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Original article

Oxidative stress and inflammation on metabolic abnormalities and renal involvement in prediabetic subjects across Europe

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ABSTRACT

Background: Studying the mechanisms involved in the transition from prediabetes to diabetes and its associated complications, such as kidney disease, is a growing challenge. This study focuses on identifying valuable biomarkers for the early detection of kidney damage, evaluating molecules associated with oxidative stress and inflammation in prediabetic individuals across Europe.

Methods: In plasma samples from individuals with prediabetes included in the ePREDICE study, we determined molecules related to oxidative stress (advance oxidative protein products-AOPP) and inflammatory biomarkers (C-reactive protein – CRP; Interleukin 6 – IL-6), and correlated them with anthropometric and biochemical data, assessing their potential for the early diagnosis of renal involvement.

Results: Among the 967 people with prediabetes, 94 presented some sign of renal impairment such as albuminuria, hyperfiltration or hypofiltration. Significant variations were identified between oxidative stress and inflammatory biomarkers (upper and lower quartiles of AOPP, CRP and IL6), and parameters associated with blood pressure, glucose metabolism, lipid

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profile, and fatty liver index. In particular, both types of biomarkers were associated with components of the metabolic syndrome. There were significant associations between AOPP and CRP, and the presence of albuminuria, but not with renal function. Overall, CRP was a better biomarker than IL-6 for most of the parameters studied.
Conclusion: These results highlight the important associations of oxidative stress and inflammation with metabolic abnormalities linked to the prediabetic state and its complications such as fatty liver and renal involvement. Although these results need to be confirmed, our study suggests that AOPP and CRP could be simple biomarkers of interest in predicting the risk of loss of renal function in people with prediabetes.

El estrés oxidativo y la inflamación en las anomalías metabólicas y la afectación renal en sujetos prediabéticos de toda Europa

RESUMEN

Antecedentes: El estudio de los mecanismos implicados en la transición de la prediabetes a la diabetes y sus complicaciones asociadas, como la enfermedad renal, es un tema de gran interés. Este estudio se centra en la identificación de biomarcadores eficaces para la detección precoz de la afectación renal, evaluando moléculas asociadas al estrés oxidativo e inflamación en individuos prediabéticos de varios países europeos.
Métodos: En muestras de plasma de individuos prediabéticos, incluidos en el estudio ePREDICE, se determinaron biomarcadores de estrés oxidativo (AOPP) e inflamación (proteína C reactiva, IL-6) y se correlacionaron con datos antropométricos y bioquímicos, evaluando su potencial en el diagnóstico precoz de la disfunción renal.
Resultados: Entre los 967 sujetos con prediabetes, 94 presentaban algún signo de afectación renal, como albuminuria, hiperfiltración o hipofiltración. Se identificaron variaciones significativas entre los biomarcadores de estrés oxidativo e inflamatorios (cuartiles superiores e inferior del AOPP, PCR e IL-6) y parámetros relacionados como la presión arterial, el metabolismo glucémico, el perfil lipídico y el índice de hígado graso. En particular, ambos biomarcadores estaban bien relacionados con los componentes del síndrome metabólico. Existieron asociaciones significativas entre la AOPP y la PCR, y la presencia de albuminuria, pero no con la función renal. En general, la PCR fue mejor biomarcador que la IL-6 para la mayoría de los parámetros estudiados.
Conclusiones: Estos resultados subrayan las importantes conexiones entre estrés oxidativo e inflamación con las anomalías metabólicas ligadas al estado prediabético y sus complicaciones como el hígado graso y la afectación renal. Aunque es necesario confirmar estos datos, nuestro estudio sugiere que los AOPP y la PCR podrían ser biomarcadores de interés en la predicción del riesgo de pérdida de la función renal en los sujetos con prediabetes.

Palabras clave:
Diabetes tipo 2
Prediabetes
Enfermedad renal crónica
Estrés oxidativo
Biomarcadores

Background

Hyperglycemia is a symptom of type 2 diabetes (T2D), a silent disease that progresses slowly. Between normoglycemia and T2D, prediabetes or intermediate hyperglycemia is a condition with several pathophysiological manifestations.[1] The rate at which people with prediabetes progress to T2D is approximately 5–10% annually[2–4] and this rate is influenced by several risk factors, as determined by the diabetes risk score, as well as the sub-phenotype of prediabetes.[5]

