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Original article

Serum retinol-binding protein 4 is associated with insulin resistance in patients with early and untreated rheumatoid arthritis

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ABSTRACT

Objective: Retinol-binding protein 4 (RBP4), systemic inflammation and insulin resistance (IR) are linked, yet the determinants of RBP4 and its impact on IR in rheumatoid arthritis (RA) are incompletely understood. The aim of this study was to explore the prevalence of IR in RA and investigate whether the serum levels of RBP4 were associated with IR in patients with RA.

Methods: In this study, 403 individuals with newly diagnosed and untreated RA were consecutively recruited. We calculated the Disease Activity Score assessed using 28-joint counts for swelling and tenderness (DAS28). Levels of serum RBP4, interleukin-6 (IL-6) and tumor necrosis factor (TNF) α were tested. IR was defined as Homeostasis model assessment for insulin resistance (HOMA-IR) index greater than or equal 2.40.

Results: In those 403 patients, 68 (16.9%) were male and the median age was 43 years (IQR: 36–52). There was an evidently positive correlation between increased serum levels of RBP4 and increasing severity of RA (DAS28) ($r = 0.403$, $P < 0.001$). Furthermore, a modest positive correlation between levels of serum RBP4 and HOMA-IR score ($r = 0.251$; $P < 0.0001$) was found. Eighty-five patients (21.1%) in patients with RA were defined as IR (HOMA-IR ≥ 2.40), which was significantly higher than in normal cases (4.7%). In the patients with IR, serum levels of RBP4 were higher when compared with those in patients free-IR $P < 0.001$. The IR distribution across the quartiles of RBP4 ranged between 5.0% (first quartile) to 39.0% (fourth quartile), P for trend < 0.001. For each 1 unit increase of RBP4, the unadjusted and adjusted risk of IR increased by 8% (OR: 1.08; 95% CI: 1.05–1.11, $P < 0.001$) and 5% (1.05; 1.02–1.09, $P = 0.001$), respectively. When RBP4 was added to the model containing established significant risk factors, AUROC (standard error) was increased from 0.768 (0.025) to 0.807(0.021). A significant difference in the AUC between the established risk factors alone and the addition of RBP4 was observed (difference, 0.039[0.004]; $P = 0.02$).

Conclusion: Elevated serum levels of RBP4 were associated with increased risk of IR and might be useful in identifying RA at risk for IR and/or impaired glucose tolerance for early prevention strategies, especially in obese and women patients

1. Introduction

Rheumatoid arthritis (RA) is a systemic, autoimmune disorder that primarily manifests as chronic synovial inflammation of multiple joints [1]. A population-based epidemiological investigation of the prevalence of RA in Beijing, china was 0.28% (95% CI: 0.19%, 0.41%) [2]. RA patients have an increased risk of cardiovascular (CV) events [3]. Systemic inflammation, insulin resistance (IR), and endothelial dysfunction have been implicated in the development of CV events in RA [4]. Increased prevalence of IR has been observed in patients with RA [5]. The modifiable factors of abdominal obesity, antihypertensive therapy, disease activity, and use of glucocorticoids appear to affect glucose metabolism in RA [6]. Festa et al. [7] showed that chronic subclinical inflammation is part of insulin resistance syndrome (IRS). Furthermore, the role of proinflammatory cytokines (including tumor necrosis factor α [TNFα] and interleukin-6 [IL-6]) in IR [8] and RA [9] had been suggested. In addition to pro-inflammatory cytokines, some proteins (adipokines secreted by adipose tissue) are associated with chronic inflammation by modulating pro-inflammatory cytokine levels [1]. Adiponectin (another adipokine) plays an important role in maintaining insulin sensitivity through its involvement in metabolic

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and anti-inflammatory regulation [10]. Serum levels of retinol-binding protein 4 (RBP4), a protein secreted by adipocytes, were increased in impaired glucose tolerance and T2DM [11]. Enhanced levels of serum RBP4 appeared to be the signal for the development of systemic IR both in experimental animals and in humans [12]. Experiments in mice also suggested that elevated RBP4 levels cause IR [13]. A previous study in human also raised the possibility that the serum RBP4 level might contribute to systemic IR [14]. RBP4, systemic inflammation and IR are linked, yet the determinants of RBP4 and its impact on IR in RA are incompletely understood. Furthermore, only few study investigated the relationship between RBP4 and impaired glucose handling and insulin sensitivity in small cohorts of untreated RA patients [15,16]. Therefore, the aim of this study was to explore the prevalence of IR in RA and investigate whether the serum levels of RBP4 were associated with IR in 403 patients with newly diagnosed and untreated RA.

