

Plasma Insulin/Erythrocytic Aldose Reductase Ratio as a Predictor for Hepatocellular Carcinoma among Type II Diabetics and Hepatitis C Virus-infected Patients

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Abstract

Background: Hepatocellular carcinoma (HCC) is a possible oncogenic progression during persistent hepatitis C-infection +/- type II diabetes mellitus (DM). We aim to investigate the plasma insulin, erythrocytic aldose reductase (AR) and sorbitol dehydrogenase (SDH) as possible predictive tools for HCC in hepatitis C-infected patients (HCV) +/- DM. Erythrocytes (RBCs) were adopted as a possible vehicle for pre-malignant variations being of short life span. **Methods:** The study included 20 healthy control and 100 patients of 48–64 years old, divided into 5 equal groups as; type II DM, HCC, HCC with DM, DM- HCV infected and non-DM HCV infected. Plasma levels of AFP and insulin were measured. **Results:** It showed an elevated AR, significant reduction of SDH in RBCs and plasma of DM patients. These values were greatly elevated among HCV, HCC, diabetic HCV, and diabetic HCC patients. All DM patients showed elevated insulin levels than normoglycemic controls. **Conclusion:** The study substantiated the use of RBCs as a vehicle for early diagnostic markers better than plasma. We recommend the use of insulin/ erythrocytic AR ratio as a new laboratory marker for predicting HCC among type II diabetics or non-treated HCV-infected patients with control insulin/ erythrocytic AR ratio by each laboratory.

Keywords: Plasma insulin/erythrocytic aldose reductase ratio- Sorbitol dehydrogenase- Type II Diabetes mellitus

Asian Pac J Cancer Prev, 23 (11), 3815-3823

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide (Yang et al., 2020), and the second cause leading to human cancer-related deaths (Wei et al., 2019). It has a poor prognosis due to its rapid infiltrating power and complicating liver cirrhosis (Xu et al., 2020). Etiological factors of the disease include infection with the hepatitis B or C virus or exposure to high levels of dietary aflatoxin B1 (Yang et al., 2020). It was proved that CCNB1, CDK1, TOP2A, RACGAP1, and ASPM genes could be potential prognostic biomarkers and therapeutic targets for HBV-associated HCC (Nguyen, 2022). Oxidative stress among these patients was also proved to aggravate hepatocarcinogenesis (Nour, 2022). Approximately, 75-80% of primary liver cancers are attributable to persistent viral infections. Cirrhosis is the major risk factor for HCC and cirrhotic patients should be screened for early detection (Rabe et al., 2001) (Shi et al., 2005). Recently, murine double minute 2 (MDM2) gene was reported to have a strong association with the development of HCC, and the GG allele may help as an

important determinant to identify the higher risk of HCC in some Far-Eastern population (Hosen, 2021).

Although ultra-sonography (US) and conventional tumor markers such as α -fetoprotein (AFP) are widely used for HCC detection, they still do not provide a satisfactory tool for early detection of HCC. Prediction of HCC is still on race, although recent trials showed that circulating cell free-DNA plays a role in the prediction of HCC despite the fact that AFP is still the commonest in the diagnosis of HCC (Radwan, 2022). As well as, its sensitivity in detecting small HCC (namely those below 1-2 cm) is rather low. The strongest information on prognosis and possibly driving treatment strategy comes only from histological examination of the tissue, which is not available at the time of diagnosis (Jain et al., 2010). The theory assuming that diabetes mellitus (DM) may increase the risk of HCC has been confirmed in some cohort and case-control studies (Li and Wang, 2017). As well as; recent investigations indicated a positive relationship between total chromosomal aberrations and the duration of diabetes; meaning that chromosomal aberrations may partly participate in worsening the

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diabetic complications(Pappuswamy, 2020).

DM was considered as an independent risk factor for hepatocellular carcinoma (HCC), despite the presence of HCV, HBV, alcoholic liver disease, or nonspecific cirrhosis. It was established that DM is associated with a 2–3-fold increase in the risk of HCC development. This finding suggests that it may account for a significant proportion of idiopathic HCC (Taura et al., 2011). Glucose is not reduced by aldose reductase (AR) under normoglycemic conditions, but it follows the normal glycolytic pathway. Increased activity of AR mediates the pathologies associated with DM and is thought to be involved in increased resistance to chemotherapeutic drugs. Thus, the use of AR inhibitors may serve as a protective therapy in cancer(Maccari and Ottanà, 2015; Penning et al., 2021).

AR is the rate-limiting enzyme in the polyol pathway (PP), in which glucose is converted to sorbitol(Yan, 2018b). It catalyzes NADPH-dependent glucose reduction into sorbitol (sorbitol pathway) and excessive deposition of sorbitol in different tissues of animal models of DM and cell lines cultured in high glucose media which was supposed as an important mediator for the pathogenesis of late diabetic complications (Singh Grewal et al., 2016). However, it was established that abnormalities of glucose metabolizing enzymes during the transformation of normal hepatocytes necessitate high glucose utilization in hepatoma cells (Abdel-Hamid et al., 2013).

In disrupted intracellular glucokinesis, sorbitol dehydrogenase (SDH) activity, an enzyme responsible for conversion of sorbitol back to fructose (Arun and Nalini, 2002) to be utilized better by cells is also disrupted. SDH, the second key enzyme in PP, catalyzes the inter-conversion of polyols, such as sorbitol and xylitol to their respective ketones. SDH deficiency leads to subsequent accumulation of sorbitol within the cells and diabetic complications (Iwata et al., 1995). Polyol profile was considered to be an index for early changes during progression to HCC in chemically induced liver cancer. To pursue early pathologic changes, red blood cells (RBCs) were suggested as a short spam vehicle for study investigations(Abdel-Hamid et al., 2011). The interference of DM with viral hepatitis C (HCV) infection was considered to be a morbidifying risk for hepatocarcinogenesis (Abdel-Hamid, 2009).

This work aimed to investigate the plasma level of blood hemoglobin, insulin, total protein, glucose, AR, SDH along with the erythrocytic content of total protein, glucose, AR, and SDH. Then correlating these variables to serum AFP, plasma, and RBCs glucose to PP enzyme concentrations, then calculating the insulin/RBCs AR content among the studied groups. Then we will try a mathematical formula from plasma insulin/RBCs AR ratio, hopefully, to find a novel early predictive non-invasive marker for HCC in HCV-infected with or without DM patients. Erythrocytes will be adopted as a possible vehicle for early pre-malignant variations due to their short life span.

