 1 and type 2 diabetics with NPDR (nonproliferative diabetic retinopathy) were significantly higher creatinine (0.73, 0.78), lower (eGFR) (76.5, 76) than type 1 and 2 diabetics without NPDR (0.68, 0.75; 82.4, 77.7; respectively). In addition there was significant larger BMI (22.7) in type 1 diabetics with NPDR than without (21.9) [31].

The present study revealed significantly higher levels of UA and MDA, while bilirubin showed significantly lower levels in diabetics compared to control. According to the ROC curve, 5.6 and 8.9 appeared to be the most suitable cut-off values for uric acid and MDA to discriminate between diabetics and healthy groups with sensitivity (64.5, 94.5), specificity (65, 100%), and effect size (0.587 and 3.554; respectively). Combinations of the three markers had nearly the same AUC, performance criteria and effect size of MDA; meaning that MDA may be used alone to the differentiation between diabetics and healthy individuals. This confirmed by logistic analysis, although higher levels of UA and MDA were associated with increased risk of DM development, higher level of MDA was suggested to be independent

predictor of DM development in healthy individuals. The observed high levels of MDA in diabetic groups reflected lipid peroxidation resulted from oxidative stress.

Similarly, the levels of serum MDA were significantly higher in type 2 diabetics than healthy control [32, 33]. Oxidative stress play a vital role in the pathogenesis of diabetes. oxidative stress harmfully affects the insulin activity through several interacting pathways and generating ROS. These could deteriorate the islets β-cells of the pancreas resulting in the reduced release of insulin. In addition, Free radical formation by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation leads to damage of enzymes, cellular injury machinery, changes in the cell membrane and increased insulin resistance which are risk for diabetes [34, 35]. MDA is a marker of lipid peroxidation which reacts with cell membrane phospholipids. The elevated level of MDA is found in different pathological diseases such as diabetes, cardiovascular diseases, renal disease, neurodegenerative disorders and cancer so that it is a good biomarker of oxidative stress and tissue damage [8]. Beside, Uric acid-mediated oxidative stress-induced lipid peroxidation, DNA damage, and activation of inflammatory factors finally lead to cellular damage [36].

Uric acid was significantly associated with the presence of DM. The high level of UA predicted DM development only in male aged < 30 years, and reducing UA had a potential effect on the prevention of future DM [37]. Another study showed that a high UA level was associated with the development of type 2 DM especially in the young group (5.5 mg/dl for women and 6.0 mg/dl for men). In addition, using uric acid as a biomarker may help in the early detection and prevention of DM [38]. In the Chinese population, higher serum uric acid level was a predictor for incident type 2 diabetes in middle-aged and elderly men and women, independent of conventional risk factors, such as family history of diabetes, blood pressure, serum creatinine, BMI and smoking. Furthermore, the ROC curves showed that a combination of serum uric acid with conventional risk factors improved the AUC for prediction of type 2 diabetes by 5% (AUC 0.65; 95% CI 0.54–0.77, $P = 0.02$) [39]. Conversely, serum uric acid level was high in healthy subjects but decrease in diabetics with increasing FBG concentrations. There was a significant negative association between the level of serum uric acid and Bangladeshi diabetics [40].

Regarding total bilirubin, increased total bilirubin was associated with 26% reduction in the risk of diabetes (OR 0.74, 95% CI 0.64–0.88) after age adjustment. Multivariate analysis, adjusting for all diabetes risk factors, confirmed this association (OR 0.80, 95% CI 0.67–0.95) [41]. Bilirubin might be correlated with the occurrence and development of type 2 DM [42].

The current study indicated that a significant positive correlation between MDA and FBG, 2HPP, HbA1c, BMI. In addition, uric acid showed significant positive correlations with HbA1c and BMI. While, bilirubin showed significant negative correlations with FBG, 2HPP, HbA1c and MDA.

These results are consistent with several studies that indicated a positive correlation between MDA and HbA1c [43, 44]. MDA as a lipid peroxidation indicator is higher in uncontrolled diabetes probably due to chronic high blood sugar followed by higher oxidative stress [45]. There was a positive correlation between HbA1C and serum uric acid levels in type 2 diabetics [10, 46]. Conversely, serum uric acid level was negatively correlated with HbA1c and PPBG glucose levels in type 2 diabetic patients [47]. Regarding total bilirubin, a negative association between serum total bilirubin and HbA1c was found in diabetics [44, 48]. In addition, the level of bilirubin showed a negative significant association with fasting blood glucose, HbA1c and cholesterol, LDL-cholesterol and triglycerides [49]. There was a negative association of serum total bilirubin with FBS and HbA1c in diabetics with retinopathy and type 2 DM without retinopathy when compared to healthy controls [12].

BMI measures the weight in relation to the height and gives a figure of total body fat. Obesity leads to oxidative stress by increasing endogenous lipid peroxides products. Uric acid and MDA showed a positive correlation with BMI [50]. Similarly, BMI showed a positive correlation with uric acid in Brazilian population [51], and with MDA in Han Chinese [52].

