ORIGINAL ARTICLE

Plasma Cellular Hypoxia, Mitochondrial Dysfunction, Disease Risk and Prognostic Factors in Type 2 Diabetic Patients in Saudi Arabia

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ABSTRACT

Objective: *This cross-sectional study evaluated the association of plasma cytochrome c (CytoC) and hypoxia-inducible factor (HIF)-1α, as mitochondrial dysfunction and cellular hypoxia biomarkers, with disease risk factors and prognosis in Type 2 diabetic patients in Saudi Arabia.*
Methods: *A total of 252 patients (94 males and 158 females) were eligible and were matched by socio-economic status, age and body mass index (BMI) with 106 healthy participants (71 males and 35 females). They were subgrouped according to BMI, disease duration and treatment. Lipid and glycaemic control indices were colorimetrically measured to calculate insulin resistance (IR) and atherogenic index of plasma (AIP). Haemoglobin A1c, C-reactive protein (CRP), CytoC and HIF-1α were measured using specific immunoassays.*
Results: *Among the patients, 50% (38.64% of males and 52.53% of females), 40.48% (43.18% of males and 40.51% of females), 4.365% (6.82% of males and 3.80% of females), 2.381% (4.55% of males and 1.90% of females), and ~0.8% (males) suffered from peripheral neuropathy, ophthalmopathy, kidney disease, myocardial infarction and ketoacidosis, respectively. The majority of complicated cases had greater age, BMI, disease duration, plasma insulin and AIP, and were on insulin. The two investigated groups were non-significantly different considering CytoC, but highly significantly different considering lipid profile (as reflected on AIP) and glycaemic control parameters (as reflected on IR, plasma CRP and HIF-1α), with significant correlations among all of them in a group-specific pattern.*
Conclusion: *Patients suffered a high rate of complications that correlated with age, BMI, disease duration, AIP, plasma insulin and insulin treatment due to poor disease control. Reduced HIF-1α and non-significant increased CytoC levels correlated negatively with bad prognostic indicators of the disease pointing to a pathogenetic implication.*

Keywords: C-reactive protein, cytochrome c, diabetic complications, hypoxia-inducible factor-1α, Type 2 diabetes

Hipoxia celular plasmática, disfunción mitocondrial, riesgo de enfermedad y factores pronósticos en pacientes diabéticos tipo 2 en Arabia Saudita

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RESUMEN

Objetivo: *Este estudio transversal evaluó la asociación del citocromo C del plasma (CitoC) y el factor inducible por hipoxia (HIF)-1α, como la disfunción mitocondrial y los biomarcadores*

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de la hipoxia celular, con los factores de riesgo y pronóstico de enfermedad en pacientes diabéticos de tipo 2 en Arabia Saudita.

Métodos: *Un total de 252 pacientes (94 varones y 158 mujeres) fueron elegidos y apareados por estado socioeconómico, edad e índice de masa corporal (IMC) con 106 participantes sanos (71 varones y 35 hembras). Se dividieron entonces en subgrupos de acuerdo con el IMC, la duración de la enfermedad y el tratamiento. Se midieron los índices de lípidos y control glucémico para calcular la resistencia a la insulina (RI) y el índice aterogénico de plasma (IAP). La hemoglobina A1C, la proteína C-reactiva (PCR), CitoC y HIF-α fueron medidos usando inmunoensayos específicos.*

Resultados: *Entre los pacientes, el 50% (38.64% de los varones y el 52.53% de las mujeres), 40.48% (43.18% de los varones y 40.51% de las mujeres), 4.365% (6.82% de los varones y 3.80% de las mujeres), 2.381% (4.55% de los varones y 1.90% de las mujeres), y ~ 0.8% (varones) padecían de neuropatía periférica, oftalmopatía, enfermedad renal, infarto de miocardio y cetoacidosis, respectivamente. La mayor parte de los casos complicados tenían mayor edad, IMC, duración de la enfermedad, insulina plasmática e IAP, y recibían tratamiento de insulina. Los dos grupos investigados no fueron significativamente diferentes considerando la CitoC, pero fueron muy significativamente diferentes en cuanto a su perfil lipídico (como se refleja en el IAP) y los parámetros de control glicémico (como se refleja en la RI, el plasma y el HIF-α), con importantes correlaciones entre todos ellos en un patrón específico de grupo.*

Conclusión: *Los pacientes tuvieron un alto índice de complicaciones que se correlacionaron con la edad, el IMC, la duración de la enfermedad, el IAP, la insulina plasmática y el tratamiento de la insulina debido al pobre control de la enfermedad. La reducción del HIF-α y el aumento no significativo de los niveles de CitoC se correlacionaron negativamente con los indicadores de mal pronóstico de la enfermedad, que apuntaban a una implicación patogénica.*

Palabras clave: Proteína C-reactiva, citocromo C, complicaciones diabéticas, factor inducible por hipoxia-1α, diabetes tipo 2