Several studies have demonstrated that people with prediabetes are more likely to experience macrovascular[6] and microvascular complications.[5,6] As observed in the Rotterdam study, individuals with prediabetes can suffer end-organ damage typical of diabetes along with systemic microvascular dysfunction.[7] Based on these data, it has been suggested that the diagnosis of prediabetes should also include determination of microvascular injury.[8,9]

The diagnosis of chronic kidney disease (CKD) traditionally relies on detecting albuminuria and a decline in estimated glomerular filtration rate (eGFR), based on serum creatinine levels. Albuminuria is a robust tool for predicting prognosis, but its correlation with structural damage and renal function is less apparent in early stages of CKD. Therefore, new biomarkers enabling earlier evaluation of renal damage in

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high-risk populations are required. Along the development of CKD, the eGFR variation is a key predictor of the renal outcome, being creatinine-based equations such as CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration)[10] and MDRD-4 (Modification of Diet in Renal Disease)[11] the most commonly used.

A systematic review and a meta-analysis found a significant association between prediabetes and the risk of CKD.[12] This was further supported by a 9-year prospective cohort study in a Korean adult population, which identified prediabetes as an independent predictor of incident CKD.[13] However, a causal inference analysis[14] suggested that while prediabetes is causally related to coronary artery disease, its causal effects on other diabetes complications, including CKD, remained unclear. Despite this, the high-risk nature of prediabetes for the development of diabetes and its association with early nephropathy and CKD are rather well-established.[15]

There is an escalating interest in the search of biomarkers for the diagnosis and prognosis of the development of diabetes and its complications.[16,17] In this context, the role of inflammation has emerged as one of pivotal markers in the pathogenesis of T2D and CKD. Systemic inflammatory markers as C-reactive protein (CRP) and interleukin-6 (IL-6) have been linked to an increased risk of T2D.[18,19] Circulating tumor necrosis factor (TNF) receptors 1 and 2 predict end-stage renal disease in both type 1 and 2 diabetes.[19,20]

Oxidative stress, intimately associated with hyperglycaemia, is a key contributor of complications in people with diabetes.[20,21] Biomarkers such as 8-hydroxy-2′deoxyguanosine, isoprostanes, malondialdehyde, and nitrotyrosine are associated to oxidative stress in people with diabetes. and further vascular damage.[20] Furthermore, oxidative stress markers have been identified as valuable indicators in prediabetes, by impacting in glucose uptake and insulin secretion.[22] Biomarkers such as advanced oxidation protein products (AOPP) and thiobarbituric acid reactive substances (TBARS), including malondialdehyde (MDA), are significant in detecting oxidative stress in diabetes and CKD.[23,24]

The Early Prevention of Diabetes Complications in People with Hyperglycaemia (ePREDICE) study is an international, multicentre, randomized, double-blind, placebo-controlled trial. Its primary goal was to prevent cardiovascular and microvascular diabetic complications (nephropathy, retinopathy and neuropathy) through lifestyle interventions and pharmacological treatments (metformin, linagliptin, and their combination).[25] The study also assessed the identification of reliable oxidative stress and inflammatory plasma biomarkers for early detection of renal dysfunction, as well as to examine their correlation with the clinical and metabolic status, in a well-characterized group of prediabetic subjects across Europe.

The aim of the present study was to determine whether oxidative stress and inflammatory biomarkers can aid in the early detection of renal dysfunction in individuals with prediabetes. This research seeks to identify tools that could help prevent or delay the development of CKD, ultimately improving the health outcomes of this population.

Materials and methods

The ePREDICE trial and the study participants

ePREDICE is an independent, multicentre, double-blind, placebo-controlled clinical trial that follows a randomization protocol. Four parallel trial arms were randomly assigned to participants with equal probability: metformin, linagliptin, a fixed-dose combination of linagliptin plus metformin, and placebo. All participants received nutrition and physical activity instructions as part of a lifestyle intervention. They were followed up throughout one-year extra observational phase after two-year active intervention.

The present study was conducted as a cross-sectional analysis examining the baseline samples from the individuals at risk of T2D and microvascular complications. This substudy aimed at identifying clinical and biochemical markers in the glucose metabolism, as well as potential oxidative and inflammatory biomarkers, that might be predictive of the early renal involvement in people with prediabetes.

Participants were adults aged 45–74 years, diagnosed of prediabetes by means of the assessment of the Impaired Fasting Glucose [IFG], Impaired Glucose Tolerance [IGT], or both. They were recruited from several centers in Austria, Bulgaria, Greece, Kuwait, Poland, Serbia, Spain, and Turkey.

