Original Article

# Role of Serum Retinol Binding protein 4(RBP4) and it's Various Correlations in Type 2 diabetes mellitus Population

**Jyoti Trivedi J[1], Juhi Aggarwal J [2], Jyoti Batra J[3], V.K. Singh[4], Rahul Arora[5]**

[1]PhD Scholar, Department of Biochemistry, Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad.

[2]Professor & HOD, Department of Biochemistry, Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad.

[3]Professor, Department of Biochemistry, Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad.

[4]Professor & HOD, Department of General Medicine, Teerthanker Mahaveer Hospital, Teerthanker Mahaveer University, Moradabad.

[5]Assistant Professor, Teerthanker College of Pharmacy, Teerthanker Mahaveer University, Moradabad.

Corresponding author- Dr. Jyoti Trivedi[1], [1]PhD Scholar (Medical Biochemistry), Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad.

**Email:** jyoti.trivedi01@gmail.com[1]

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## Abstract

Diabetes mellitus is gaining the status of a potential epidemic and is the most common endocrine disorder, although major driving factors behind this epidemic are poor nutrition and lifestyle transitions, growing evidence supports a role of recently proposed adipokine i.e. Retinol binding protein 4 in the pathogenesis of type 2 diabetes mellitus Objective: To estimate serum RBP4 concentrations in type 2 diabetes mellitus patients and to correlate it with diabetic parameters like FPG, HbA1c, and BMI Methodology: 113 participants aged 35-55 years, both males and females were included, who were diagnosed and confirmed by the estimation of FPG (≥126mg/dl) and HbA1c level (≥6.5%) as type 2 diabetes mellitus patients. An equal number of age and sex-matched pre-confirmed non-diabetic individuals from hospital staff were recruited as controls. Serum RBP4 (Human) concentration was measured by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technology using Bioassay Technology Laboratory, ELISA kit Result: The concentration of serum RBP4 in the blood plasma of type 2 diabetes mellitus patients(76.53±26.96ng/ml) was found to be significantly higher than the healthy controls(53.77±12.62ng/ml), (P<0.001). Positive correlations were found between serum RBP4 with FPG, HbA1c, and BMI in the diabetic groups (p<0.001) Conclusion: The concentration of serum RBP4 was found to be increased in Type 2 diabetes mellitus patients, and related to various clinical parameters of diabetes, suggesting a role in the pathogenesis of Type 2 diabetes mellitus.

**Keywords:** Insulin resistance, Retinol binding protein 4, Type 2 diabetes mellitus.

## INTRODUCTION

Diabetes mellitus has considered a potential epidemic and is the most common endocrine disorder.[1] Although major driving factors behind this epidemic are poor nutrition and lifestyle transitions, growing evidence suggests a role of adipokines and they may also be suggested as good markers of metabolic syndrome and unregulated production of these cytokines may change metabolic homeostasis, insulin sensitivity, immune response, and cardiovascular disease.[2]

Retinol binding protein 4 (RBP4) is a recently proposed adipokine, reported by contributing to insulin resistance and T2DM, belongs to the lipocalins family, and transports vitamin A (retinol) from the liver to peripheral tissues. The protein encoded gene is located on chromosome 10 (10q23.33) near regions encoding genes linked to increased levels of fasting glucose, such as gene TCF7L2 (10q25.3) which controls CREB and Fox01 genes.[3]

Experimental studies have suggested that RBP4 is upregulated in insulin-resistant mouse models and in subjects with insulin resistance or type 2 diabetes mellitus. Furthermore, genetic knockout of GLUT4 expression in adipose tissue of mice results in reciprocal changes in adipose RBP4 expression and circulating RBP4 levels.[3] Moreover, increasing serum RBP4 may induce

hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and found to impair insulin signaling in muscle.[4] Also, RBP4 has been known as a negative acute-phase inflammatory reactant. It has recently been suggested that inflammation produced by RBP4 may induce insulin resistance and cardiovascular risk.[5]

Fenretinide, a synthetic retinoid that increases urinary RBP4 excretion, reduces serum RBP4 levels, and found to improve insulin action in obese mice, thus may have a therapeutic potential role in T2DM.[6] However, the link between RBP4 and insulin resistance in humans is still under discussion. Hence, RBP4 seems to affect insulin resistance, therefore in view of the above statements, the present study was designed to measure the levels of RBP4 in type 2 diabetes mellitus patients and controls and correlate with various parameters and also find associating relationship with obesity.