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INTRODUCTION

In Type 2 diabetes (T2DM), mitochondrial dysfunction pathogenetically impairs insulin release and action, systematically and at pancreatic β-cell level (1, 2). Cellular adaptation to hypoxia, energy homeostasis, metabolism and mitochondrial biogenesis are mastered by the transcriptional regulator, hypoxia-inducible factor (HIF)-1α (3). Hypoxia-inducible factor-1α is not only a major glucose homeostasis regulator (4), a vascular health and angiogenic activator, but it also rejuvenates the cells of the whole body through activating autophagy (5) and thus is responsible for the health benefits of dieting and exercise (6). However, obliterating HIF-1α-induced vascular reorganization protects against diabetic complications (7). Moreover, aerobic inactivation of HIF-1α promotes mitochondria biogenesis, thermogenesis, brown adipocyte differentiation and vascular health (8). Reduction in the body weight correlates reduction in HIF-1α and inflammation and their dependent genes and inducers (9). Cytochrome c (CytoC) oxidase couples generation of adenosine triphosphate with electron transport. Leakage of CytoC signals dysfunctional mitochondria and instigates cell dismissal (10). However, CytoC activity positively correlates with autophagy, expected to maintain healthy endothelial cells (11).

Cellular hypoxia and mitochondria dysfunction are causal in diabetic vascular dysfunction. To test such hypothesis, this study aimed to measure in T2DM patients the plasma levels of each of HIF-1α and CytoC and to clinically correlate them with C-reactive protein (CRP), insulin resistance (IR), atherogenic index of plasma (AIP) and disease prognostic indicators.

SUBJECTS AND METHODS

The subjects of this cross-sectional study consisted of consented patients and healthy controls who attended the Diabetes Care Unit of Prince Meteb General Hospital, and College of Medicine, Jouf University, Sakaka, Saudi

Arabia, from November 1, 2015 to February 20, 2017. Ethical approval was obtained from the bioethics committees of the Ministry of Health Directorate and of Jouf University. Normal healthy participants matched by socio-economic status, age and BMI (n = 106; age range: 25–83 years; mean ± standard deviation (SD): 42.57 ± 19.53) and patients with T2DM (n = 252; age range: 23–85 years; mean ± SD: 48.13 ± 26.06) voluntarily enrolled. Controls and patients were subdivided according to gender (71 male and 35 female controls *versus* 94 male and 158 female patients) and BMI (< 25: 32 controls and 88 patients; ≥ 25: 74 controls and 164 patients), respectively. Patients were further subdivided according to treatment (non-insulin: 102; insulin: 150) and disease duration (< 5 years: 125; ≥ 5 years: 127), respectively. Demographic data, anthropometric indices and disease history were anonymously recorded for each participant. Patients' exclusion criteria included immobilization for any reason, renal or hepatic failure, autoimmune diseases, acute infections, congenital and haemolytic anaemias, and systemic inflammatory diseases. In patients, the disease severity score (no complications = 0, one = 1, two = 2, three = 3, and four or more = 4) and treatment score (no treatment = 0, metformin = 1, hypoglycaemic ± metformin = 2, and insulin ± metformin = 3) were related to the investigated biomarkers (12).

Age- and gender-stratified BMI (www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/metric_bmi_calculator/bmi_calculator.html) and A Body Shape Index (ABSI; http://absi-calc.appspot.com) were calculated. Fasting plasma lipid and glycaemic control indices were colorimetrically assayed (Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany) to calculate AIP and homeostatic model assessment of IR (HOMA-IR), respectively. Atherogenic index of plasma was calculated as log (triglycerides/high-density lipoprotein cholesterol; TAGs/HDL-C) in mM/L. Homeostatic model assessment of IR was calculated as glucose (mg/dL) x insulin (mIU/L)/405. Specific enzyme-linked immunosorbent assay kits from Cloud-Clone Corp (Wuhan, Hubei, People's Republic of China; cat# CEA190Hu, CEA448Hu, SEA821Hu, SEA798Hu and SEA594Mi) were used to quantitate human haemoglobin A1c (HbA1c), insulin, CRP, HIF-1α and CytoC.

Statistical analysis

Data are presented as n, range and mean ± SD. Statistical analysis used Prism 6.0 GraphPad (GraphPad Software, Inc, La Jolla, CA, USA), applying unpaired two-tailed Student's t-test and one-way analysis of variance with Newman-Keuls Multiple Comparison Test. Spearman's non-parametric correlation analysis was done for parameters within groups. Significance limit was set at p value of ≤ 0.05 at 95% confidence interval.

RESULTS

Demographics and complications

Comparing the whole group of healthy controls *versus* the whole group of diabetic patients, there were non-significant differences considering age, BMI, ABSI and plasma CytoC (Table 1). However, they were very highly significantly different considering AIP, HOMA-IR, plasma CRP and HIF-1α ($p < 0.001$ except for HIF-1α, where $p < 0.05$). Among patients, 50% (n = 126) suffered from peripheral neuropathy (constituting 38.64% of males and 52.53% of females), 40.48% (n = 102) suffered from ophthalmopathy (constituting 43.18% of males and 40.51% of females), 4.365% (n = 11) experienced kidney disease (constituting 6.82% of males and 3.80% of females), 2.381% (n = 6) were diagnosed with myocardial infarction (constituting 4.55% of males and 1.90% of females), and ketoacidosis was diagnosed in ~0.8% of males (n = 2). In relation to BMI, only 5% of males with complications and 14.58% of females with complications had BMI < 25. The overwhelming majority of patients on insulin treatment suffered from complications (85.185%) that were mostly multiple, while 57.5% of those on non-insulin treatment were complication-free.