Exclusion criteria were comprehensive, encompassing conditions like T1D, recent cardiovascular events, severe obesity, and certain chronic diseases, among others. Detailed criteria are described in Gabriel et al.[25]

Plasma, serum, and urine samples were obtained from all patients at the time of recruitment (baseline). The samples were aliquoted, stored at −80 °C, and sent on dry ice to the central laboratory (IIS-FJD) for biomarker determination.

Anthropometric, biochemical variables, and lifestyle questionnaires

In the ePREDICE study, anthropometric and biochemical data were collected at each participating center, and the participants completed questionnaires that have been utilized in this study. Key variables included plasma glucose (PG), serum total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density (LDL) cholesterol, liver function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gamma-glutamyl transpeptidase (GGT); fatty liver index (FLI); Matsuda Index; Oral Glucose Insulin Sensitivity (OGIS); Insulinogenic Index and other components of glucose metabolism. The study of renal function and assessment of oxidative stress, as well as inflammatory biomarkers, were performed at Fundación Jimenez Díaz, as the ePREDICE central laboratory in Madrid, Spain. The diagnostic assessment of the estimated glomerular filtration rate (eGFR) was carried out using the Modification of Diet in Renal Disease 4 (MDRD4) formula. The clinical classification was performed in accordance with the Kidney Disease: Improving Global Outcomes (KDIGO) Nephrology Guidelines.[26,27]

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Table 1 – Classification of individuals with prediabetes according with renal involvement based on the urine albumin/creatinine ratio (UACR) and estimated glomerular filtration (eGFR) using the MDRD-4 formula.

Urine albumin/creatinine ratio (mg/g)	Individuals, $n = 967$	Percentage
<30	923	95.3%
30–300	43	4.6%
>300	1	0.1%
eGFR MDRD-4 (mL/min/1.73 m^2)	**Individuals**	**Percentage**
>130	20	2.1%
130-60	918	94.9%
59-45	29	3%

Oxidative stress

Advance oxidation protein products (AOPP) were measured spectrophotometrically in plasma samples at 340 nm, and the results were expressed in chloramine-T equivalents, following the methodology described by Selmeci et al.[23,28] Malondialdehyde (MDA) was assessed using the Thiobarbituric Acid Reactive Substances (TBARS) assay.[29]

Inflammatory biomarkers

High sensitivity C-reactive protein (Hs-CRP) was assessed by latex-enhanced immunoturbidimetry (ADVIA 2400 Chemistry System, Siemens, Germany). Serum IL-6 was quantified by ELISA (R&D Systems, MIN, USA) in accordance with the manufacturer's protocol.

Statistical analysis

Data were managed using OpenClinica. Descriptive statistics, median and interquartile ranges for quantitative variables, and percentages for qualitative variables are presented. Non-parametric data were analyzed using the Kruskal–Wallis and Mann–Whitney–Wilcoxon tests. Correlation matrices evaluated linear dependencies among quantitative variables. Pearson's χ^2 test was used for qualitative data. Statistical significance was set at p-value < 0.05. Analyses were performed using R software (v4.0.2) in R Studio (v1.4), with libraries such as ggplot2, jmv, finalfit, and gtsummary.

Results

Identification of renal involvement in prediabetic subjects

Out of the 967 participants included in the study, the analysis of renal function data revealed that 20 individuals exhibited an eGFR exceeding 130 mL/min/1.3 m^2, and they were considered as having hyperfiltration. Additionally, 29 individuals displayed an eGFR below 60 mL/min/1.3 m^2 and were listed as having chronic kidney disease. The rest of participants were considered to have renal function within the "normal range".

Based on albumin urinary excretion (albumin/creatinine ratio), 44 subjects had albuminuria within the ratio range of 30–300 mg/g, corresponding to stage A2, according to the KDIGO guidelines. Notably, one person exhibited an albuminuria level of 510 mg/g (stage A3) (Table 1).

Surprisingly, we only identified two persons with hyperfiltration in conjunction with albuminuria at stage A2, while another three people displayed hypofiltration along with albuminuria stage A2. These findings depicted valuable insights into the spectrum of renal involvement within this prediabetic population. Unfortunately, no further clinical and follow-up information was gathered about those individuals' outcome.

Oxidative stress biomarkers and clinical and metabolic status

For statistical analysis, AOPP data were categorized into quartiles, as detailed in Table 2. Our data revealed that the individuals in the upper quartile of AOPP (Q4) had higher weight, height, systolic and diastolic blood pressure, insulin sensitivity, serum total cholesterol, triglycerides and HDL, ALT and the AST/ALT ratio, GGT, metabolic syndrome, and FLI compared with those with the lowest AOPP level (Q1).