2. Methods

2.1. Study population

In this study, individuals with newly diagnosed and untreated RA (duration of symptoms < 12 months at entry) aged from 30 to 75 years were consecutively recruited from January 2014 to December 2017 at the Clinical Rheumatology Center of Zhengzhou Central Hospital Affiliated to Zhengzhou University (Zhengzhou, Henan, China). To verify the diagnosis, the ACR/EULAR 2010 criteria [17] for RA were used. None of the RA patients had previously received disease-modifying antirheumatic drug (DMARD) therapy or oral glucocorticoids. The exclusion criteria were as following: (1) a history or presence of malignant disease; (2) known liver or kidney abnormalities, major complicating diseases such as amyloidosis or heart or lung disease; (3) metabolic syndrome, diabetes mellitus and impaired glucose metabolism; (4) drugs which may influence lipid or glucose metabolism; (5) hypertension, hyperuricemia and cardio-cerebrovascular disease; (6) pregnant or breastfeeding, serious psychiatric disorders. Patients with hypothyroid were also excluded since a study identified an independent association between hypothyroidism and markers of insulin sensitivity in RA; (7) subjects with incomplete data or lost written informed consent. At the same time, 128 apparently healthy, non-smoker volunteers, proportionally matched for age, sex and body mass index (BMI) to the patient group were enrolled in the study as controls. The exclusion criteria were the same as for the RA patients. Written informed consent was obtained after having provided verbal and written information to participants or nearest relatives when relevant. Ethics approval was approved by The Ethics Committee for Medical Research at Zhengzhou Central Hospital Affiliated to Zhengzhou University.

2.2. Clinical assessment

Demographic characteristics (age, sex and BMI), smoking history, waist circumference, erosive osteoarthritis, duration of disease and family history of T2DM were assessed based on self-reported information. Disease activity variables comprised the C-reactive protein (CRP) level, the erythrocyte sedimentation rate (ESR), the swollen joint count (in 28 joints), the tender joint count (in 28 joints), and the patient's assessment of disease activity [measured on a 0–100 mm visual analog scale (VAS); "no pain" (left end 0 mm) and "excruciating pain" (right end 100 mm)] [18]. In addition, we calculated the Disease Activity Score assessed using 28-joint counts for swelling and tenderness (DAS28; 0–2.6 low disease activity; 2.6–5.1 moderate disease activity; > 5.1 high disease activity; www.das-score.nl) [6]. Furthermore, the RA disease activity score also assessed by DAS28-ESR and DAS28-CRP [19]. Physical function using the anglicized version of the Health Assessment Questionnaire (HAQ) [20].

2.3. Laboratory tests

Morning fasting blood samples were collected in 5 mL BD Vacutainer® Rapid Serum Tube (New Jersey, USA) from each participant. Those samples were quickly centrifuged to separate the serum from the cells and were then immediately frozen at −80 °C until analysis. All samples and indicators examination were detected and analyzed in duplicates. Those blood tested were blinded to the clinical outcome. Levels of RBP4 in the serum were batch analyzed using a commercially available ELISA assay (R & D Systems, Minneapolis, MN). The lower detection limit was 2.0 ng/mL, and the detection range was 2.0–100 ng/mL. Inter-assay and intra-assay coefficients of variation were 6.0%–9.0% and 5.0–7.5%, respectively. Due to the high levels of RBP4(μg/mL in the serum sample, 1:100 dilution was used in this study. Immunometric enzyme immunoassays were used to measure serum levels of interleukin-6 (IL-6) (high sensitivity Quantikine, detection limit 0.2 pg/mL; R&D Systems, Minneapolis, MN), and tumor necrosis factor (TNF) (high sensitivity Quantikine, detection limit 0.2 pg/mL; R& D Systems). Routine serum biomarkers, for instance, triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), CRP, creatinine, rheumatoid factor(RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, fasting serum glucose (FSG) and fasting serum insulin (FSI) were tested using standard detection methods. In addition, undetectable levels were defined as equal to the lower limit in the experiment. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated with the formula as follows: HOMA-IR = [fasting serum insulin (μIU/mL) × fasting serum glucose (mmol/L) 22.5]. IR was defined as HOMA-IR index greater than or equal 2.40 [16]. Estimated glomerular filtration rate (eGFR) was calculated using modification of diet in renal disease study equation. Atherogenic Index of Plasma (AIP) was calculated using log(TG/HDL-C).

2.4. Statistical analysis

The results are expressed as *n* (%) for categorical variables and as median [interquartile range (IQR)] for the continuous variables. Proportions were compared using the Chi^2 test, and the Mann–Whitney test was used to compare continuous variables between groups. Correlations among continuous variables were assessed by the spearman rank-correlation coefficient.

The influence of RBP4 on IR was performed by univariate and multivariate binary logistic regression analysis. We used crude models and multivariate models adjusted for all significant markers and reported odds ratios (ORs) and 95% confidence intervals (CI). For multivariate analysis, we chosen confounders including age, sex, BMI, waist circumference, erosive osteoarthritis, duration of disease, current smoker, family history of T2DM, disease activity, anti-CCP, No. of swollen joints, No. of tender joints, DAS28, physical activity, and blood levels of CRP, IL-6, TNFα, RF, total cholesterol, triglyceride, HDL, LDL and eGFR. For a more detailed exploration of the RBP4 and IR, we also used multivariate analysis models to estimate adjusted OR and 95% CIs of IR for RBP4 quartiles (with lowest quartile of RBP4 as reference).