Materials and Methods

Patient groups

In this study, we recruited 120 persons (48–64 years old), classified into 6 equal groups as follows: healthy control, type II DM, HCC, type II DM with HCC, type II DM having HCV infection, and normoglycemic with HCV infection. HCC patients were first grade (without metastasis) and diagnosed by liver biopsy as listed in their hospital files at Heart and Liver Diseases Research Center in Kafrelsheikh, Kafrelsheikh Governorate, Egypt, during 2019-2020. Samples were collected after approval of the Committee of Ethical Guidelines, Kafrelsheikh University, and written informed consents were taken from participating patients by their inspective medical team.

Chemicals

All chemicals were of analytical grade, purchased from Sigma-Aldrich, USA.

Blood samples

Five ml venous blood samples were obtained from each individual after fasting for 8 hours and collected into heparinized tubes. Blood hemoglobin was measured firstly, then all blood samples were subsequently centrifuged at 2000 rpm/min for 10 min then plasma was kept in sterile screw-capped vials at -20°C right biochemical measurements, the sedimented erythrocytes were washed three times with approximately 2 times its volume cold saline, centrifuged, then the washed RBCs (WRBCs) were kept for biochemical investigations at -5°C.

Preparation of red blood cell samples for AR and SDH determination

The washed RBCs were transferred into tubes containing 1 ml of acid-citrate-dextrose solution (23 mM citric acid, 45 mM sodium citrate, 82 mM dextrose) and stored at 4 °C for less than 7 days. No significant change in AR level had been found during 7 days of storage. The samples were mixed well, centrifuged at 1,000 rpm for 10 min, the supernatant was removed and the resultant RBCs were washed twice with 10 vol of ice-cold PBS (137 mM NaCl, 2.7 KCl, 4.3 mM Na₂HP0₄, 1.4 mM KH₂PO₄, pH 7.3), centrifuged for 5 minutes at 2000 rpm and the supernatant was discarded completely. The washed RBCs were frozen at -20°C(Nishimura et al., 1993).

Erythrocytic AR and SDH extraction

Erythrocytes were hemolyzed by adding an equal volume of phosphate buffer (pH 7.0) and by two cycles of freezing and thawing using a dry ice acetone bath. The supernatant fraction of the hemolysate was obtained by centrifugation at 15,000 rpm for 15 min and stored at -80 °C for erythrocytic AR and SDH determination by ELISA (Nishimura et al., 1993).

Extraction of glucose from RBCs

An aliquot of saline-washed frozen RBCs was used for extraction of glucose by adding 2 ml of cold 6% perchloric acid (1.7 ml of pure perchloric acid in 50 ml of distilled water) to 200 µl of washed RBCs. The protein

was precipitated and the erythrocytes were centrifuged at 3000 rpm/min at 4°C for 10 min. The supernatant was neutralized at 4°C with 1 M K₂CO₃ (Vinson et al., 1989; Wang et al., 1995) and then used for measurement of RBCs glucose content.

Extraction of total protein from RBCs

The extraction of total protein was done by haemolysis of RBCs. The cytoplasm of the RBCs contains dissolved salts and sugars. Since the cytoplasm of the RBCs has a higher amount of distilled water, there is a net flow of water into the cell by osmosis. The cell would swell up and eventually bursts and therefore the content of total protein in the erythrocytes has been determined. Two ml of distilled water were added to 200 µl of washed RBCs and shaken gently, then centrifuged at 3000 rpm for 15 minutes and the supernatant was used to determine RBCs total protein content (Chapman and Sussdorf, 1967).

Determination of plasma insulin level

Plasma insulin level was determined by enzyme-linked immunosorbent assay (ELISA) according to the method described before (Clark and Hales, 1994), using a commercially available kit from Shanghai Sunred Biological Technology Co., Ltd, China.

Determination of AFP level

Plasma AFP level was determined by ELISA (Abelev, 1974; Sell, 1990) using a commercially available kit from Cell Biolabs, Inc., San Diego, USA.

Quantitative determination of plasma and RBCs glucose content

This was carried out using a commercial kit obtained from SPINREACT diagnostics, Egypt (Kaplan, 1984).

Quantitative determination of total protein in plasma and RBCs hemolysate

Total protein was determined according to the method mentioned elsewhere (Gornall et al., 1949), using a commercial kit purchased from Elitech Clinical Systems Co, Italy.

Determination of SDH levels in plasma and RBCs

SDH concentration was determined by ELISA (Hellgren et al., 2007), using a commercially available kit from Shanghai Sunred Biological Technology Co., Ltd, China.

Determination of AR levels in plasma and RBCs

AR was determined by ELISA (Li et al., 2012) using a kit provided from Cloud Clone, USA. Both AR and SDH contents in RBCs were calculated as ng/mg Hb.

Determination of blood Hb% levels

Hemoglobin was measured spectrophotometrically (Van Kampen and Zijlstra, 1961) using a commercial kit obtained from SPINREACT diagnostics, Egypt.

For each group; plasma insulin was subdivided by erythrocytic AR (bad enzyme) concentration. The resultant insulin/ erythrocytic AR value was the same for

both DM and control groups. The insulin/ erythrocytic AR value was then used to figure out a final value that lets us predict progression into HCC in DM or non-treated persistent HCV infected patients. This was done by dividing this figure of DM by the control group which was equal to 1.0. When this figure for the HCV group was divided by the DM group (both are non-HCC-affected), the resultant value was 0.69. This value will be the cut-off value for non-HCC developing individuals. However, this ratio in the HCC group, when divided by both DM or control ratio, the result was the same which was 0.49, taking into account that HCC patients are early, not metastatic groups. Below 0.49 patients will be considered as going to HCC progression.

Thus; this ratio of 0.49 or less is a suggested predictor of HCC development among type II diabetics or non-treated HCV-infected patients.

Statistical analysis

The data were expressed as Mean ± standard deviation (SD). The difference between groups was determined by using one-way analysis of variance (ANOVA) followed by Tukey's-HSD multiple range post hoc test. GraphPad Prism software version 6.0, USA was used for statistical evaluation of the obtained data, and the statistical significance was considered at P < 0.05. Spearman correlation was used to evaluate the associations between different variables.