We also observed a significant correlation between AOPP and the following variables: systolic blood pressure ($R^2 = 0.132$, $p = 0.002$), diastolic blood pressure ($R^2 = 0.144$, $p < 0.001$), OGIS ($R^2 = -0.088$, $p = 0.043$), serum total cholesterol ($R^2 = 0.194$, $p < 0.001$), serum triglycerides ($R^2 = 0.388$, $p < 0.001$), the metabolic syndrome status ($R^2 = 0.194$, $p < 0.001$), HDL ($R^2 = -0.169$, $p < 0.001$), LDL ($R^2 = 0.095$, $p = 0.03$), ALT ($R^2 = 0.129$, $p = 0.003$), AST/ALT ratio ($R^2 = -0.183$, $p < 0.001$), GGT ($R^2 = 0.149$, $p < 0.001$), and FLI ($R^2 = 0.132$, $p = 0.002$).

Association of AOPP with the metabolic syndrome components

In accordance with the NCEP ATP III guidelines,[30] the metabolic syndrome was defined when an individual met three or more of the following criteria: overweigh (waist circumference exceeding 102 cm for men and 88 cm for women), hypertension (exceeding 130/85 mmHg), fasting serum triglycerides (exceeding 150 mg/dL), and lower HDL (below 40 mg/dL for men or 50 mg/dL for women) and hyperglycemia (fasting PG over 100 mg/dL).

Our analysis revealed a significant association between elevated AOPP and each of the components of the metabolic syndrome: 698 out of 967 people were overweight or obese, with AOPP levels averaging 2675 ± 1168 μmol/L compared with to 2612 ± 1208 μmol/L in non-obese individuals (p-value = not significant). People with hypertension ($n = 484$) had AOPP of 2794 ± 1275 versus 2517 ± 1051 in people with normotension ($p = 0.0071$).

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Table 2 – Quartile analysis of AOPP for the main study variables: the Kruskal–Wallis's test was used for quartile analysis (*p*-value), with the Mann–Whitney–Wilcoxon test applied for comparisons between the Q1 and Q4 quartiles (*p*-value2).