Second, receiver operating characteristic curves (ROC) was used to test the overall diagnostic accuracy of the RBP4 and other biomarkers and results were reported as area under the curve (AUC). Integrated discrimination improvement (IDI) and net reclassification improvement (NRI) indices were calculated to determine the clinical utility of the addition of RBP4 to established risk factors and the ability of RBP4 to improve IR association [21].

Third, we also conducted analyses separately among cases who experienced women and obesity. Obesity has been more precisely defined by the National Institutes of Health (the NIH) as a BMI of 30 and above [22]. All statistical analysis was performed with SPSS for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA) and the ROCR package (version 1.0-2). Statistical significance was defined as $P<0.05$.

2.5. Role of the funding source

The funding organizations had no role in the design and concept of the study; the collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

3. Results

3.1. Patient demographics

In our study, there was a sum of 403 patients with RA that had serum samples were included. In those patients, 68 (16.9%) were male and the median age was 43 years (IQR: 36–52). Out of the 403 patients, 77(19.1%) were with high disease activity (DAS28 > 5.1). The baseline characteristics between RA patients and control cases were shown in Table 1.

3.2. The correlation between serum levels of RBP4 and other factors

As shown in Appendix A, Fig. S1a [See the supplementary material associated with this article online], when compared to healthy controls, serum levels of RBP4 was significantly higher in patients with RA [13.8(IQR: 10.5–18.3) μg/mL vs. 29.6(IQR: 20.3–40.7) μg/mL; $P<0.001$]. There was an evidently positive correlation between increased serum levels of RBP4 and increasing severity of RA (DAS28) ($r=0.403$, $P<0.001$). Interestingly, we found that the association between RBP4 and DAS28 CRP ($r=0.465$, $P<0.001$) was stronger than RBP4 and DAS28 ESR ($r=0.413$, $P<0.001$). Serum levels of RBP4 in high disease activity (34.7; IQR: 24.5–45.9 μg/mL) were significantly higher when compared with levels in low disease activity and moderate disease activity (26.1; 18.2–35.1 μg/mL and 31.3; 21.3–40.4 μg/mL). In addition, there were significantly positive correlations between serum levels of RBP4 and BMI ($r=0.128$, $P=0.01$), CRP ($r=0.133$, $P=0.008$), IL-6 ($r=0.172$, $P=0.001$), TNFα ($r=0.178$, $P<0.001$). Furthermore, a modest positive correlation between levels of serum RBP4 and HOMA-IR score ($r=0.251$; $P<0.001$) [Appendix A, Fig. S2] was found. Interestingly, this positive trend still exists even adjusted for possible confounders in multivariate ordered logistic regression ($P=0.015$). The positive correlation was more pronounced in FSI than in FSG ($r=0.305$ vs. $r=0.203$). On the other hand, there was no influence of age, sex, waist circumference, duration of disease, current smoker, family history of T2DM, No. of swollen joints, No. of tender joints, physical activity, blood levels of total Cholesterol, triglyceride, HDL, LDL and eGFR on RBP4 in RA patients, with no statistical difference (all $P>0.05$). However, there was significantly positive correlation between serum levels of RBP4 and AIP ($r=0.136$, $P=0.02$).

3.3. The prevalence of IR in RA

Eighty-five patients (21.1%; 95% CI: 17.1%–25.1%) in patients with RA were defined as IR (HOMA-IR ≥ 2.40), which was significantly higher than in normal cases (4.7%). Interestingly, when compared with controls, incidence of IR in patients with RA was increased by 444% (OR: 5.44; 95% CI: 2.31–12.77).

Table 1
Demographic and laboratorial characteristics of the included subjects[a].