Results

Blood hemoglobin, plasma, and erythrocytic total protein levels among the studied groups

Blood hemoglobin concentration in both HCC and DM with HCC groups was significantly decreased, and this level among HCV and DM with HCV groups was less significantly decreased than both control and DM groups. Plasma and erythrocytic total protein levels in both HCC and DM with HCC groups were significantly decreased; while this level was significantly higher among HCV and DM with HCV groups than both control and DM groups (Table 1).

Plasma insulin and alpha-fetoprotein levels among the studied groups

Plasma insulin concentration was significantly increased in DM, HCC, DM with HCC, DM with HCV, and HCV groups than the control group. Plasma AFP concentration in both HCC and DM with HCC groups was significantly increased, and this level was less significantly increased among HCV and DM with HCV groups than both control and DM groups (Table 1).

Plasma and erythrocytic glucose content among the studied groups

The level of plasma and erythrocytic glucose among DM, DM with HCC, and DM with HCV groups was significantly increased (P < 0.001), compared to healthy control. HCC and HCV-infected groups showed no significant change in blood plasma glucose level when compared to control, being both normoglycemic. But HCC

Table 1. Variations in Blood Levels of Hb, Alpha Fetoprotein, Insulin, and Both Plasma and Erythrocyte Levels of Glucose, Total Protein, Aldose Reductase and Sorbitol Dehydrogenase among Studied Groups (Values are expressed as mean± SD, N=20)

Parameter	Group					
	Control	Diabetic	HCC	Diabetic HCC	Diabetic HCV	HCV
Whole blood Hb (g/dl)	15.2 ± 0.9	14.9 ± 0.6	8.0 ± 0.5 ^{a,b}	8.0 ± 0.6 ^{a,b}	12.6 ± 0.4 ^{a,b,c,d}	13.3 ± 0.4 ^{a,b,c,d}
Plasma total protein (g/dl)	7.5 ± 1.1	7.6 ± 0.5	5.3 ± 0.7 ^{a,b}	5.7 ± 0.7 ^{a,b}	10.1 ± 0.3 ^{a,b,c,d}	9.0 ± 0.3 ^{a,b,c,d}
Erythrocyte total protein (g/dl)	5.5 ± 0.5	4.7 ± 0.2 ^a	3.7 ± 0.6 ^{a,b}	3.4 ± 0.6 ^{a,b}	7.0 ± 0.4 ^{a,b,c,d}	7.5 ± 0.4 ^{a,b,c,d}
Plasma insulin (mU/l)	13.4 ± 2.4	25.5 ± 6.9 ^a	16.6 ± 2.2 ^b	28.6 ± 4.6 ^{a,c}	28.2 ± 4.7 ^{a,c}	16.4 ± 1.9 ^{b,d,e}
Plasma AFP (ng/ml)	2.6 ± 0.3	2.4 ± 0.2	12.8 ± 6.2 ^{a,b}	15.0 ± 6.4 ^{a,b}	7.2 ± 1.2 ^{c,d}	6.2 ± 1.2 ^{a,c,d}
Plasma glucose (mg/dl)	91.8 ± 5.7	322.3 ± 53.0 ^a	69.0 ± 2.4 ^b	287.1 ± 48.8 ^{a,c}	305.6 ± 53.3 ^{a,c}	100.9 ± 6.7 ^{b,d,e}
Erythrocyte glucose (µmol/l)	20.3 ± 1.4	45.4 ± 5.3 ^a	14.1 ± 0.8 ^{a,b}	36.8 ± 4.9 ^{a,b,c}	42.6 ± 4.8 ^{a,c,d}	19.9 ± 1.5 ^{b,c,d,e}
Plasma AR (ng/ml)	0.5 ± 0.1	4.4 ± 2.1 ^a	6.8 ± 1.7 ^{a,b}	9.9 ± 1.8 ^{a,b,c}	5.1 ± 1.4 ^{a,d}	3.5 ± 1.7 ^{a,c,d}
Erythrocyte AR (ng/mg Hb)	4.2 ± 0.6	8.1 ± 2.1 ^a	11.5 ± 1.5 ^{a,b}	15.2 ± 2.0 ^{a,b,c}	10.1 ± 2.2 ^{a,c,d}	7.6 ± 1.7 ^{a,c,d,e}
Plasma SDH (ng/ml)	3.9 ± 0.4	2.2 ± 1.7 ^a	10.3 ± 1.7 ^{a,b}	5.2 ± 1.0 ^{b,c}	8.90 ± 1.1 ^{a,b,d}	12.8 ± 1.2 ^{a,b,c,d,e}
Erythrocyte SDH (ng/mg Hb)	5.1 ± 0.5	1.9 ± 1.9 ^a	13.5 ± 1.4 ^{a,b}	7.1 ± 1.2 ^{a,b,c}	10.1 ± 1.1 ^{a,b,c,d}	16.0 ± 1.2 ^{a,b,c,d,e}

Significant difference vs. ^a, normal control; ^b, Diabetic; ^c, HCC; ^d, Diabetic HCC; ^e, Diabetic HCV; Significant differences between groups were analyzed by one-way ANOVA test followed by Tukey's-HSD multiple range post hoc test, significance was considered at P < 0.05. HCC, Hepatocellular carcinoma; HCV, Hepatitis C virus; AR, Aldose reductase; SDH, Sorbitol dehydrogenase; AFP, Alpha fetoprotein; All diabetic patients were of type II.

group exhibited a significant decrease in the erythrocytic content of glucose by 30.4% (P < 0.01) when compared with the control group (Table 1).

Plasma and erythrocytic content of AR and SDH among the studied groups

The content of plasma and erythrocytic AR in DM with

HCV, HCC, and DM with HCC groups was significantly increased (P < 0.001) compared to healthy control. As well, HCV-infected and DM groups exhibited a significant increase in the plasma and erythrocytic AR concentration, compared to the control group (Table 1).