## MATERIALS AND METHODS

### *Study Design*

This Hospital-based, cross-sectional type of observational study was conducted at Santosh Medical College & Hospital, Ghaziabad, in collaboration with Teerthanker Mahaveer medical college & Research Center, Moradabad, Uttar Pradesh, India from August 2021- February 2022.

### *Inclusion criteria*

113 participants aged 35-55 years, both males and females were included, who was diagnosed and confirmed by the estimation of FPG (≥126mg/dl) and HbA1c level (≥6.5%) as type 2 diabetes mellitus patients. An equal number of age and sex-matched pre-confirmed non-diabetic individuals from hospital staff were recruited as controls. The study was approved by the Institute Ethics Committee of the Santosh Deemed to be University, Ghaziabad {F. No.SU/2021/2131[4]}.

All participants were informed about the study protocol and written informed consent was obtained from all the participants under study.

### *Exclusion Criteria* [7,8]

Type 1 Diabetes mellitus, Chronic kidney disease, Chronic liver disease, Thyroid dysfunction, Patient on weight reduction therapy, hypoglycemic drugs, insulin therapy, and statins were excluded.

### *Sample Size Calculation*

$n = Z^2 \alpha/2 \times p\,(1-p) \times D / E^2$

$Z^2 \alpha/2$: Standard normal Variate = 1.96 at 5% type 1 error

P: prevalence rate [9] = 8.03

D: design effect= 1

E: margin of error = 5%

**Sample size (n)** = $(1.96)^2 \times 8.03(100-8.03) \times 1 / (5)^2$

= 113

### *Sample collection*

- After overnight fasting for 8-10 hours, about 5 ml of venous blood was drawn under aseptic conditions from the antecubital vein of all the type 2 diabetics and controls
- EDTA vial was used for glycosylated hemoglobin (HbA1c)
- Fluoride oxalate vial for fasting plasma glucose (FPG) estimation
- Plain vial was taken for serum RBP4, serum iron, serum ferritin, and UIBC
- Serum samples were stored at 2-6ºC and processed within one month period
- Repeated thaw cycles were avoided

### *Methodology*

Serum **RBP4** concentration was measured by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technology using Bioassay Technology Laboratory, ELISA kit.[10] **Fasting plasma glucose** (FPG) estimation was done by the Glucose Oxidase–Peroxide (GOD-POD) Method.[11] **HbA1c** estimation was done by the Particle enhanced immunoturbidimetric method.[12] BMI was calculated by dividing body weight (Kg) by the square of height (meters)

### *Statistical Analysis*

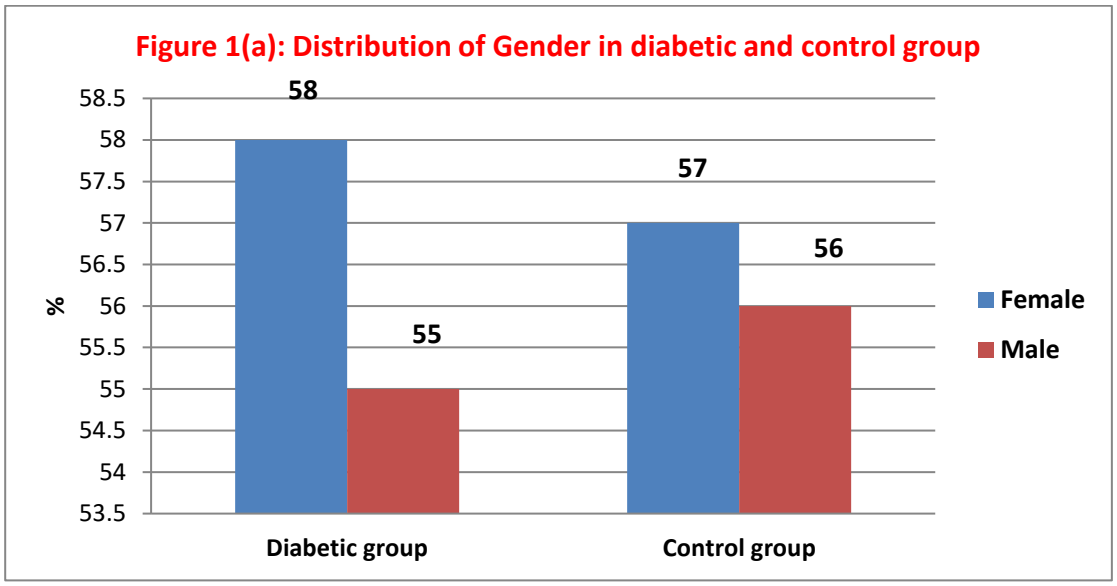
Statistical Package for the Social Sciences (SPSS) version 28.0 was used for the analysis of data. Qualitative data were represented as frequency and percentage whereas quantitative data were expressed as Mean±Standard Deviation (SD) for both case and control groups. **Independent Student's t-test** was used to compare the values of serum RBP4 and BMI between cases and control groups. Correlation between serum RBP4 and diabetic parameters and BMI in diabetic cases was done by **using Pearson's coefficient (r-value). ANOVA** was used to compare more than two groups. **Linear regression** was done to show the relationship between variables. The $p<0.05$ was considered to be statistically significant and the p-value <0.001 is highly significant.