	RA	Control cases
n	403	128
Age, years	43(36–52)	43(35–52)
Sex, male	68(16.9)	21(16.4)
BMI, kg/m^2	27.7(25.2–29.3)	27.5(25.3–28.7)
< 25	88(21.8)	26(20.3)
25–30	222(55.1)	75(58.6)
> 30	93(23.1)	27(21.1)
Waist circumference, cm	89(85–94)	88(85–93)
Disease duration, months	6.5(4.0–8.0)	–
Current smoker	84(20.8)	15(11.7)*
Family history of T2DM	54(13.4)	12(9.4)
RF positive	311(77.2)	–
Anti-CCP positive	268(66.5)	–
Erosive Osteoarthritis	48(11.9)	
No. of swollen joints (28 assessed)	2(1–4)	–
No. of tender joints (28 assessed)	3(2–4)	–
Disease activity (by VAS), mm	41(35–48)	–
DAS28[b]	3.4(2.5–4.8)	–
Low disease activity	201(49.9)	–
Moderate disease activity	125(31.0)	–
High disease activity	77(19.1)	–
HAQ	1.7(1.1–2.4)	–
ESR, mm/h	38(27–55)	9(5–13)***
Serum CRP, mg/L	8.2(4.2–13.2)	3.8(2.8–6.6)***
Serum IL-6, pg/mL	20.8(14.5–30.8)	4.8(1.9–7.9)***
Serum TNFα, pg/mL	11.6(8.5–15.2)	2.5(1.1–3.7)***
RBP4, μg/mL	29.6(20.3–40.7)	13.8(10.5–18.3)***
Serum TG, mmol/L	1.1(1.3–1.8)	0.9(0.7–1.3)**
Serum TC, mmol/L	4.2(3.4–5.1)	3.9(3.3–4.8)*
Serum HDL-C, mmol/L	1.5(1.2–1.8)	1.4(1.2–1.7)
Serum LDL-C, mmol/L	2.1(1.3–2.7)	1.8(1.1–2.2)*
eGFR, mL/min/1.73 m^2	81(75–93)	88(81–97)**
AIP[c]	0.39(0.31–0.48)	0.32(0.27–0.41)**
FSG, mmol/L	5.13(4.83–5.77)	4.98(4.61–5.42)**
FSI, μIU/mL	5.77(4.50–8.11)	1.92(1.33–3.26)***
HOMA-IR	1.31(0.95–2.05)	0.31(0.24–0.68)***
HOMA-IR ≥ 2.40	85(21.1)	6(4.7)***

BMI: body mass index; FSG: fasting serum glucose; FSI: fasting serum insulin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; RBP4: retinol-binding protein 4; HOMA-IR: homeostasis model assessment of insulin resistance; RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate; TNF-alpha: tumor necrosis factor alpha; IL-6: interleukin-6; DAS28: Disease Activity Score in 28 Joints; VAS: Visual Analogic Scale; RF: rheumatoid factor; HAQ: Health Assessment Questionnaire; TG: triglyceride; eGFR: estimated glomerular filtration rate; T2DM: type 2 diabetes mellitus; Anti-CCP: anti-cyclic citrullinated peptide; AIP: Atherogenic Index of Plasma.

[a] Results are summarized in median (inter quartile) or *n* (%); *P* was tested by Mann–Whitney tests or Fisher's exact test.

[b] DAS28, 0–2.6 low disease activity; 2.6–5.1 moderate disease activity; > 5.1 high disease activity.

[c] AIP was calculated using log (TG/HDL-C).

* $P<0.05$.

** $P<0.01$.

*** $P<0.001$.

3.4. The correlation between serum levels of RBP4 and IR

In the 85 patients with IR, serum levels of RBP4 were higher when compared with those in patients free-IR 39.0(IQR: 30.1–46.4) μg/mL vs. 26.4(IQR: 18.3–37.7) μg/mL; $P<0.001$) [Appendix A, Fig. S1b]. The probability of IR increased gradually with increasing RBP4 quartiles (Fig. 1). The IR distribution across the quartiles of RBP4 ranged between 5.0% (first quartile) to 39.0% (fourth quartile).

In univariate and multivariate logistic regression analysis, we calculated the ORs of RBP4 as compared with IL-6, CRP, TNFα and other risk factors to diagnose IR (Table 2). For each 1unit increase of RBP4, the unadjusted and adjusted risk of IR increased by 8% (OR: 1.08; 95% CI: 1.05–1.11, $P<0.001$) and 5% (1.05; 1.02–1.09, $P=0.001$), respectively. For a more detailed exploration of the relationship between RBP4 and IR, multivariate analysis models to estimate adjusted OR and 95% CIs of IR for RBP4 quartiles (with

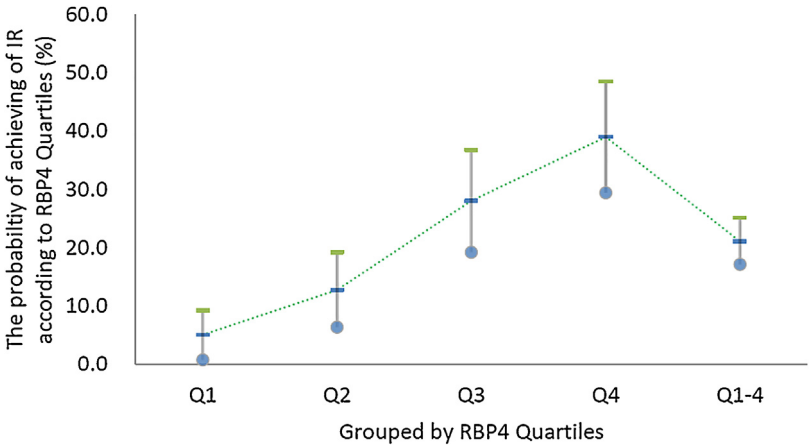

Fig. 1. The incidence for IR (95% CI) according to the baseline RBP4 quartiles. RBP4: retinol-binding protein 4; IR: insulin resistance.

Table 2
Demographic and laboratorial characteristics of the RA with and without IR[a].