Plasma and erythrocytic SDH expression were significantly increased in HCV-infected, HCC, DM with

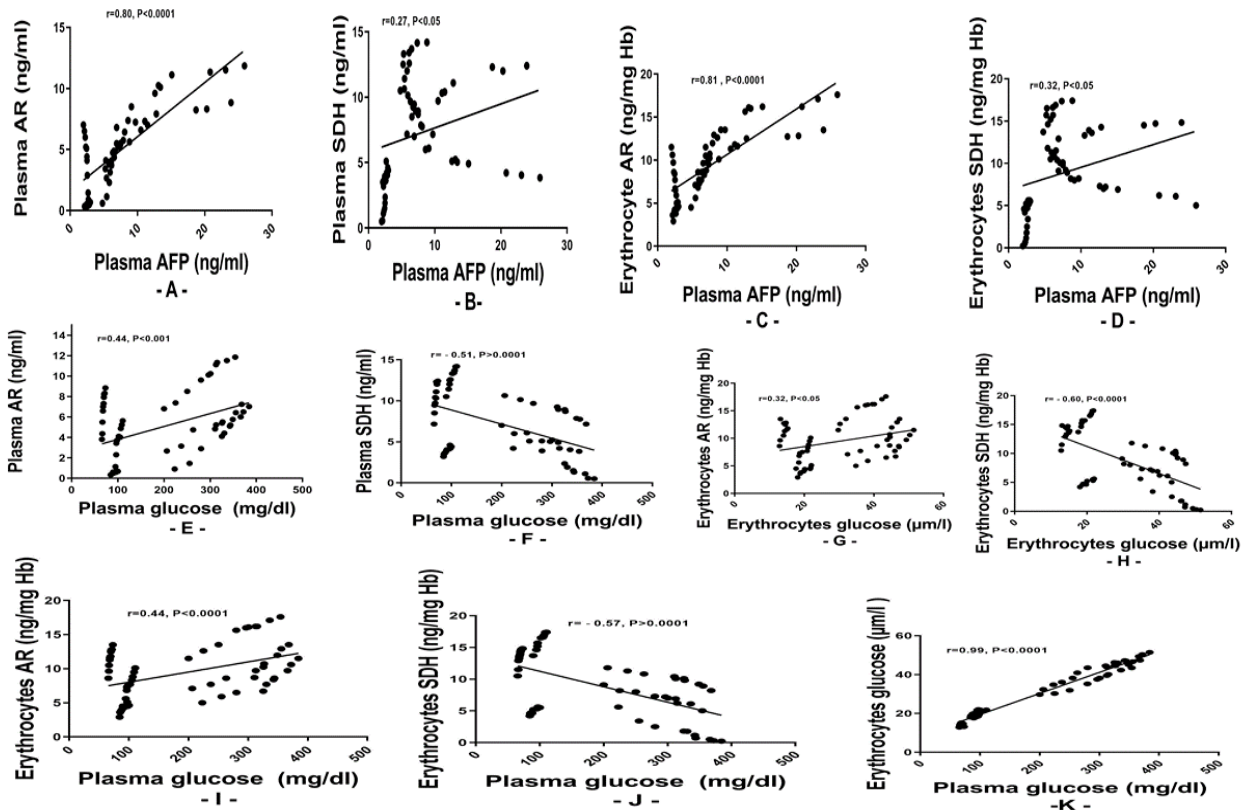


Figure 1. Correlation Studies between AFP and Both Plasma and Erythrocyte Aldose Reductase (AR) and Sorbitol Dehydrogenase (SDH), (A-D). Correlations were also calculated between both plasma and erythrocyte glucose against both plasma and erythrocyte AR and SDH, (E-H). Plasma glucose level was also correlated to erythrocyte AR, SDH, and glucose (I-K).

HCC, and DM with HCV groups, compared to healthy control ($P < 0.001$), while in DM, it was significantly decreased (Table 1).

Correlation between AFP and polyol enzymes in plasma and erythrocytes among the studied groups

AFP was significantly and positively correlated with plasma AR (Figure 1 A, $P < 0.0001$, $r = 0.838$), and plasma SDH (Figure 1 B, $P < 0.05$). As well as, significant positive correlations between plasma AFP and AR content in erythrocytes (Figure 1 C, $P < 0.0001$), and erythrocytic SDH (Figure 1 D, $P < 0.05$) were observed.

The associations of glucose and polyol enzymes in plasma and erythrocytes among the studied groups

Plasma glucose was correlated positively with plasma AR (Figure 1 E, $P < 0.01$), and negatively with plasma SDH (Figure 1 F, $P < 0.001$). Also, there is a significant positive correlation between erythrocytic glucose and erythrocytic AR content (Figure 1 G, $P < 0.01$), and a negative correlation with erythrocytic SDH content (Figure 1 H, $P < 0.0001$).

Plasma glucose was significantly correlated with erythrocytic AR content (Figure 1 I, $P < 0.0001$), while significantly and negatively correlated with erythrocytic SDH content (Figure 1 J, $P < 0.0001$). Also, there is a highly significant correlation between plasma glucose level and erythrocytic glucose content (Figure 1 K, $P < 0.0001$).

Discussion

The majority of studies conclude that AFP is not a useful diagnostic test for the detection of HCC (Sherman, 2001; Tong et al., 2001; Abdel-Hamid, 2008; Park et al., 2017; Wang and Zhang, 2020), but AFP continues to be used as the common tumor marker (Sauzay et al., 2016). Current studies examining the test characteristics of AFP for diagnosing HCC in patients with one or more risk factors have substantial methodological limitations, making it difficult to distinguish between falsely elevated AFP levels and tumors in benign liver diseases (Fujiyama et al., 2002; Soresi et al., 2003; Hanif et al., 2022).

In our study, the AFP level was identified as a risk factor for HCC development in HCV-infected patients, where its level is between 6 and 20 ng/ml. The present study showed that the level of AFP was increased in the early stages of HCC, but not in all HCC cases. AFP lacks its sensitivity in a small portion of early-detected HCC patients with an elevation in AFP level (Fattovich et al., 2004). It's a non-invasive predictive tumor marker of the progression of HCC in HCV patients, but it is difficult to estimate the sensitivity and specificity for this test clearly (Roberts, 2019). We need more straightforward non-invasive serological biomarkers that can be combined with / or replace AFP to significantly improve HCC diagnosis. The main objective is to uncover new biomarkers because of the low AFP sensitivity and specificity in diagnosis and follow-up of HCC among HCV-infected patients with or without DM.

Our results showed that type II DM patients had a

significant increase in both plasma glucose and insulin levels. It means that our DM subjects suffer insulin resistance, due to elevated insulin levels despite the increased glucose level.