The present study found that, UA and MAD showed significantly higher levels, while bilirubin showed significantly lower levels in DR when compared to those without DR. According to the ROC curve, 0.67, 4.6 and 16.7 appeared to be the most suitable cut-off value for bilirubin, uric acid and MDA for discrimination between diabetics with and without DR; sensitivity (54.3, 88.6, 91.4), specificity (75, 60, 100%) and effect size (0.439, 1.008 and 3.108); respectively. The combination of the three markers increased the accuracy and effect size in differentiation between diabetes with and without DR. Furthermore, UA and MDA were suggested to be independent predictors of DR development in diabetics.

Prolonged hyperglycemia causes oxidative stress and increase the production of ROS that leads to the disruption of normal cellular physiology. The four major mechanisms involved in increased intracellular oxidative stress as a result of hyperglycemia, are polyol pathway, the hexosamine pathways, protein kinase C (PKC) and advanced glycation end-products (AGEs) [53]. Activation of this pathways lead to increase the production of ROS that cause vascular injury including pericyte loss, microaneurysm formation, platelet aggregation, loss of capillary endothelial cells, apoptosis, thickening of the basement membrane,and neuroglial damage which collectively contribute to the initiation and progression of DR. Uric acid is closely related to these pathological changes due to it may act as a prooxidant to

trigger oxidative stress in cells and contribute to endothelial dysfunction and damage via triggering oxidative and endoplasmic reticulum stress and inducing mitochondrial dysfunction and mitochondrial DNA damage. Furthermore, hyperuricemia increase the development of diabetic retinopathy by increasing ROS production, which leads to inflammation of the retina, dysfunction in the vessel [36, 54] and cellular damage via DNA modification, mitochondrial damage, protein misfolding, lipid peroxidation, cell death and impairments in the antioxidant defense system. The levels of lipid peroxide is increased in the retina causing endothelial dysfunction and leading to diabetic retinopathy. MDA is highly toxic compound formed by lipid peroxidation due to free radical damage. Free radicals and oxidative stress are responsible for the development of diabetic microangiopathy leading to diabetic retinopathy [55, 56]. Conversely, bilirubin is a potential endogenous anti-oxidant and it has anti-inflammatory effects [48]. The ant-inflammatory effect and anti-oxidative effect may contribute to the protective effect of bilirubin on vascular damage [57]. Thus, high total bilirubin could inhibit oxidative stress and inflammation processes and delay or interrupt development of DR [58].

MDA might help to predict the risk of diabetic retinopathy [59]. Estimation of MDA was related to oxidative stress in diabetic and this estimation can be effective in control and prevention of DR development [60]. Conversely, MDA levels did not show any significant correlation between diabetics with and without retinopathy. While the level of serum total bilirubin was lower in diabetic retinopathy patients than diabetics without retinopathy [24]. Total bilirubin concentration is lower in patients with type 2 diabetes with severer retinopathy. Thus, bilirubin might protect against retinopathy in type 2 diabetic patients [61], and in type 1 diabetics [62]. The elevated serum bilirubin levels might have an independent protective effect against DR among diabetics [63]. Serum uric acid concentration is associated with the increase in the severity of DR [64]. Conversely, another study indicated that there was no significant difference of serum UA level in DR group. While patients with microvascular complications had higher levels of serum UA compared to those without complications [10].

Several studies used logistic regression for the prediction of DR and there was different factor associated with DR; age, duration of diabetes, HbA1c, hypertension, triglycerides, LDL and family history of DR had a significant role in the occurrence of DR [65]. Similarly, other studies suggested that age, duration of diabetes, HbA1C levels, poor glycaemic control, dyslipidemia, hypertension and insulin treatment were as risk factors of DR among diabetics patients [66, 67]. The limitation of this study is that first, the limited number of subjects. Second, we were unable to classify DR types into non-proliferative diabetic retinopathy (NDPR) and proliferative diabetic retinopathy (PDR), and the subgroup analyses based on DR types were not conducted. A strength of this study is, this work is one of few studies that investigated the relationship between the combination of simple, easily measurable and inexpensive three markers with diabetic retinopathy in diabetic patients in the Egyptian population.

In conclusion, this study revealed that higher levels of UA and MDA were associated with increased risk of DM and DR development, while bilirubin was not associated with decreased risk of DM or DR. However, the combination of MDA, uric acid and bilirubin may be a valuable addition to current options for the prognosis of diabetic retinopathy, and this is simple, easily measurable and inexpensive. In addition, MDA was suggested to be independent predictor of DM and DR development. Beside, UA may be used as independent biomarker to predict the risk of DR. Duration of diabetes is associated with DR. Furthermore, further prospective multicenter studies involving larger sample sizes are warranted to validate the usefulness of this finding in clinical practice.

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## Compliance with ethical standards

**Conflict of interest** The Authors Hadeel Ahmed Shawki, Rasha Elzehery, Maha Shahin, Ekbal M.Abo-hashem, Magdy M. Youssef declared that they have no conflict of interest. The authors alone are responsible for the content and writing of this article.

**Ethical approval** This study was ethically approved by Mansoura University Ethics Committee at the faculty of Science (Sci-ch-ph-2020-28) (10-5-2020).

**Informed consent** An informed consent was obtained from all participant.

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