	All	IR	No-IR
n	403	85	318
Age, years	43 (36–52)	45 (39–56)	43 (36–52)
Sex, male	68 (16.9)	15 (17.6)	53 (16.7)
BMI, kg/m^2	27.7 (25.2–29.3)	28.5 (25.9–30.2)	27.3 (25.0–28.8)*
Waist circumference, cm	89 (85–94)	91 (88–96)	88 (85–93)*
Disease duration, months	6.5 (4.0–8.0)	7.5 (5.0–9.0)	6.5 (3.5–7.5)
Anti-CCP positive	268 (66.5)	60 (70.6)	208 (65.4)
Erosive osteoarthritis	48 (11.9)	15 (17.6)	33 (10.4)
Current smoker	84 (20.8)	33 (38.8)	51 (16.0)***
Family history of T2DM	54 (13.4)	12 (14.1)	42 (13.2)
RF positive	311 (77.2)	67 (78.8)	244 (76.7)
No. of swollen joints (28 assessed)	2 (1–4)	3 (2–5)	2 (1–3)**
No. of tender joints (28 assessed)	3 (2–4)	4 (3–5)	3 (1–4)**
Disease activity (by VAS), mm	41 (35–48)	48 (41–55)	40 (34–48)**
DAS28	3.4 (2.5–4.8)	4.0 (2.9–5.5)	3.2 (2.4–4.6)**
HAQ	1.7 (1.1–2.4)	1.3 (0.8–1.6)	1.8 (1.3–2.5)***
ESR, mm/h	38 (27–55)	48 (33–67)	35 (24–49)***
Serum CRP, mg/L	8.2 (4.2–13.2)	10.4 (6.6–15.6)	7.8 (3.6–12.6)**
Serum IL-6, pg/mL	20.8 (14.5–30.8)	24.8 (16.8–34.9)	18.8 (12.1–27.7)**
Serum TNFα, pg/mL	11.6 (8.5–15.2)	13.8 (9.9–18.4)	10.9 (8.2–14.6)***
RBP4, μg/mL	29.6 (20.3–40.7)	39.0 (30.1–46.4)	26.4 (18.3–37.7)***
Serum TG, mmol/L	1.1 (1.3–1.8)	1.3 (1.5–2.0)	1.0 (1.2–1.8)
Serum TC, mmol/L	4.2 (3.4–5.1)	4.4 (3.4–5.3)	4.1 (3.3–5.0)
Serum HDL-C, mmol/L	1.5 (1.2–1.8)	1.2 (0.9–1.4)	1.6 (1.3–1.8)
Serum LDL-C, mmol/L	2.1 (1.3–2.7)	2.4 (1.5–3.0)	2.0 (1.1–2.6)
AIP[b]	0.39 (0.31–0.48)	0.55 (0.43–0.75)	0.33 (0.27–0.41)***
eGFR, mL/min/1.73 m^2	81 (75–93)	75 (71–84)	82 (77–95)*
FSG, mmol/L	5.13 (4.83–5.77)	5.63 (5.50–6.04)	4.98 (4.71–5.33)***
FSI, μIU/mL	5.77 (4.50–8.11)	11.7 (9.5–14.1)	5.44 (4.33–6.38)***
HOMA-IR	1.31 (0.95–2.05)	2.94 (2.49–3.52)	1.22 (0.91–1.51)***

BMI: body mass index; FSG: fasting serum glucose; FSI: fasting serum insulin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; RBP4: retinol-binding protein 4; HOMA-IR: homeostasis model assessment of insulin resistance; RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate; TNF-alpha: tumor necrosis factor alpha; IL-6: interleukin-6; DAS28: Disease Activity Score in 28 Joints; VAS: Visual Analogic Scale; RF: rheumatoid factor; HAQ: Health Assessment Questionnaire; TG: triglyceride; eGFR: estimated glomerular filtration rate; T2DM: type 2 diabetes mellitus; IR: insulin resistance; anti-CCP: anti-cyclic citrullinated peptide; AIP: Atherogenic Index of Plasma.

[a] Results are summarized in median (inter quartile) or *n* (%); *P* was tested by Mann–Whitney tests or Fisher's exact test; IR was defined as HOMA-IR ≥ 2.40.

[b] AIP was calculated using log(TG/HDL-C).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$

Q1 as reference) was also presented. Comparing the Q2, Q3 and Q4 quartiles against the Q1 of the RBP4 (Table 3), RBP4 in Q3 and Q4 were associated with IR, and increased risk of IR by 209% (OR: 3.09; 95% CI: 1.89–6.78) and 602% (7.02; 3.94–12.33).