Insulin resistance can unfortunately worsen the disease state of the patient when associated with metabolic disease (Sasaki et al., 2020). As well as, fasting blood glucose levels were higher in HCV-infected patients than healthy controls, an observation that was reported before (Bose and Ray, 2014; Li et al., 2019b). This hyperglycemia could increase oxidative stress in the cells, which may initiate cancer development (Matthews et al., 1985; Li et al., 2014; Imai et al., 2019).

In our study, blood glucose level was decreased in HCC patients, this showed an accordance with some previously reported observations (Khattab et al., 2012; Radwan et al., 2019). They revealed that the HCC group showed higher levels of insulin. Previous studies proved that chronic HCV infection may damage the pancreatic beta cells, leading to the death of these cells through multiple mechanisms (Masini et al., 2005; Albert et al., 2006; Wang et al., 2012).

As well, insulin resistance is commonly associated with chronic liver diseases (Bugianesi et al., 2005; Irshad et al., 2011). Our study showed an agreement with some previous data assuming that insulin levels were higher in diabetic HCV infected patients and diabetic HCC patients, than patients with type II DM (Kawaguchi and Sata, 2010; Radwan et al., 2019). Early studies suggested that hyperinsulinemia can accelerate the progression of HCC rather than hyperglycemia (Baba et al., 2017), and even the treatment with insulin constitutes a risk factor for HCC (Kawaguchi et al., 2010; Schlesinger et al., 2013).

It was reported that hyperglycemic conditions would lead to a reduction in the average lifespan of RBCs and consequently would reduce RBCs count (Nada, 2015). We pursued biochemical pathways of glucose conversion into sorbitol, a polyol pathway (PP), in addition to AR and SDH expression to check whether they are involved in the pathogenesis of HCC among DM patients infected with HCV. The same parameters, in addition to plasma insulin, were used to prove the role of RBCs in the early prediction of HCC progression. Here, we called the AR and SDH as polyol profile, that was studied in DM type II in both plasma and RBCs to follow the variations in PP during the disease development and to see whether this profile can help as a possible early index for progression to HCC in diabetic patients infected with hepatitis C virus. As glucose uptake by the liver is not dependent on insulin, the glycation reaction would be enhanced in the liver by hyperglycemia (Myint et al., 1995; Abdel-Hamid et al., 2013).

AR is the rate-limiting enzyme in the polyol pathway, converting glucose to sorbitol using NADPH as a co-factor (Yan, 2018b). The second enzyme of the PP is SDH, catalyzing the conversion of sorbitol to fructose using NAD^+ as a co-factor (Ighodaro, 2018).

Our result showed that AR expression was significantly increased in DM (Yan, 2018a), Glucose to sorbitol conversion leads to NADPH cellular depletion which can induce oxidative stress by impairing glutathione

metabolism(Cumbie and Hermayer, 2007; Ravindranath et al., 2009) increasing the cell death, and accumulation of intracellular sorbitol(Kador and Kinoshita, 1985). It was reported that AR activity was found to be significantly higher among HCV-infected and HCC patients(Lee et al., 2001; Semmo et al., 2015).

The hyperglycemic condition results in activation of the PP and increases the activity of SDH, leading to the formation of a large amount of fructose(Figueroa-Romero et al., 2008), and the increase of NADH: NAD⁺ ratio (Nishikawa et al., 2000; Yan, 2018b). In our study, SDH content was increased with increasing glucose levels in both plasma and RBCs.

Increasing NADH/NAD⁺ ratio under hyperglycemic conditions, and excess NADH can be used as the substrate for NADH oxidase, which can lead to oxidative stress(Araki and Nishikawa, 2010; Yan, 2014).

SDH converts glucose to fructose in the pp and excessive metabolites as fructose-3-phosphate and 3-deoxyglucosone are produced, being more effective non-enzymatic glycation agents than glucose(HOSHI et al., 1996). The flux of glucose through the PP would increase the production of AGEs, causing oxidative stress(Devaraj et al., 2005; Sánchez-Pérez et al., 2005; Nowotny et al., 2015). Also, fructose is a stronger glycation sugar than glucose (Hershko and Ciechanover, 1998; Gugliucci, 2017; Amani and Fatima, 2020). In our study SDH activity decreases in the diabetic group along with increased plasma and RBCs' glucose levels.

In diabetes, fructose is overproduced in the body, as the PP consumes approximately 30% of blood glucose(Yan, 2018b). Overproduction of fructose leads to metabolic consequences. Thus, excess fructose can glycate proteins(Gugliucci, 2017), causing protein dysfunction. In the current work, plasma glucose level was decreased in the HCC group, and RBCs as well. High blood glucose level encourages sorbitol accumulation, preventing its conversion to fructose (Chung et al., 2003; Ighodaro, 2018).

Our results showed that SDH activity was increased in HCV-infected patients, which is following with another registered study(Wiesner et al., 1965). In our results, SDH contents were higher in HCV-infected patients than HCC patients, our results are in accordance with a previous study (Li et al., 2019a), suggesting that activity of SDH promotes migration and invasion of HCC-cells. A previous study suggested a critical tumor-suppressive role for SDH in HCC.

RBCs' AR content was significantly higher in all groups than in plasma even in the control group. It was postulated that the contents of erythrocytic AR, as well as sorbitol may have a value as a quantitative trait to be included with other markers to establish a risk profile for the development of late diabetic complications(Reddy et al., 2008). There was a linear correlation between serum AFP levels and both AR and SDH contents in plasma and RBCs of studied groups. It was noticed that serum SDH levels of more than 15 ng/ml was associated with shorter recurrence-free survival after surgical interventions to patients with HCC. Moreover, baseline serum SDH and alpha-fetoprotein (AFP) levels might better predict

the recurrence of HCC, so, incorporating serum SDH along with AFP levels in clinical practice may elevate the predictability of prognosis in HCC patients(Jeon et al., 2021).

As well as, our work displayed a negative correlation between elevated plasma glucose with plasma SDH levels. The same was noticed in RBCs' glucose and SDH values in the studied groups. Here we can translate these correlations in a way that RBCs can offer the same correlation instead of plasma, but a given benefit arises by using the short life-spanned vehicle (RBCs) which is greatly expressive for early changes than plasma. We previously assumed this benefit(Abdel-Hamid et al., 2011). In all cases, defective SDH expression might adversely affect the prognosis towards late diabetic complications.