Atherogenic index of plasma

Among the healthy controls, all comparisons of the whole group *versus* gender and BMI subgroups showed non-significant differences in AIP. However, healthy female controls had lower AIP than healthy male controls ($p < 0.05$). Among the diabetic patients, all possible comparisons of the whole group *versus* gender, BMI, treatment and disease duration subgroups showed non-significant differences in AIP, except for those with BMI < 25 showing lower AIP compared to the whole group ($p < 0.05$) and to patients with BMI ≥ 25 ($p < 0.01$). Comparing healthy controls *versus* patients as whole groups and as respective subgroups, there were highly significant increases in AIP in patients ($p < 0.001$).

Insulin resistance

Among healthy controls, HOMA-IR was significantly lower in those with BMI < 25 compared to the whole

Table 1: Characteristics and clinico-investigational indices of healthy controls and Type 2 diabetic patients in Saudi Arabia

Parameters	Healthy controls (n = 106) Mean ± SD (range)	Diabetic patients (n = 252) Mean ± SD (range)	Student's t-test *p*
Age (years)	44.90 ± 14.13 (25.0–83.0)	48.13 ± 15.55 (23.0–85.0)	Non-significant
A Body Shape Index	0.070 ± 0.010 (0.051–0.089)	0.069 ± 0.008 (0.051–0.105)	Non-significant
Body mass index	27.96 ± 5.961 (18.58–41.1)	29.15 ± 4.913 (18.26–42.46)	Non-significant
Total cholesterol (mg/dL)	143.7 ± 31.36 (76.23–200.4)	174.1 ± 38.83 (100.3–292.7)	< 0.001
Low-density lipoprotein cholesterol (mg/dL)	63.10 ± 20.53 (27.01–160)	102.6 ± 27.01 (51.0–189.0)	< 0.001
High-density lipoprotein cholesterol (mg/dL)	58.96 ± 11.93 (30.04–80.65)	35.93 ± 7.751 (20.0–61.0)	< 0.001
Triglycerides (mg/dL)	139.5 ± 33.42 (67.77–200.8)	210.1 ± 86.44 (107.1–539.9)	< 0.001
Atherogenic index of plasma	0.011 ± 0.139 (-0.386–0.330)	0.390 ± 0.174 (0.076–0.869)	< 0.001
Haemoglobin A1c (%)	6.755 ± 0.967 (4.509–8.935)	8.080 ± 1.024 (6.254–12.90)	< 0.001
Glucose (mg/dL)	90.26 ± 13.24 (53.76–110.7)	169.2 ± 51.29 (91.04–411.0)	< 0.001
Insulin (mIU/L)	4.129 ± 1.871 (1.993–13.59)	9.212 ± 5.024 (0.778–26.52)	< 0.001
HOMA-IR	0.927 ± 0.492 (0.40–3.40)	3.868 ± 2.549 (0.30–13.70)	< 0.001
C-reactive protein (mg/dL)	1.697 ± 0.845 (0.179–4.028)	3.404 ± 1.063 (0.527–6.948)	< 0.001
Cytochrome c (ng/mL)	6.827 ± 11.46 (0.0–105.2)	7.742 ± 9.813 (0.0–39.13)	Non-significant
Hypoxia-inducible factor-1α (pg/mL)	58.61 ± 36.46 (15.40–267.2)	45.90 ± 59.83 (1.0–546.8)	< 0.05
Treatment score		2.079 ± 0.969 (0.0–3.0)	–
Complication score		0.985 ± 0.944 (0.0–4.0)	–
Disease duration (years)		6.969 ± 6.172 (0.0–30.0)	–

group ($p < 0.01$) and those with BMI ≥ 25 ($p < 0.001$). Among patients, the whole group had lower HOMA-IR than each of those with a disease duration of > 5 years, those on non-insulin treatment and those on insulin treatment ($p < 0.001$). In patients, there was higher HOMA-IR among those with > 5 years of disease duration than those with ≤ 5 years of disease duration ($p < 0.001$), among those with non-insulin treatment than those with insulin treatment ($p < 0.01$), and among those with BMI < 25 than those with ≥ 25 ($p < 0.01$). Comparing healthy controls and patients as whole groups and respective gender and BMI subgroups, there was significantly higher HOMA-IR in patients ($p < 0.001$).