Using ROC curves, RBP4 level at 26.5 μg/mL diagnosed the development of IR with the highest sensitivity and specificity [88.5% and 50.7%, respectively; area under the curve (AUC) = 0.733, 95% CI: 0.680–0.787; $P < 0.001$]. RBP4 levels had a higher diagnostic accuracy as compared to CRP [AUC: 0.610; 95% CI: 0.544–0.675; $P < 0.001$], TNFα [AUC: 0.650; 0.586–0.715; $P < 0.001$] and IL-6 [AUC 0.618; 0.558–0.682; $P < 0.001$]. The combined model (RBP4/CRP/IL-6/TNFα) improved those biomarkers alone (AUC of the combined model, 0.786; 95% CI: 0.736–0.835), Fig. 2. This improvement was stable in an internal 5-fold cross validation that resulted in an average AUC (standard error) of 0.73 (0.037) for the RBP4 and 0.83 (0.027) for the combined model, corresponding to a difference of

Table 3
Logistic regression model for serum levels of RBP4 according to quartiles of the distributions using IR as the dependent variables.

Dependent variables[b]	IR/RA	OR unadjusted (95% CI)	P[c]	OR adjusted (95% CI)[a]	P[c]
Quartile 1	5/96	Reference	–	Reference	–
Quartile 2	13/89	2.80 (0.96–8.18)	0.05	1.75(0.83–3.63)	0.15
Quartile 3	28/72	7.47 (2.75–20.28)	< 0.001	3.09 (1.89–6.78)	0.002
Quartile 4	39/61	12.76 (4.59–32.87)	< 0.001	7.02 (3.94–12.33)	< 0.001

OR: odds ratio; CI: confidence interval; BMI: body mass index; FSG: fasting serum glucose; FSI: fasting serum insulin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; RBP4:retinol-binding protein 4; HOMA-IR: homeostasis model assessment of insulin resistance; ESR: erythrocyte sedimentation rate; TNF-alpha: tumor necrosis factor alpha; IL-6: interleukin-6; DAS28: Disease Activity Score in 28 Joints; VAS: Visual Analogic Scale; RF: rheumatoid factor; HAQ: Health Assessment Questionnaire; TG: triglyceride; eGFR: estimated glomerular filtration rate; T2DM: type 2 diabetes mellitus; RA: rheumatoid arthritis; IR: insulin resistance; anti-CCP: anti-cyclic citrullinated peptide.

[a] Adjust for age, sex, BMI, waist circumference, erosive osteoarthritis, duration of disease, anti-CCP, current smoker, family history of T2DM, disease activity, No. of swollen joints, No. of tender joints, DAS28, physical activity, and blood levels of CRP, IL-6, TNFα, RF, total cholesterol, triglyceride, HDL, LDL and eGFR. IR was defined as HOMA-IR ≥ 2.40.

[b] Serum RBP4 levels in Quartile 1 (< 20.3 μg/mL), Quartile 2 (20.3–29.6 μg/mL), Quartile 3 (29.7–40.7 μg/mL), and Quartile 4 (> 40.7 μg/mL).

[c] *P* tread < 0.001.

Table 4
Serum levels of RBP4 at admission diagnosis of IR with AUROC.

IR	AUROC					NRI (*P*) IDI (*P*)
	RBP4	Risk factors[a]	Risk factors with RBP4	Incremental area (*P*)[b]		
At admission	0.733	0.768	0.807	0.039 (0.02)	0.106 (0.002)	0.041 (0.01)

BMI: body mass index; FSG: fasting serum glucose; FSI: fasting serum insulin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; RBP4: retinol-binding protein 4; HOMA-IR: homeostasis model assessment of insulin resistance; ESR: erythrocyte sedimentation rate; TNF-alpha: tumor necrosis factor alpha; IL-6: interleukin-6; DAS28: Disease Activity Score in 28 Joints; VAS: visual analogic scale; RF: rheumatoid factor; HAQ: Health Assessment Questionnaire; TG: triglyceride; eGFR: estimated glomerular filtration rate; T2DM: type 2 diabetes mellitus; IR: insulin resistance.

[a] Established risk factors including: age, sex, BMI, waist circumference, duration of disease, current smoker, family history of T2DM, disease activity, No. of swollen joints, No. of tender joints, DAS28, physical activity, and blood levels of CRP, IL-6, TNFα, RF, total cholesterol, triglyceride, HDL, LDL and eGFR; IR was defined as HOMA-IR ≥ 2.40.

[b] Comparison of AUROCs: established risk factors without RBP4 levels vs. established risk factors with RBP4 levels.