Conversely, a positive correlation between elevated plasma glucose with plasma AR level was observed. The same was noticed in RBCs' glucose and AR values in studied groups. It was reported that high glucose levels elevated AR protein expression; although, this was not accompanied by apparent enzyme activation(Lewko et al., 2011). Correlations in our study were calculated by plotting each parameter against the other parameter as a stream in all groups to guess the association between these parameters as an absolute correlation in the studied groups.

Interestingly, our study revealed that a strong positive correlation is found between plasma and RBCs glucose in the studied groups. Recent literature agree that RBCs of DM patients are greatly affected by hyperglycemia in a manner that a plethora of adverse changes occurs in the RBCs including a correlative increased glucose content to hyperglycemia and late diabetic complications(Wang et al., 2021).

Cancer activates the conversion of sorbitol to fructose promoting SDH expression. Previous studies also revealed that SDH expression was significantly elevated in HCV and HCC patients and elevations were prominent in RBCs than plasma, adding that SDH activity is significantly reduced in diabetic patients(HOSHI et al., 1996).

Study limitations

These results to be more popularized, repetition on large scale multicenter is necessitated. Further studies including single nucleotide polymorphisms (SNPs) and RNA expression of both AR and SDH in both type I and type II DM patients to predict the future of late diabetic complications of these patients especially patients comorbidified by hepatitis C or B infections.

In conclusion, AFP lacks a satisfactory value for the prediction of the HCC among risky subjects as diabetics and persistently non-treated HCV-infected patients. Type II DM is known to cause definite biochemical abnormalities which worsen prognosis and treatment outcomes in HCV-infected patients. Chronic HCV is significantly associated with high expression of AR and SDH in plasma and RBCs.

Our study substantiated the use of the studied RBCs variables as early detection markers for HCC among chronic HCV-infected patients +/- DM, in addition, PP can be advised as a follow-up test among HCV-infected patients and type II diabetics.

The use of insulin/ erythrocytic AR ratio was the same for both DM and control groups. This ratio is useful to predict progression into HCC in DM or non-treated persistent HCV infected patients. The value of 0.69 would be the cut-off value for non-HCC developing individuals. Below 0.49, patients will be considered as going to HCC progression. We recommend the use of insulin/ erythrocytic AR ratio as a non-invasive test, suggested for the first time to predict HCC development among type II diabetics or non-treated HCV-infected patients.

Author Contribution Statement

The manuscript was conceptualized and reviewed, commented on previous versions of the manuscript by Nabil MA, who also assumed the predictive equation. Material preparation, data collection and analysis were performed by Asmaa EA and Moustafa SA. Sherif MH supervised logistics of the research. The first draft of the manuscript was written by Asmaa EA and Nabil MA. All authors read and approved the final manuscript.

Acknowledgments

Statements and Declarations

Funding statement

This work was self-funded; no any funding body funded the work.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University of Zagazig (Date 20 Jan 2017 /No 09). Informed consent was obtained from all individual participants included in the study.

Conflicts of interest

There are no conflicts of interest.

References

- Abdel-Hamid M (2008). Priority considerations in early laboratory diagnosis of hepatocellular carcinoma. *IJIB*, **3**, 196-201.
- Abdel-Hamid N, Nazmy M, Abdel-Bakey A (2011). Polyol profile as an early diagnostic and prognostic marker in natural product chemoprevention of hepatocellular carcinoma in diabetic rats. *Diabetes Res Clin Pr*, **92**, 228-37.
- Abdel-Hamid N, Ramadan M, Amgad S (2013). Glycoregulatory enzymes as early diagnostic markers during premalignant stage in hepatocellular carcinoma. *Am J Cancer Prev*, **1**, 14-9.
- Abdel-Hamid NM (2009). Recent insights on risk factors of hepatocellular carcinoma. *World J Hepatol*, **1**, 3.
- Abelev GI (1974). Alpha-fetoprotein as a marker of embryo-specific differentiations in normal and tumor tissues. *Transplant Rev*, **20**, 3-37.
- Albert L, Cristina H, Joan G (2006). Glucose abnormalities with hepatitis C virus infection. *Diabetes Care*, **29**, 1140.
- Amani S, Fatima S (2020). Glycation with fructose: the bitter side of nature's own sweetener. *Curr Diabetes Rev*, **16**, 962-70.
- Araki E, Nishikawa T (2010). Oxidative stress: a cause and therapeutic target of diabetic complications. *J Diabetes Investig*, **1**, 90-96.
- Arun N, Nalini N (2002). Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. *Plant Food Hum Nutr*, **57**, 41-52.
- Baba H, Kurano M, Nishida T, et al (2017). Facilitatory effect of insulin treatment on hepatocellular carcinoma development in diabetes. *BMC Res Notes*, **10**, 1-7.
- Bose SK, Ray R (2014). Hepatitis C virus infection and insulin resistance. *World J Diabetes*, **5**, 52.
- Bugianesi E, McCullough AJ, Marchesini G (2005). Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology*, **42**, 987-1000.
- Chapman SE, Sussdorf DH (1967). Changes in subcellular and circulating antibody levels during the hemolysin response in rabbits. *J Immunol*, **98**, 860-7.
- Chung SS, Ho EC, Lam KS, et al (2003). Contribution of polyol pathway to diabetes-induced oxidative stress. *J Am Soc Nephrol*, **14**, 233-36.
- Clark P, Hales C (1994). How to measure plasma insulin. *Diabetes Metab Rev*, **10**, 79-90.
- Cumbie BC, Hermayer KL (2007). Current concepts in targeted therapies for the pathophysiology of diabetic microvascular complications. *Vasc Health Risk Manag*, **3**, 823.
- Devaraj S, Venugopal SK, Singh U, et al (2005). Hyperglycemia induces monocytic release of interleukin-6 via induction of protein kinase C- α and- β . *Diabetes*, **54**, 85-91.
- Fattovich G, Stroffolini T, Zagni I, et al (2004). Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology*, **127**, 35-50.
- Figuroa-Romero C, Sadidi M, Feldman EL (2008). Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. *Rev Endocr Metab Disord*, **9**, 301-14.
- Fujiyama S, Tanaka M, Maeda S, et al (2002). Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. *Oncology*, **62**, 57.
- Gornall AG, Bardawill CJ, David MM (1949). Determination of serum proteins by means of the biuret reaction. *J Biol Chem*, **177**, 751-66.
- Gugliucci A (2017). Formation of fructose-mediated advanced glycation end products and their roles in metabolic and inflammatory diseases. *Adv Nutr*, **8**, 54-62.
- Hanif H, Ali MJ, Susheela AT, et al (2022). Update on the applications and limitations of alpha-fetoprotein for hepatocellular carcinoma. In (pp 216–29). WJG.
- Hellgren M, Kaiser C, de Haij S, et al (2007). A hydrogen-bonding network in mammalian sorbitol dehydrogenase stabilizes the tetrameric state and is essential for the catalytic power. *Cell Mol Life Sci*, **64**, 3129-38.
- Hershko A, Ciechanover A (1998). The ubiquitin system. *Annu Rev Biochem*, **67**, 425-79.
- Hosen MB, Khaleque N, Chakraborty S, Mahtab MA, Kabir Y (2021). MDM2 (T309G) Gene Polymorphism Determines the Susceptibility of Hepatocellular Carcinoma in Bangladesh. *Asian Pac J Cancer Biol*, **6**, 213-17.
- Hoshi A, Takahashi M, Fujii J, et al (1996). Glycation and inactivation of sorbitol dehydrogenase in normal and diabetic rats. *Biochem J*, **318**, 119-23.
- Ighodaro OM (2018). Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomed Pharmacother*, **108**, 656-62.
- Imai K, Takai K, Hanai T, et al (2019). Homeostatic model assessment of insulin resistance for predicting the recurrence of hepatocellular carcinoma after curative treatment. *Int J Mol Sci*, **20**, 605.
- Irshad M, Irshad K, Nag P (2011). Insulin resistance in liver diseases. *Macedonian J Med Sci*, **4**, 201-9.
- Iwata T, Popescu NC, Zimonjic DB, et al (1995). Structural