Plasma level of C-reactive protein, cytochrome c and hypoxia-inducible factor-1α

Comparing the whole group of healthy controls and their gender subgroups showed non-significant differences in plasma CRP. However, the two control BMI subgroups showed BMI-dependent increases ($p < 0.05$). Likewise, T2DM patients as a whole group and as gender subgroups showed significant differences in CRP. However, their BMI subgroups *versus* each other were significantly different ($p < 0.01$), and the whole group *versus* patients with BMI < 25 was also significantly higher ($p < 0.05$). Significantly higher CRP was observed when comparing patients and healthy controls as whole groups and as respective gender and BMI subgroups ($p < 0.001$).

Comparing the whole group of healthy controls and their gender and BMI subgroups showed non-significant differences in plasma CytoC content. Likewise, T2DM patients as a whole group and as gender, BMI and disease duration subgroups showed significant differences in CytoC. However, comparing patients on insulin *versus* non-insulin treatment showed significantly lower CytoC (5.985 ± 1.007 *versus* 9.046 ± 10.170 ng/mL respectively; $p < 0.05$). Comparing control *versus* T2DM males, there was significantly lower CytoC (5.601 ± 6.210 *versus* 7.746 ± 8.165 ng/mL respectively; $p < 0.05$).

Comparing the whole group of healthy controls and their gender subgroups showed non-significant differences in plasma HIF-1α content. However, the healthy BMI < 25/≥ 25 subgroups were significantly different in HIF-1α (69.93 ± 53.02 *versus* 53.72 ± 25.26 pg/mL respectively; $p < 0.05$). Likewise, T2DM patients as a whole group and as gender and disease duration subgroups showed significant differences in HIF-1α. However, their BMI < 25/≥ 25 (50.82 ± 61.46 *versus* 44.96 ± 59.74 respectively; $p < 0.05$) and insulin/non-insulin treatment subgroups (32.78 ± 4.487 *versus* 54.99

± 68.87 mIU/L respectively; $p < 0.01$) were significantly different. The whole group of patients showed significantly reduced HIF-1α levels *versus* the whole group of healthy controls ($p < 0.05$). Using a cut-off level of 131.53 pg/mL (*ie* normal mean ± 2 SD), there were 11 cases with higher levels of circulating HIF-1α, among which there were seven free of any complications. A similar analysis for CytoC (cut-off value of 29.75 ng/mL) did not reveal a clear demarcation.

Trend of complication and treatment

As expected, longer disease duration ($p < 0.001$) and a higher BMI ($p < 0.05$) showed a higher rate of disease complications than shorter disease duration and a lower BMI, respectively. Longer disease duration ($p < 0.001$) and male patients ($p < 0.01$) significantly tended to have insulin treatment, compared with shorter disease duration and female patients, respectively.

Correlation analysis

Hypoxia-inducible factor-1α showed positive correlation *versus* age (in controls), ABSI (in patients), BMI (in patients), CytoC (in all patient and healthy control subgroups) and complication score (in patient subgroups), but negative correlation *versus* AIP (in all patient and healthy control subgroups), CRP (in all patient and healthy control subgroups), HOMA-IR (in patients), disease duration (in patient subgroups) and treatment scores (in patient subgroups) (Table 2).

Cytochrome c showed positive correlation *versus* each of BMI, ABSI and HIF-1α, but negative correlation *versus* AIP, HOMA-IR and CRP in all patient and healthy control subgroups, and positive correlation *versus* complication score and negative correlation *versus* disease duration and treatment scores in patients' subgroups (Table 3).

C-reactive protein showed positive correlation *versus* each of age, ABSI, BMI, AIP and HOMA-IR, but negative correlation *versus* CytoC and HIF-1α in all patient and healthy control subgroups (Table 4).

Atherogenic index of plasma showed positive correlation *versus* each of age and BMI (particularly in patients' subgroups), HOMA-IR and CRP, but negative correlation *versus* CytoC and HIF-1α in all patient and healthy control subgroups, and positive correlation *versus* disease complication and treatment scores in patients' subgroups (Table 5).

Homeostatic model assessment of IR showed positive correlation *versus* each of age, ABSI, BMI, AIP and CRP (particularly in controls) but negative correlation *versus* CytoC and HIF-1α in all patient and healthy control subgroups and negative correlation *versus* disease duration and treatment scores in patients' subgroups (Table 6). In all patients' subgroups, complication scores correlated very strongly positively with age, ABSI, BMI, disease duration, treatment score (0.478/0.001), CytoC and HIF-1α (0.264/0.01; particularly in males and non-insulin treated patients).

DISCUSSION

It was reported that Al-Jouf (to which this sample of patients belonged) and Makkah held the highest disease and complication prevalence in Saudi Arabia (13). Reportedly, internationally diabetic morbidity increased with age and disease duration, particularly among males, smokers, poor glycaemic control, hypertension, presence of other complications, hyperlipidaemics and insulin users (14–16). This applied to this sample of patients. However, higher BMI and ABSI but not male gender also conferred increased complication risk in the patients. A strong correlation was observed between AIP and complication score, as previously confirmed (14, 17). The rate of complications in the present study was among the highest compared to these studies.