Characteristic	Q1 N = 130 (25%)	Q2 N = 176 (33%)	Q3 N = 88 (17%)	Q4 N = 132 (25%)	*p*-Value	*p*-Value2
Sex					0.92	0.89
Male	57 (44%)	73 (41%)	36 (41%)	59 (45%)		
Female	73 (56%)	103 (59%)	52 (59%)	73 (55%)		
Age (yr)	59 (53, 65)	59 (53, 65)	58 (53, 65)	59 (52, 65)	>0.99	0.92
Weight (kg)(M vs F)	87 (77, 96)	91 (82, 100)	90 (85, 104)	89 (80, 101)	0.38	0.42
	74 (66, 86)	78 (68, 92)	83 (70, 90)	83 (73, 90)	0.12	0.02
Body mass index	28.8 (26.0, 33.5)	30.2 (27.1, 33.8)	30.7 (27.8, 34.0)	30.1 (27.7, 33.1)	0.23	0.17
Height (cm) (M vs F)	172 (167, 177)	174 (169, 180)	175 (171, 178)	175 (170, 178)	0.33	0.18
	158 (154, 162)	161 (155, 165)	159 (156, 164)	161 (156, 166)	0.063	0.017
Waist circumference (cm) (M vs F)	108 (100, 115)	106 (100, 114)	107 (99, 113)	106 (96, 113)	0.84	0.39
	99 (89, 108)	100 (96, 110)	101 (94, 109)	99 (94, 108)	0.56	0.46
Hip circumference (cm) (M vs F)	108 (100, 114)	107 (102, 112)	109 (102, 114)	106 (100, 110)	0.45	0.12
	110 (102, 117)	111 (104, 121)	111 (104, 117)	108 (104, 120)	0.91	0.51
Systolic blood pressure (mmHg)	129 (118, 141)	128 (120, 141)	136 (127, 146)	136 (123, 146)	0.003	0.014
Diastolic blood pressure (mmHg)	80 (74, 87)	80 (74, 87)	84 (76, 91)	84 (80, 90)	<0.001	0.001
Fasting plasma glucose (mg/dL)	116 (112, 121)	115 (112, 122)	115 (112, 123)	115 (112, 121)	0.86	0.63
2 h plasma glucose (mg/dL)	147 (121, 171)	142 (123, 162)	145 (124, 176)	148 (128, 177)	0.26	0.4
Fasting serum insulin (UI)	10 (7, 14)	11 (8, 15)	12 (8, 15)	11 (8, 16)	0.49	0.14
Matsuda Index	2.81 (2.08, 4.03)	2.75 (1.80, 3.83)	2.58 (1.94, 3.34)	2.43 (1.85, 3.45)	0.16	0.03
Homeostatic Model Assessment for Insulin Resistance (HOMA IR)	2.95 (2.10, 4.06)	3.10 (2.22, 4.51)	3.36 (2.41, 4.22)	3.27 (2.22, 4.64)	0.51	0.17
Oral Glucose Insulin Sensitivity	336 (316, 364)	336 (307, 359)	324 (294, 349)	320 (296, 349)	0.002	<0.001
Prediabetes criteria					0.15	0.16
IGT	29 (22%)	40 (23%)	28 (32%)	43 (33%)		
IFG	51 (39%)	80 (45%)	32 (36%)	42 (32%)		
IGT + IFG	50 (38%)	56 (32%)	28 (32%)	47 (36%)		
HbA1c (%)	5.80 (5.50, 6.10)	5.80 (5.60, 6.10)	5.80 (5.60, 6.00)	5.84 (5.60, 6.07)	0.84	0.51
Mean eGFR, MDRD4 (mL/min/1.73 m^2)	86 (75, 96)	86 (71, 100)	83 (72, 91)	86 (76, 99)	0.26	0.83
eGFR stages MDRD-4 (mL/min/1.73 m^2)					0.09	0.11
G1	36 (28%)	65 (37%)	24 (27%)	50 (38%)		
G2	80 (62%)	91 (52%)	54 (61%)	69 (52%)		
G3a	4 (3.1%)	9 (5.1%)	7 (8.0%)	8 (6.1%)		
HF	10 (7.7%)	11 (6.2%)	3 (3.4%)	5 (3.8%)		
Urinary albumin/creatinine ratio (mg/g)	3 (2, 5)	4 (1, 8)	3 (1, 9)	2 (1, 5)	0.011	0.015
Serum total cholesterol (mg/dL)	198 (179, 219)	197 (174, 222)	205 (179, 230)	205 (183, 236)	0.026	0.043
Serum triglycerides (mg/dL)	95 (74, 131)	116 (83, 144)	125 (101, 169)	146 (97, 205)	<0.001	<0.001
Serum HDL (mg/dL) (M vs F)	50 (43, 59)	46 (41, 52)	45 (41, 54)	42 (37, 48)	0.002	<0.001
	56 (47, 66)	52 (46, 61)	52 (43, 62)	48 (40, 61)	0.0025	0.005
Serum LDL (mg/dL)	123 (105, 144)	121 (100, 142)	129 (102, 149)	129 (108, 151)	0.15	0.19
Metabolic syndrome (Y)	62 (48%)	92 (52%)	58 (66%)	101 (77%)	<0.001	<0.001
Platelet count	228 (200, 262)	236 (203, 282)	235 (200, 271)	243 (210, 278)	0.29	0.051
ALT (SGPT) (UI)	20 (17, 28)	22 (17, 30)	25 (20, 36)	23 (18, 32)	0.005	0.039
AST (SGOT) (UI)	22 (17, 26)	19 (16, 24)	21 (14, 25)	20 (16, 25)	0.11	0.23
AST/ALT ratio	0.97 (0.80, 1.21)	0.89 (0.68, 1.08)	0.80 (0.57, 1.00)	0.85 (0.64, 1.10)	0.008	0.007
Gammaglutamyl transferase (GGT)	24 (15, 35)	22 (16, 32)	24 (16, 44)	30 (20, 42)	0.008	0.002
Fatty liver index	64 (34, 85)	69 (43, 85)	74 (52, 88)	75 (49, 90)	0.014	0.003
Alcohol consumption (Y)	67 (53%)	107 (62%)	51 (59%)	77 (59%)	0.48	0.37
Smoking (Y)	58 (45%)	87 (49%)	33 (38%)	74 (56%)	0.045	0.064
CRP (ng/mL)	0.22 (0.08, 0.57)	0.20 (0.05, 0.55)	0.23 (0.08, 0.70)	0.31 (0.14, 0.56)	0.25	0.2
IL-6 (pg/mL)	3 (1, 5)	3 (1, 9)	3 (2, 13)	3 (1, 5)	0.19	0.29

Pearson's χ^2 test was used for qualitative variable analysis. Y denotes Yes.