- organization of the human sorbitol dehydrogenase gene (SORD). *Genomics*, **26**, 55-62.
- Jain S, Singhal S, Lee P, et al (2010). Molecular genetics of hepatocellular neoplasia. *Am J Transl Res*, **2**, 105.
- Jeon D, Choi WM, Kim JS, et al (2021). Serum sorbitol dehydrogenase as a novel prognostic factor for hepatocellular carcinoma after surgical resection. *Cancers (Basel)*, **13**.
- Kador PF, Kinoshita JH (1985). Role of aldose reductase in the development of diabetes-associated complications. *Am J Med*, **79**, 8-12.
- Kaplan L (1984). Glucose. *Clin Chem*, **30**, 1032-36.
- Kawaguchi T, Sata M (2010). Importance of hepatitis C virus-associated insulin resistance: therapeutic strategies for insulin sensitization. *World J Gastroenterol*, **16**, 1943.
- Kawaguchi T, Taniguchi E, Morita Y, et al (2010). Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection. *Liver Int*, **30**, 479-86.
- Khatab MA, Eslam M, Mousa YI, et al (2012). Association between metabolic abnormalities and hepatitis C-related hepatocellular carcinoma. *Ann Hepatol*, **11**, 487-94.
- Lee KW, Ko BC, Jiang Z, et al (2001). Overexpression of aldose reductase in liver cancers may contribute to drug resistance. *Anti-Cancer Drug*, **12**, 129-32.
- Lewko B, Latawiec E, Maryn A, et al (2011). Osmolarity and glucose differentially regulate aldose reductase activity in cultured mouse podocytes. *Exp Diabetes Res*, **2011**, 278963.
- Li J, Liang N, Long X, et al (2019). SDHC-related deficiency of SDH complex activity promotes growth and metastasis of hepatocellular carcinoma via ROS/NFκB signaling. *Cancer Lett*, **461**, 44-55.
- Li L, Li Z, Cheng H, et al (2012). Racial difference in aldose reductase C-106T genetic polymorphism and association with essential hypertension. *Zhong nan da xue xue bao. Yi xue ban= J Cent South Univ. Med Sci*, **37**, 156-60.
- Li WQ, Park Y, McGlynn KA, et al (2014). Index-based dietary patterns and risk of incident hepatocellular carcinoma and mortality from chronic liver disease in a prospective study. *Hepatology*, **60**, 588-97.
- Li X, Wang X (2017). Diabetes mellitus and risk of hepatocellular carcinoma. *Biomed Res Int*, **2017**, 5202684.
- Li Y, Wang X, Yu G, et al (2019). The association of hepatitis c virus infection status with serum glucose levels. *BMC Gastroenterol*, **19**, 1-6.
- Maccari R, Ottanà R (2015). Targeting aldose reductase for the treatment of diabetes complications and inflammatory diseases: new insights and future directions. *J Med Chem*, **58**, 2047-67.
- Masini M, Campani D, Boggi U, et al (2005). Hepatitis C virus infection and human pancreatic β-cell dysfunction. *Diabetes Care*, **28**, 940-41.
- Matthews DR, Hosker J, Rudenski A, et al (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **28**, 412-19.
- Myint T, Hoshi S, Ookawara T, et al (1995). Immunological detection of glycosylated proteins in normal and streptozotocin-induced diabetic rats using anti hexitol-lysine IgG. *Biochim Biophys Acta*, **1272**, 73-79.
- Nada AM (2015). Red cell distribution width in type 2 diabetic patients. *Diabetes Metab Syndr Obes*, **8**, 525.
- Nguyen T, Vu N, Tran T, Hoang H, Bui H (2022). Screening and identification of key genes in hepatitis B virus-related hepatocellular carcinoma through an integrated bioinformatics approach. *Asian Pac J Cancer Biol*, **7**, 143-49.
- Nishikawa T, Edelstein D, Du XL, et al (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*, **404**, 787-90.
- Nishimura C, Furue M, Ito T, et al (1993). Quantitative determination of human aldose reductase by enzyme-linked immunosorbent assay: Immunoassay of human aldose reductase. *Biochem Pharmacol*, **46**, 21-8.
- Nour H, Maroua C, Samir D (2022). A study of the relationship between oxidative stress and risk of developing hepatocellular carcinoma in people with hepatitis B infection; A Systematic Review Study. *Asian Pac J Cancer Biol*, **4**, 316-20.
- Nowotny K, Jung T, Höhn A, et al (2015). Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules*, **5**, 194-222.
- Pappuswamy M, Rajesh N, Maria Philip A (2020). Analysis of chromosomal aberrations and micronuclei in type 2 diabetes mellitus patients. *Asian Pac J Cancer Biol*, **5**, 15-8.
- Park SJ, Jang JY, Jeong SW, et al (2017). Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. *Medicine*, **96**.
- Penning TM, Jonnalagadda S, Trippier PC, et al (2021). Aldose reductases and cancer drug resistance. *Pharmacol Rev*, **73**, 1150-71.
- Rabe C, Cheng B, Caselmann WH (2001). Molecular mechanisms of hepatitis B virus-associated liver cancer. *Digestive Dis*, **19**, 279-87.
- Radwan HA, Elsayed EH, Saleh OM (2019). Significance of serum adiponectin and insulin resistance levels in diagnosis of Egyptian patients with chronic liver disease and HCC. *Asian Pac J Cancer Prev*, **20**, 1833.
- Radwan N, Abdelkhalik H, Elgayar D, Elsharkawy M (2022). Study the role of cell free DNA in the diagnosis of hepatocellular carcinoma, an Egyptian study. *Asian Pac J Cancer Biol*, **7**, 3-8.
- Ravindranath TM, Mong PY, Ananthakrishnan R, et al (2009). Novel role for aldose reductase in mediating acute inflammatory responses in the lung. *J Immunol*, **183**, 8128-37.
- Reddy GB, Satyanarayana A, Balakrishna N, et al (2008). Erythrocyte aldose reductase activity and sorbitol levels in diabetic retinopathy. *Mol Vis*, **14**, 593-601.
- Roberts LR (2019). Current status of the GALAD and BALAD biomarker models for hepatocellular carcinoma. *Gastroenterol Hepatol*, **15**, 672.
- Sánchez-Pérez Y, Carrasco-Legleu C, García-Cuellar C, et al (2005). Oxidative stress in carcinogenesis. Correlation between lipid peroxidation and induction of preneoplastic lesions in rat hepatocarcinogenesis. *Cancer Lett*, **217**, 25-32.
- Sasaki N, Ozono R, Higashi Y, et al (2020). Association of insulin resistance, plasma glucose level, and serum insulin level with hypertension in a population with different stages of impaired glucose metabolism. *J Am Heart Assoc*, **9**, e015546.
- Sauzay C, Petit A, Bourgeois A-M, et al (2016). Alpha-fetoprotein (AFP): A multi-purpose marker in hepatocellular carcinoma. *Clin Chim Acta*, **463**, 39-44.
- Schlesinger S, Aleksandrova K, Pischon T, et al (2013). Diabetes mellitus, insulin treatment, diabetes duration, and risk of biliary tract cancer and hepatocellular carcinoma in a European cohort. *Ann Oncol*, **24**, 2449-55.
- Sell S (1990). Cancer markers of the 1990s. Comparison of the new generation of markers defined by monoclonal antibodies and oncogene probes to prototypic markers. *Clin Lab Med*, **10**, 1-37.
- Semmo N, Weber T, Idle J, et al (2015). Metabolomics reveals that aldose reductase activity due to AKR 1B10 is upregulated in hepatitis C virus infection. *J Viral Hepatitis*, **22**, 617-24.
- Sherman M (2001). Alpha-fetoprotein: an obituary. *J Hepatol*,