The utility of plasma HIF-1α as a systemic cellular hypoxia biomarker in different clinical settings was previously confirmed (18). However, a few discrepant studies ascribed it in diabetes and other diseases (19–22). It is well established that diabetes is characterized by defective angiogenesis, vasculopathy and poor wound-healing partly due to relative tissue hypoxia and reduced autophagy. Therefore, it was empirical to expect a complex picture for plasma HIF-1α, with responsive patients expected to have better prognosis. Actually, not only the reduced HIF-1α but also the increased CytoC positively correlated with HDL-C and hepatocyte growth factor (the β-cell mitogen; unpublished data), while the three of them (HIF-1α, CytoC and HDL-C) negatively correlated with BMI, CRP, AIP, HOMA-IR, disease duration and insulin treatment. These poor prognostic indices positively correlated with each other and the disease complications. Since both CytoC and HIF-1α activity positively correlated with the cell-rejuvenating autophagy, they were expected to be cytoprotectants, particularly for endothelial cells (4, 5, 11). In confirmation, both of them were higher in those with lower BMI and in patients on non-insulin treatment. However, hyperglycaemia-induced myocardial apoptosis implicates CytoC release and autophagy of mitochondria (10, 23).

Table 2: Correlation analysis for plasma hypoxia-inducible factor (HIF)-1α *versus* characteristics and investigated parameters in participating healthy controls and Type 2 diabetic patients in Saudi Arabia

HIF-1α	Healthy controls					Diabetic patients								
	Whole group	Males	Females	BMI < 25	BMI ≥ 25	Whole group	Males	Females	BMI < 25	BMI ≥ 25	Insulin	Non-insulin	DD ≤ 5 years	DD > 5 years
	Correlation coefficient (*p*)					Correlation coefficient (*p*)								
Age	ns	0.284 (< 0.02)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
ABSI	ns	0.553 (< 0.001)	ns	ns	ns	ns	ns	0.152 (< 0.05)	0.425 (< 0.01)	ns	ns	ns	ns	ns
BMI	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.154 (< 0.05)	ns	ns	ns	ns
AIP	-0.346 (< 0.001)	-0.341 (< 0.004)	ns	-0.435 (< 0.01)	-0.321 (< 0.005)	ns	-0.356 (< 0.02)	ns	ns	ns	ns	ns	ns	ns
HOMA-IR	ns	ns	ns	ns	ns	ns	-0.326 (< 0.04)	ns	ns	ns	ns	-0.185 (< 0.05)	-0.248 (< 0.03)	ns
CytoC	0.361 (< 0.001)	ns	0.600 (< 0.001)	0.590 (< 0.001)	0.237 (< 0.04)	0.673 (< 0.001)	0.648 (< 0.001)	0.679 (< 0.001)	0.737 (< 0.001)	0.663 (< 0.001)	0.513 (< 0.001)	0.758 (< 0.001)	0.739 (< 0.001)	0.625 (< 0.001)
CRP	ns	ns	ns	-0.347 (< 0.05)	-0.273 (< 0.02)	ns	ns	ns	ns	ns	-0.211 (< 0.05)	ns	ns	-0.181 (< 0.05)
CS	NA	NA	NA	NA	NA	ns	ns	ns	ns	ns	ns	0.236 (< 0.01)	ns	ns
DD	NA	NA	NA	NA	NA	-0.208 (< 0.003)	ns	-0.159 (< 0.05)	-0.357 (< 0.03)	ns	ns	ns	ns	ns
TS	NA	NA	NA	NA	NA	-0.139 (< 0.05)	ns	-0.268 (< 0.001)	-0.489 (< 0.002)	-0.159 (< 0.05)	ns	ns	ns	-0.247 (< 0.006)

ABSI = A Body Shape Index, BMI = body mass index, AIP = atherogenic index of plasma, HOMA-IR = homeostatic model assessment of insulin resistance, CytoC = cytochrome c, CRP = C-reactive protein, CS = complication score, DD = disease duration, TS = treatment score, ns = non-significant relationship, and NA = non-applicable.

Table 3: Correlation analysis for plasma cytochrome c *versus* characteristics and investigated parameters in participating healthy controls and Type 2 diabetic patients in Saudi Arabia