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AOPP was significantly elevated among the 279 people with hypertriglyceridemia compared with those with normal triglycerides: 3177 ± 1307 versus 2462 ± 1063 ($p < 0.001$). For 304 people with low HDL (below 40 mg/dL for men or 50 mg/dL for women), compared with those with normal HDL, AOPP was higher, 2878 ± 1244 versus 2551 ± 1131 ($p = 0.003$).

No significant difference in AOPP concentration was observed by the hyperglycemia status (2665 ± 1184 versus 2515 ± 1059).

Association of AOPP with renal involvement

The association between AOPP and renal involvement (albuminuria) was not significant. Thus, in people with albuminuria AOPP was 2650 ± 901 compared with 2659 ± 1198 in those without. However, when AOPP was categorized into quartiles, individuals in the upper quartile of AOPP had significantly higher albuminuria/creatinine ratio than those in the lowest AOPP quartile (Table 2).

People with hypofiltration (<60 mL/min/1.73 m^2) had AOPP 2887 ± 1426, while those with hyperfiltration (>130 mL/min/1.73 m^2) had 2278 ± 910 and in people with normal eGFR) (60–130 mL/min/1.73 m^2) it was 2656 ± 1168. Furthermore, no significant differences in eGFR was noted between the AOPP extreme quartiles (Q1 and Q4). These data underscore the lack of significant association between AOPP and different stages of renal function in the prediabetic population.

Inflammatory biomarkers and clinical and metabolic characteristics

We analyzed two well-known inflammatory markers: CRP and IL-6. Table 3 depicts the data assessed by Q4 vs Q1 quartiles and the different clinical and metabolic characteristics.

People in the Q4 of CRP showed significant differences in several anthropometric variables such as age, weight, height, waist circumference, and BMI compared with those in the Q1. Elevated CRP was also associated with a higher diastolic blood pressure and various parameters associated with greater severity of insulin resistance (fasting insulin, Matsuda, HOMA-IR, or OGIS), markers of metabolic syndrome and hepatic steatosis (Table 3). Among renal parameters, only the presence of albuminuria was directly associated with CRP ($p = 0.014$).

Regarding IL-6 people in the Q4 displayed statistically significant differences with anthropometric variables such as weight, height, waist circumference, BMI and some metabolic variables compared with those in the Q1. Among renal parameters, IL-6 was associated with eGFR decline ($p = 0.017$), and a trend with albuminuria ($p = 0.075$).

No correlation was observed between inflammatory biomarkers and oxidative stress (CRP vs AOPP, $R^2 = 0.074$, $p = 0.09$; IL6 vs AOPP, $R^2 = 0.072$, $p = 0.322$).

Discussion

This work explored the potential of oxidative and inflammatory biomarkers to predict early renal injury and their association with metabolic abnormalities in well-characterized cohort of people with prediabetes. As in previous studies, an appreciable number (94/967) of the participants in the ePREDICE study displayed renal abnormalities manifested as albuminuria, hyperfiltration, or kidney failure at the first examination.[31,32]

One striking observation was that AOPP values were remarkably associated with a range of clinical and metabolic abnormalities in these people with prediabetes. These included high blood pressure, a variety of parameters indicative of suboptimal glycemic control, dyslipidemia, and the metabolic syndrome components either individually or in combination. In fact, oxidative stress has been associated to hypertension,poor glycemic control and dyslipidemia,[33–38] supporting the hypothesis that increased levels of advanced glycation end products and pro-oxidants could contribute to a proinflammatory milieu facilitating renal injury. In addition, a recent study (39) in >1.5 million participants has pointed out that 57% of women and 53% of men with incident cardiovascular disease presented several modifiable risk factors (BMI, systolic blood pressure, non-HDL, current smoking, and diabetes), as depicted in the present ePREDICE study.

However, despite the remarkable correlation between AOPP values and the different components of the metabolic syndrome and fatty liver, the association with renal involvement was less marked. Thus, although there was a significant association between elevated AOPP (Quartile 4 vs Quartile 1) and the presence of albuminuria, no differences with eGFR stages was observed. In, contrast with our data in prediabetic individuals. It has been recently reported that AOPP were markers for advanced CKD stages 4–5, probably indicating the value of persistent elevated AOPP blood levels at long term.

The association between inflammation and T2D and its complications has been pointed out in both clinical and preclinical studies.[21,41–43] Therefore, we also examined CRP and Q3 IL-6 blood levels to know their potential clinical value in the early detection of renal involvement. The association between CRP and IL-6 and renal involvement was less marked than in studies including people with T2D. In general, CRP was a better biomarker than IL-6 for most of the parameters studied. There was a significant association between CRP (Q4 vs Q1) and the presence of albuminuria, but not with renal function.