- 34, 603-05.
- Shi J, Zhu L, Liu S, et al (2005). A meta-analysis of case-control studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma in China. *Br J Cancer*, **92**, 607-12.
- Singh Grewal A, Bhardwaj S, Pandita D, et al (2016). Updates on aldose reductase inhibitors for management of diabetic complications and non-diabetic diseases. *Mini Rev Med Chem*, **16**, 120-62.
- Soresi M, Magliarisi C, Campagna P, et al (2003). Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. *Anticancer Res*, **23**, 1747-53.
- Taura N, Ichikawa T, Miyaaki H, et al (2011). Prevalence of type 2 diabetes mellitus in Japanese patients with hepatocellular carcinoma. *Exp Ther Med*, **2**, 81-4.
- Tong MJ, Blatt LM, Kao VW (2001). Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. *J Gastroen Hepatol*, **16**, 553-9.
- Van Kampen E, Zijlstra W (1961). Standardization of hemoglobinometry II. The hemoglobinocyanide method. *Clin Chim Acta*, **6**, 538-44.
- Vinson JA, Staretz ME, Bose P, et al (1989). In vitro and in vivo reduction of erythrocyte sorbitol by ascorbic acid. *Diabetes*, **38**, 1036-41.
- Wang H, Zhang ZB, Wen RR, et al (1995). Experimental and clinical studies on the reduction of erythrocyte sorbitol-glucose ratios by ascorbic acid in diabetes mellitus. *Diabetes Res Clin Pract*, **28**, 1-8.
- Wang Q, Chen J, Wang Y, et al (2012). Hepatitis C virus induced a novel apoptosis-like death of pancreatic beta cells through a caspase 3-dependent pathway. *PLoS One*, **7**, e38522.
- Wang T, Zhang K-H (2020). New blood biomarkers for the diagnosis of AFP-negative hepatocellular carcinoma. *Front Oncol*, **10**, 1316.
- Wang Y, Yang P, Yan Z, et al (2021). The relationship between erythrocytes and diabetes mellitus. *J Diabetes Res*, **2021**, 6656062.
- Wei L, Wang X, Lv L, et al (2019). The emerging role of microRNAs and long noncoding RNAs in drug resistance of hepatocellular carcinoma. *Mol Cancer*, **18**, 147.
- Wiesner IS, Rawnsley HM, Brooks FP, et al (1965). Sorbitol dehydrogenase in the diagnosis of liver disease. *Am J Dig Dis*, **10**, 147-51.
- Xu Y, Sun C, Han B, et al (2020). High KIAA1522 expression predicts a poor prognosis in patients with hepatocellular carcinoma. *Oncol Lett*, **20**, 509-16.
- Yan L-J (2014). Pathogenesis of chronic hyperglycemia: from reductive stress to oxidative stress. *J Diabetes Res*, **2014**.
- Yan L (2018). Redox imbalance stress in diabetes mellitus: role of the polyol pathway. *Anim Models Exp Med*, **1**, 7-13.
- Yang WS, Zeng XF, Liu ZN, et al (2020). Diet and liver cancer risk: a narrative review of epidemiological evidence. *Br J Nutr*, **124**, 330-40.



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