CytoC	Healthy controls					Diabetic patients								
	Whole group	Males	Females	BMI < 25	BMI ≥ 25	Whole group	Males	Females	BMI < 25	BMI ≥ 25	Insulin	Non-insulin	DD ≤ 5 years	DD > 5 years
	Correlation coefficient (*p*)					Correlation coefficient (*p*)								
Age	ns	ns	ns	ns	ns	ns	ns	ns	ns			0.184 (< 0.05)	ns	ns
ABSI	0.236 (< 0.05)	0.525 (< 0.002)	ns	ns	ns	0.185 (< 0.02)	ns	0.159 (< 0.05)	0.521 (< 0.001)		0.194 (< 0.05)	0.205 (< 0.05)	ns	0.210 (< 0.04)
BMI	ns	ns	ns	0.346 (< 0.05)	ns	0.189 (< 0.01)	ns	0.211 (< 0.009)	ns	0.217 (< 0.01)	0.295 (< 0.007)	0.199 (< 0.04)	ns	0.239 (< 0.01)
AIP	-0.309 (< 0.001)	-0.314 (< 0.008)	ns	-0.398 (< 0.025)	-0.248 (< 0.03)	ns	-0.601 (< 0.001)	ns	ns	ns	ns	ns	ns	ns
HOMA-IR	ns	-0.236 (< 0.05)	ns	ns	-0.271 (< 0.05)	ns	-0.310 (< 0.05)	ns	ns	ns	ns	ns	-0.236 (< 0.04)	ns
HIF1α	0.361 (< 0.001)	ns	0.600 (< 0.001)	0.590 (< 0.001)	0.237 (< 0.04)	0.673 (< 0.001)	645 (< 0.001)	0.679 (< 0.001)	0.737 (< 0.001)	0.663 (< 0.001)	0.511 (< 0.001)	0.758 (< 0.001)	0.739 (< 0.001)	0.625 (< 0.001)
CRP	ns	ns	ns	-0.330 (< 0.05)	ns	ns	ns	ns	ns	ns	-0.188 (< 0.02)			-0.179 (< 0.05)
CS	NA	NA	NA	NA	NA	ns	0.406 (< 0.007)	ns	ns	ns	ns	0.264 (< 0.01)		0.180 (< 0.05)
DD	NA	NA	NA	NA	NA	-0.138 (< 0.05)	-0.183 (< 0.05)	-0.176 (< 0.03)	ns	-0.158 (< 0.05)	ns	ns		
TS	NA	NA	NA	NA	NA	-0.219 (< 0.002)	ns	-0.251 (< 0.002)	-0.384 (< 0.02)	-0.186 (< 0.02)	ns	ns	-0.227 (< 0.05)	-0.228 (< 0.01)

ABSI = A Body Shape Index, BMI = body mass index, AIP = atherogenic index of plasma, HOMA-IR = homeostatic model assessment of insulin resistance, CytoC = cytochrome c, HIF-1α = hypoxia-inducible factor-1α, CRP = C-reactive protein, CS = complication score, DD = disease duration, TS = treatment score, ns = non-significant relationship, and NA = non-applicable.

Table 4: Correlation analysis for plasma C-reactive protein (CRP) *versus* characteristics and investigated parameters in participating healthy controls and Type 2 diabetic patients in Saudi Arabia

CRP	Healthy controls					Diabetic patients								
	Whole group	Males	Females	BMI < 25	BMI ≥ 25	Whole group	Males	Females	BMI < 25	BMI ≥ 25	Insulin	Non-insulin	DD ≤ 5 years	DD > 5 years
	Correlation coefficient (*p*)					Correlation coefficient (*p*)								
Age	0.185 (< 0.05)	ns	0.342 (< 0.05)	ns	ns	ns	0.313 (< 0.04)	0.157 (< 0.05)	0.298 (< 0.05)	ns	ns	ns	0.219 (< 0.05)	ns
ABSI	ns	ns	ns	ns	0.293 (< 0.05)	ns	0.504 (< 0.05)	ns	ns	ns	ns	ns	ns	ns
BMI	0.289 (< 0.004)	0.361 (< 0.004)	ns	ns	0.319 (< 0.009)	0.314 (< 0.001)	0.392 (< 0.02)	0.286 (< 0.001)	ns	0.277 (< 0.001)	0.317 (< 0.004)	0.256 (< 0.006)	0.348 (< 0.02)	0.306 (< 0.001)
AIP	0.210 (< 0.03)	ns	ns	ns	0.279 (< 0.02)	0.158 (< 0.03)	ns	0.166 (< 0.04)	ns	ns	ns	0.181 (< 0.05)	ns	0.182 (< 0.05)
HOMA-IR	0.319 (< 0.001)	0.294 (< 0.01)	0.372 (0.03)	ns	0.408 (< 0.001)	ns	ns	ns	ns	ns	ns	ns	ns	ns
HIF1α	ns	ns	ns	-0.330 (< 0.05)	-0.273 (< 0.02)	ns	ns	ns	ns	ns	-0.211 (< 0.05)	ns	ns	-0.181 (< 0.05)
CytoC	ns	ns	ns	-0.347 (< 0.05)	ns	ns	ns	ns	ns	-0.188 (< 0.02)	ns	ns	ns	-0.179 (< 0.05)
CS	NA	NA	NA	NA	NA	ns	ns	ns	ns	ns	ns	ns	ns	ns
DD	NA	NA	NA	NA	NA	ns	ns	ns	ns	ns	ns	ns	ns	ns
TS	NA	NA	NA	NA	NA	ns	ns	ns	ns	ns	ns	ns	ns	ns

ABSI = A Body Shape Index, BMI = body mass index, AIP = atherogenic index of plasma, HOMA-IR = homeostatic model assessment of insulin resistance, CytoC = cytochrome c, HIF-1α = hypoxia-inducible factor-1α, CRP = C-reactive protein, CS = complication score, DD = disease duration, TS = treatment score, ns = non-significant relationship, and NA = non-applicable.