Similar to AOPP, the present data also highlight the importance of CRP (better than IL-6) as a useful biomarker of the metabolic dysregulation in people with prediabetes, and also potentially of incipient kidney damage (albuminuria), reinforcing the idea that a certain degree of inflammation and oxidative stress may also facilitate renal injury in individuals with prediabetes.

The reason why in our population AOPP was a reliable biomarker of oxidative stress, while other biomarkers of oxidative stress as MDA (data not shown) is unclear. Two major reasons could be envisioned: first, the limited stability of those compounds. Even though samples were properly shipped from different European countries and stored at −80° without breaking the cold chain, the average time from basal extraction to determination was almost 10 months, so it is likely that the radicals had a much shorter life under the collection conditions. The second reason might arise from the nature of AOPP, which represents the sum of all oxidative

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Table 3 – Comparison of several study variables between the quartile 1 and 4 for the inflammatory biomarkers IL-6 and CRP.

Characteristic	IL-6 Q1	IL-6 Q4	IL-6 *p*-value	CRP Q1	CRP Q4	CRP *p*-value
Sex (F)	28 (55%)	27 (48%)	0.49	90 (49%)	134 (69%)	<0.001
Age (yr)	59 (52, 64)	59 (52, 65)	0.54	60 (54, 66)	57 (51, 63)	0.008
Weight (kg) (M vs F)	93 (88, 194)	86 (72, 94)	0.11	86 (78, 96)	91 (83, 103)	0.008
	86 (80, 100)	76 (67, 89)	0.031	72 (65, 81)	86 (76, 98)	<0.001
BMI	30.3 (26.4, 33.6)	32.1 (29.6, 36.5)	0.011	28.3 (25.6, 31.2)	32.9 (28.8, 37.0)	<0.001
Height (cm) (M vs F)	174 (171, 177)	170 (165, 177)	0.1	174 (168, 179)	173 (170, 176)	0.52
	160 (157, 168)	158 (154, 161)	0.11	159 (155, 165)	160 (156, 166)	0.41
Waist circumference (cm) (M vs F)	110 (103, 116)	102 (98, 113)	0.075	103 (96, 110)	108 (101, 117)	0.003
	110 (100, 113)	99 (94, 109)	0.066	96 (88, 102)	103 (95, 113)	<0.001
Hip circumference (cm) (M vs F)	108 (102, 112)	106 (100, 111)	0.4	104 (100, 110)	108 (102, 116)	0.016
	115 (104, 126)	111 (105, 119)	0.42	105 (199, 112)	115 (106, 124)	<0.001
Systolic blood pressure (mmHg)	133 (126, 142)	134 (122, 143)	0.69	133 (122, 142)	133 (121, 147)	0.8
Diastolic blood pressure (mmHg)	84 (78, 90)	82 (75, 89)	0.51	81 (76, 88)	85 (77, 91)	0.031
Fasting glucose (mg/dL)	115 (112, 122)	115 (112, 121)	0.88	115 (112, 122)	115 (112, 119)	0.44
2 h plasma glucose (mg/dL)	144 (128, 166)	160 (132, 172)	0.14	144(121, 166)	142(122, 162)	0.5
Fasting Insulin (UI)	11.0 (8.2, 15.6)	12.8 (9.3, 18.2)	0.12	10 (7, 14)	12 (9, 17)	<0.001
Matsuda Index	2.76 (1.95, 3.32)	2.43 (1.64, 3.26)	0.36	3.01 (2.11, 4.32)	2.50 (1.76, 3.37)	<0.001
HOMA IR	3.13 (2.32, 4.29)	3.54 (2.62, 5.34)	0.13	2.70 (1.86, 3.87)	3.49 (2.44, 4.86)	<0.001
Oral Glucose Insulin Sensitivity	330 (300, 350)	326 (297, 346)	0.43	339 (310, 363)	327 (298, 354)	0.021
Prediabetic criteria			0.39			0.42
IGT	20 (39%)	15 (27%)		54 (30%)	46 (24%)	
IFG	15 (29%)	19 (34%)		73 (40%)	87 (45%)	
IGT + IFG	16 (31%)	22 (39%)		56 (31%)	60 (31%)	
HbA1c	5.80 (5.60, 6.00)	5.90 (5.68, 6.20)	0.084	5.80 (5.50, 6.00)	5.89 (5.60, 6.10)	0.22
Mean eGFR MDRD4 (mL/min/1.73 m^2)	89 (78, 99)	79 (71, 92)	0.017	85 (75, 95)	86 (76, 95)	0.75
eGFR stages (MDRD-4) (mL/min/1.73 m^2)			0.053			0.35
G1	1 (1.9%)	1 (1.8%)		2 (1.1%)	7 (3.6%)	
G2	23 (44%)	18 (32%)		67 (37%)	69 (36%)	
G3a	28 (54%)	32 (56%)		106 (58%)	112 (58%)	
HF	0 (0%)	6 (11%)		8 (4.4%)	5 (2.6%)	
Urine albumin/creatinine ratio (mg/g)	3 (2, 6)	5 (2, 15)	0.075	2 (1, 5)	3 (1, 8)	0.014
Total cholesterol (mg/dL)	202 (188, 218)	196 (171, 226)	0.39	201 (180, 225)	205 (176, 227)	0.81
Triglycerides (mg/dL)	129 (98, 191)	128 (85, 162)	0.47	115 (85, 148)	117 (87, 153)	0.47
HDL (mg/dL) (M vs F)	43 (41, 47)	51(42, 58)	0.027	48 (42, 58)	45 (39, 52)	0.11
	52 (40, 60)	51 (44, 58)	0.71	58 (47, 66)	52 (46, 61)	0.013
LDL (mg/dL)	122 (101, 140)	124 (96, 143)	0.93	125 (103, 145)	125 (104, 146)	0.61
Metabolic syndrome (Y/N)	32 (63%)	37 (66%)	0.72	94 (51%)	128 (66%)	0.003
Platelets	220 (193, 256)	227 (198, 272)	0.33	224 (198, 255)	241 (210, 293)	<0.001
ALT (SGPT)	25 (18, 30)	26 (20, 40)	0.12	22 (17, 29)	22 (17, 30)	0.45
AST (SGOT)	22 (6, 25)	22 (16, 29)	0.14	21 (17, 25)	19 (16, 25)	0.3
AST/ALT ratio	0.89 (0.25, 1.06)	0.80 (0.58, 0.96)	0.52	0.95 (0.71, 1.13)	0.89 (0.68, 1.05)	0.034
Gammaglutaryl transferase (GGT)	28 (18, 37)	28 (20, 44)	0.64	24 (17, 36)	26 (18, 38)	0.13
Fatty liver index	71 (41, 89)	84 (71, 94)	0.027	58 (35, 77)	79 (60, 93)	<0.001
Alcohol consumption (Y/N)	24 (47%)	33 (59%)	0.22	106 (58%)	119 (62%)	0.46
Smoke (Y/N)	25 (49%)	32 (57%)	0.4	82 (45%)	94 (49%)	0.45