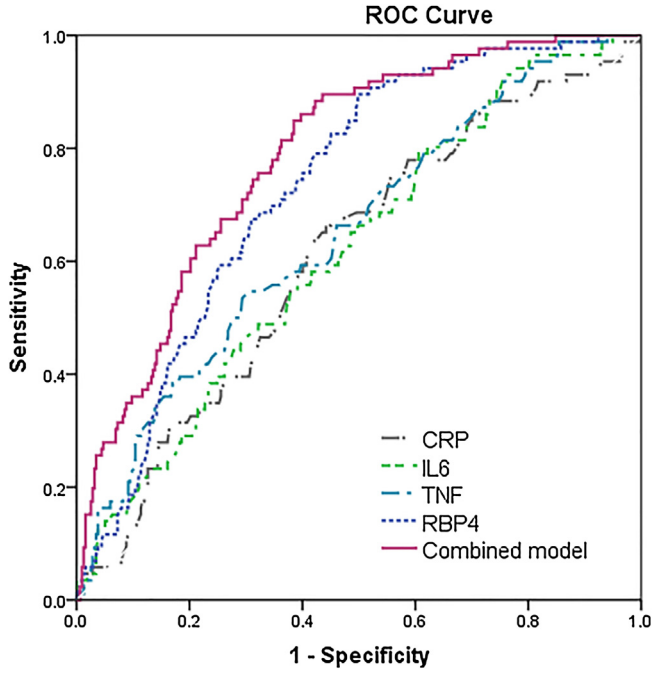

Fig. 2. Receiver operator characteristic curve demonstrating sensitivity as a function of 1-specificity for diagnosing the IR based on the model incorporating all 4 biomarkers (RBP4/CRP/IL-6/TNF-alpha) and the relative contribution of each biomarker alone (initial cohort). RBP4: retinol-binding protein 4; IR: insulin resistance; CRP: C-reactive protein; TNF-alpha: tumor necrosis factor alpha; IL-6: interleukin-6.

0.10(0.010). When RBP4 was added to the model containing established significant risk factors (Table 4), AUROC (standard error) was increased from 0.768 (0.025) to 0.807(0.021). As shown in the Table 4, the NRI statistic (*P* = 0.002) and the IDI statistic (*P* = 0.01) found that the RBP4 level provided obviously increased discrimination between RA with and without IR.

3.5. Subgroup analysis

Furthermore, we also conducted analyses separately among cases who experienced women and obesity. In the multivariate regression analysis, the date suggested that for each 1 μg/mL increase of serum level of RBP4, the association was stronger among women (OR = 1.06, 95% CI: 1.02–1.11; *P* < 0.001) versus man (OR = 1.03, 95% CI: 1.00–1.08; *P* = 0.008). In this study, 93 patients (23.1%) were defined as obesity, and 30 of those patients classified as IR. The incidence of IR in RA patients with obesity was nearly two times when compared with RA patients without obesity (32.3% vs. 17.7%). Interestingly, the diagnostic value of RBP4 to diagnose IR with obesity (OR = 1.08, 95% CI: 1.04–1.14) was stronger than to diagnose IR (1.05; 1.02–1.09).

4. Discussion

In this study, we measure RBP4 serum levels in newly diagnosed and untreated RA and further assess its association with IR. Our main findings were as the following: (1) serum levels of RBP4 were higher in RA than in controls; (2) serum levels of RBP4 were positively associated with severity of RA (defined by DAS28); (3) elevated serum levels of RBP4 were associated with increased risk of IR and might be useful in identifying RA at risk for IR and/or T2DM for early prevention strategies; (4) this correlation was more pronounced in obese and women patients. Furthermore, most of the correlations presented between RBP4 and patient characteristics (DAS28 and HOMA-IR) were modest, and the association had a high clinical pertinence in obese and women patients.

Consistent with our findings, a previous study showed that high serum RBP4 might have clinical implications for lipid metabolism and insulin action in adolescents [23], while another indicated a relationship between RBP4, insulin sensitivity, and percent trunk fat in individuals who may not have features of IR [24]. Furthermore, Graham et al. [14] reported that RBP4 was an adipocyte-secreted molecule that was elevated in the serum before

the development of frank diabetes and appears to identify IR. However, another study finished by Ferraz-Amaro et al. [25] did not find correlation between RBP4 and the presence of IR and β-cell function in patients with RA. In addition, in the above study [25], the data described that RBP4 levels were significantly lower in the RA patients than in controls [13.99 (9.78–19.88) vs. 21.50 (10.28–32.59) μg/mL, $P < 0.01$]. Differences in methodologies used to assess IR and included patients make comparison the previous studies with the present study difficult.

Previous studies had suggested that patients with RA are likely to have IR and have increased risk of cardiovascular disease (CVD) [1]. High-grade systemic inflammation was implicated in the development of IR in RA patients [5]. It has recently been suggested that inflammation produced by RBP4 induces IR and CVD [26]. Similarly, in this study we found that elevated serum levels of RBP4 were associated with increased risk of IR in patients with RA.

The increased IR seen in RA is closely linked to the systemic inflammation induced by certain pro-inflammatory cytokines such as TNFα and IL-6 [16]. TNFα has also been implicated in the development of IR, and a previous study in a model of obese mice found that knockout mice lacking expression of TNFα or TNFα receptors were more sensitive than controls to insulin [27]. Similar to TNFα, IL-6 and the IL-6 receptor also had been suggested play a role in RA [28], IR and the development of type 2 DM [29]. Similarly, in this study, we also found that elevated serum levels of TNFα and IL-6 were associated with increased risk of IR in patients with RA.