Table 5: Correlation analysis for the atherogenic index of plasma (AIP) *versus* characteristics and investigated parameters in participating healthy controls and Type 2 diabetic patients in Saudi Arabia

AIP	Healthy controls					Diabetic patients								
	Whole group	**Males**	**Females**	**BMI < 25**	**BMI ≥ 25**	**Whole group**	**Males**	**Females**	**BMI < 25**	**BMI ≥ 25**	**Insulin**	**Non-insulin**	**DD ≤ 5 years**	**DD > 5 years**
	Correlation coefficient (*p*)					**Correlation coefficient (*p*)**								
Age	ns	ns	0.320 (< 0.05)	ns	ns	0.204 (< 0.004)	ns	0.261 (< 0.001)	ns	0.148 (< 0.05)	ns	0.247 (< 0.007)	0.346 (< 0.002)	
ABSI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
BMI	ns	ns	ns	ns	ns	0.333 (< 0.001)	ns	0.394 (< 0.001)	0.284 (< 0.05)	0.287 (< 0.001)	0.319 (< 0.004)	0.360 (< 0.001)	0.334 (< 0.003)	0.351 (< 0.001)
HOMA-IR	ns	ns	ns	0.350 (< 0.05)	ns	0.159 (< 0.025)	ns	0.156 (< 0.05)	ns	ns	0.219 (< 0.05)	0.182 (< 0.05)	ns	0.180 (< 0.05)
HIF-1α	-0.346 (< 0.001)	-0.314 (< 0.008)	ns	-0.435 (< 0.01)	-0.321 (< 0.005)	ns	-0.356 (< 0.02)	-0.166 (< 0.04)	ns	ns	ns	ns	ns	
CytoC	-0.309 (< 0.001)	-0.341 (< 0.004)	ns	-0.398 (< 0.025)	-0.248 (< 0.03)	ns	-0.601 (< 0.001)	ns	ns	ns	ns	ns	ns	
CRP	0.210 (< 0.03)	ns	ns	ns	0.279 (< 0.02)	0.158 (< 0.03)	ns	ns	ns	ns	ns	0.181 (< 0.05)	ns	0.182 (< 0.05)
CS	NA	NA	NA	NA	NA	0.168 (< 0.02)	ns	0.269 (< 0.001)	ns	ns	ns	ns	ns	
DD	NA	NA	NA	NA	NA	ns	ns	ns	ns	ns	ns	ns	ns	ns
TS	NA	NA	NA	NA	NA	ns	ns	0.155 (< 0.05)	ns	ns	ns	ns	ns	ns

ABSI = A Body Shape Index, BMI = body mass index, AIP = atherogenic index of plasma, HOMA-IR = homeostatic model assessment of insulin resistance, CytoC = cytochrome c, HIF-1α = hypoxia-inducible factor-1α, CRP = C-reactive protein, CS = complication score, DD = disease duration, TS = treatment score, ns = non-significant relationship, and NA = non-applicable.

Table 6: Correlation analysis for plasma homeostatic model assessment of insulin resistance (HOMA-IR) *versus* characteristics and investigated parameters in participating healthy controls and Type 2 diabetic patients in Saudi Arabia

HOMA-IR	Healthy controls					Diabetic patients								
	Whole group	Males	Females	BMI < 25	BMI ≥ 25	Whole group	Males	Females	BMI < 25	BMI ≥ 25	Insulin	Non-insulin	DD ≤ 5 years	DD > 5 years
	Correlation coefficient (*p*)					Correlation coefficient (*p*)								
Age	0.253 (< 0.01)	0.480 (< 0.005)	0.547 (< 0.001)	ns	0.225 (< 0.05)	ns	ns	ns	ns	ns	ns	0.188 (< 0.04)	0.224 (< 0.05)	ns
ABSI	ns	0.486 (< 0.001)	0.390 (< 0.03)	ns	ns	0.157 (< 0.05)	ns	ns	0.324 (< 0.05)	ns	0.312 (< 0.007)	ns	ns	0.279 (< 0.005)
BMI	0.558 (< 0.001)	ns	0.675 (< 0.001)	ns	0.545 (< 0.001)	0.282 (< 0.001)	ns	0.289 (< 0.001)	ns	0.215 (< 0.007)	ns	0.416 (< 0.001)	0.412 (< 0.001)	0.237 (< 0.01)
AIP	ns	ns	ns	0.350 (< 0.05)	ns	0.159 (< 0.025)	ns	0.156 (< 0.05)	ns	ns	0.219 (< 0.05)	0.182 (< 0.05)		0.180 (< 0.05)
HIF-1 α	ns	ns	ns	ns	ns	ns	-0.326 (< 0.04)	ns	ns	ns	ns	-0.185 (< 0.05)	-0.248 (< 0.03)	ns
CytoC	ns	-0.236 (< 0.05)	ns	ns	-0.271 (< 0.05)	ns	-0.310 (< 0.05)	ns	ns	ns	ns	ns	-0.236 (< 0.04)	ns
CRP	0.319 (< 0.001)	0.294 (< 0.01)	0.372 (< 0.03)	ns	0.408 (< 0.001)	ns	ns	ns	ns	ns	ns			ns
CS	NA	NA	NA	NA	NA	ns	ns	ns	ns	ns	ns	ns		ns
DD	NA	NA	NA	NA	NA	-0.137 (< 0.05)	ns	ns	ns	-0.149 (< 0.05)	ns	ns	ns	ns
TS	NA	NA	NA	NA	NA	-0.158 (< 0.03)	ns	-0.220 (< 0.006)	ns	-0.158 (< 0.05)	ns	ns	ns	-0.178 (< 0.05)