The Mann–Whitney–Wilcoxon test was employed to assess differences between the first (Q1) and fourth (Q4) quartiles. For the analysis of qualitative variables, Pearson's Chi-square (χ^2) test was utilized. Y denotes Yes and N denotes No.

elements that target proteins with different lifespan in the bloodstream; thus albumin, the most abundant plasma protein, has a half-life of 20 days.[38] Therefore, AOPP could be considered as an analog of HbA1c, in the sense that they reflect systemic exposure over a long period.

The cross-sectional nature of our analysis limits our ability to establish causality between elevated biomarker levels and the development of renal injury among people with prediabetes. Additionally, the study mainly focused on European populations, which may hamper the extrapolation

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of our findings to other ethnicities and geographical locations.

Although further studies are needed to identify novel biomarkers in people with prediabetes, this area of research holds a promising avenue for early renal damage detection and therapeutic interventions. By unveiling reliable and valuable markers of early renal damage, clinicians could potentially identify individuals at risk of developing early kidney injury, allowing for timely and targeted interventions that could delay or prevent the onset of T2D and its renal complications.

Conclusions

Biomarkers of oxidative stress and inflammatory present remarkable association with the complex metabolic abnormalities among people with prediabetes, and they may be early indicators of renal dysfunction. Although further studies are needed, both AOPP and CRP are reliable biomarkers of potential interest in the evaluation of the health status of people with prediabetes.

Limitations: The lack of CKD-EPI formula implementation (as the current standard for eGFR measurement) in all centers during ePREDICE's baseline data collection limits our ability to compare it with MDRD4; however, given the high correlation (R = 0.88) where both formulas were available, we consider that the analysis performed remains valid.

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Conflict of interest

The authors declare no conflicts of interest.

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