In this study, we found that 21.1% of the patients with RA were defined as IR. Higher figures were reported by Douglas et al. [30] (37%) and La Montagna et al. [31] (88.9%). The contradiction in prevalence of IR in RA patients in different studies might likely to depend on differences in study design, methods used to assess IR, genetic and racial background of the studied subjects. A previous study showed that HOMA-IR was found to be higher in Blacks and Hispanics compared to whites and Chinese [32]. More importantly, the patients in this study was newly diagnosed and untreated RA, while previous studies had been conducted in RA patients with longstanding illness. In fact, the development of diseases and changes in lifestyle can exacerbate the occurrence of IR. Interestingly, disease activity seemed moderate in our population of recent untreated RA (mean DAS 3.4 with only 19.1% of patients with high disease activity), whereas 55.5% of patients had high activity in ESPOIR cohort (mean DAS 5.11) [33]. However, the HAQ in our study (median: 1.7; IQR: 1.1–2.4) was significantly higher than in ESPOIR cohort (mean: 0.98; SD: 0.68) [33]. These differences could be caused by the difference in racial and lifestyles. Further studies should be carried out to explain differences.

In this study, the BMI seems to be quite elevated for this population (median BMI: 27.7) and 93 patients (23.1%) were defined as obesity. In addition, there was significantly positive correlations between serum levels of RBP4 and BMI ($r = 0.128$, $P = 0.01$). It was suggested that visceral obesity was an important component of the insulin resistance syndrome [34]. Thus, the association between RBP4 and IR might be mediated by obesity and adipose tissue. However, this association still existed even adjusted for BMI in multivariate logistic regression, other mechanisms that need to be considered. Two important pathophysiological mechanisms of IR involved in patients with RA were inflammation and oxidative stress [35]. RBP4 might play role in the IR through these two pathways. First, RBP4 gene expression in humans was associated with inflammatory markers [36]. In this study, we also found that RBP4 were associated with IL-6, CRP and TNFα. However, after adjusted for those biomarkers, RBP4 were still associated with risk of IR. Second, a pervious study demonstrates increased levels of Hs-CRP, endothelial dysfunction, and the relation with IR in young and normal-weight women with polycystic ovary syndrome [37]. Recent molecular investigations had suggested that impaired insulin receptor signaling in insulin responsive tissues, oxidative stress, and endoplasmic reticulum (ER) stress play role in the IR [38]. RBP4 deteriorates endothelial mitochondrial function and promotes vascular oxidative stress by suppressing phosphatidyl inositol 3-kinase (PI3K)/Akt signaling [39]. Third, increased serum RBP4 levels in humans might contribute to impaired insulin-stimulated glucose uptake in muscle and elevated hepatic glucose production [40]. Last, regions near the RBP4 locus on human chromosome 10q have been linked to hyperinsulinemia or early onset of type 2 diabetes [41].

5. Strengths and limitations

Strengths of our study include the fact that the individuals with newly diagnosed and untreated RA were consecutively recruited. Most of the previous studies have been conducted in RA patients with longstanding illness, which might influence the metabolism, including IR [10,42]. Second, some factors (including diabetes mellitus, hypertension, hyperuricemia and cardio-cerebrovascular disease) that affect the serum levels of RBP4 have been eliminated. Third, we chose different strategies using the quartile of RBP4 and cut-off value. In addition, IDI and NRI indices were calculated.

Some limitations of our study should be considered. First, though we adjusted for a comprehensive panel of potential confounders, residual confounding and other unmeasured potential confounders cannot be ruled out. Retinol, transthyretin and sex hormones plays an important role in adipose tissue (RBP4) metabolism. In addition, the natriuretic peptide system including B-type natriuretic peptide (BNP) and the N-terminal fragment of its prohormone (NT-pro BNP) plays an important role in adipose tissue metabolism, which might influence the secretion of adiponectin and RBP4 [43]. However, we did not obtain that information. Second, the overwhelming majority of our sample was Chinese, limiting the generalizability to other ethnicities. Third, it has been argued that commercially available Elisa kits are unreliable for measuring circulating RBP4, as compared to Western blotting [44]. Fourth, there are well-recognized limitations of HOMA as an estimate of IR compared with the more accurate timed oral glucose tolerance test or hyperinsulinemic euglycemic clamp. Finally, our comparisons of associations between RBP4 and IR were cross-sectional and were thus subject to uncertainties in causality.

Our studies suggested that elevated serum levels of RBP4 were associated with increased risk of IR in newly diagnosed and untreated RA patients, independent of established conventional risk factors. The ability to assess the risk of IR (impaired glucose tolerance) before the onset of T2DM in patients with RA would provide a rational means for implementing preventive lifestyle interventions or pharmacologic treatment.

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Patient consent

Written informed consent was obtained after having provided verbal and written information to participants or nearest relatives when relevant.

Ethics approval

Ethics approval was approved by The Ethics Committee for Medical Research at Zhengzhou Central Hospital Affiliated to Zhengzhou University.

Statement

No additional unpublished data are available.

Disclosure of interest

The authors declare that they no competing interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jbspin.2018.07.002.

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