ABSI = A Body Shape Index, BMI = body mass index, AIP = atherogenic index of plasma, CytoC = cytochrome c, HIF-1α = hypoxia-inducible factor-1α, CRP = C-reactive protein, CS = complication score, DD = disease duration, TS = treatment score, ns = non-significant relationship, and NA = non-applicable.

Serum HIF-1α increases and negatively correlates survival in non-diabetic patients with acute decompensated heart failure (20). Moreover, serum level of HIF-1α in T2DM patients is significantly higher than that in healthy controls and correlates with kidney function and disease duration (22). Specific allelic polymorphism in HIF-1α gene prevails in diabetic patients and complicated diabetes than healthy controls leading to a pathogenetic decrease in HIF-1α messenger ribonucleic acid expression irrespective of the oxygen conditions of cells (24). Diabetic hyperglycaemia affects both HIF-1α stability and activation in a complex way, resulting in suppression of expression of HIF-1α target genes essential for wound-healing both *in vitro* and *in vivo* (25). On the contrary, diabetes anti-angiogenic phenotype correlates with reduction in the total and nuclear HIF-1α protein levels (26). However, increased expression of HIF-1α correlates with diabetic retinal and kidney damage (27, 28). Hypoxia-inducible factor-1α is a central regulator of adipocyte lipid catabolism and energy expenditure by interfering with the function of sirtuin 2. Inactivation of HIF-1α reduces metabolic phenotype, promotes adipocyte browning and induces mitochondrial biogenesis and function in visceral adipocytes, culminating to protection from obesity and IR (29, 30). Synthesis of the anti-insulin, anti-angiogenic glucocorticoids is down-regulated by HIF-1α (31). The significant reduction in HIF-1α levels in the current sample of patients was pathogenetic since induction of HIF-1α is a protective factor for β-cells against apoptosis (32). Reduction in HIF-1α and its weak inverse association with poor prognosis in the current sample of patients was not applied to CytoC since the former is expected to serve a chronic complex protective role while the latter is a marker of acute cellular damage that needs longitudinal rather than cross-sectional dissection. Insulin effect on hepatocytes in high glucose condition and in experimental T2DM is mediated by HIF-1α. Knockdown of HIF-1α abrogates insulin effects (33).

The very high AIP in the patients *versus* controls reflected their dyslipidaemia. Atherogenic index of plasma correlated with BMI, glycaemic control indices and disease complication scores in the patients of this study, as previously reported (34). The low HDL-C/high TAGs pattern is associated with the degree of hyperglycaemia and significantly predicts the incidence of vascular events in coronary patients with T2DM (35). Similar to the patients of this study, IR contributes to such atherogenic profile with increased free fatty acid and very low-density lipoprotein flux at the liver (36). Differences in insulin and glucose in the patients of this study were reflected on their HOMA-IR. However, opposite to AIP, plasma insulin and insulin treatment, HOMA-IR did not correlate with complication scores in the patients of this study. C-reactive protein associates higher insulin and HbA1c, pointing to a role for inflammation in IR and glucose intolerance (37). Correlation among CRP, HbA1c, insulin, HOMA-IR, inflammation and hyperglycaemia jointly contribute to cardiovascular disease in T2DM patients (38). In this study, CRP was higher in patients than in controls, particularly for those on insulin. Among the patients, correlation of plasma insulin and insulin use as a treatment with AIP but not correlation of HOMA-IR with worse prognostic markers and complication score particularly supports the hypothesis raised by Nolan *et al* (39) that IR is a natural protective strategy. The absence of a negative correlation between treatment scores and AIP may confirm poor diabetic control that could be due to several reasons, most probably patient incompliance. This is further confirmed by a strong positive association between AIP and glycaemic control in the present investigation, similar to a study by Zhu *et al* (40), but still contrary to the one by Tan *et al* showing no correlation (41).

CONCLUSION

A comparatively very high rate of complications was evident in the patients of this study that correlated positively with their AIP, dyslipidaemia, age, disease duration, CRP, insulin as treatment and BMI, but not gender and HOMA-IR. The reduction in plasma HIF-1α and non-significant increases in CytoC in patients correlated with risk and poor prognostic indicators, pointing to a pathogenetic implication that requires confirmation in prospective longitudinal studies.

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