## RESULTS

**Table 1**: Percentage and group-wise distribution of type 2 diabetes mellitus patients and controls

| **S.No.** | **Demographical variables** | **Category** | **Diabetic cases (n=113)** | **Controls (n=113)** |
|---|---|---|---|---|
| a | Gender | Female | 58(51.3%) | 57(50.4%) |
| | | Male | 55(48.7%) | 56(49.6%) |
| b | BMI | Normal weight(n=119) | 50(44.2%) | 69(61.1%) |
| | | Overweight(n=75) | 41(36.3%) | 34(30.1%) |
| | | Obesity (n=32) | 22(19.5%) | 10(8.8%) |

- An equal distribution of gender-matched individuals was taken into study
- The maximum percentage of participants were normal in weight in both cases and the control group as compared to overweight and obese individuals



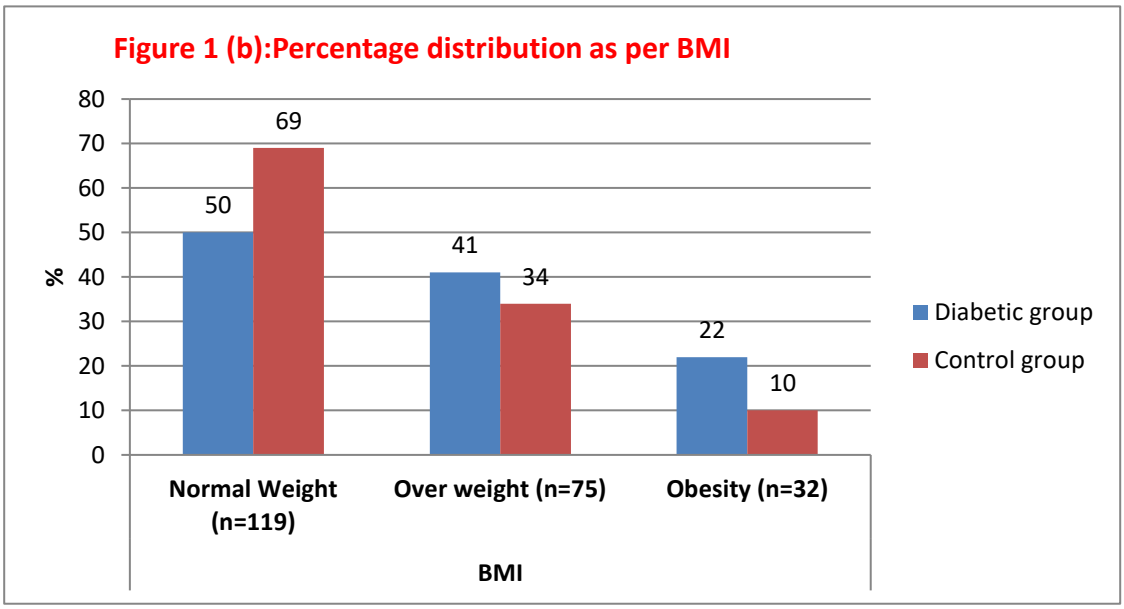




**Table 2**: Comparison between diabetic and control groups on various demographical variables (Independent Student's t-test)

| **Demographical variables** | **Diabetic Cases (Mean ± SD)** | **Controls (Mean ± SD)** | **P-value** |
|---|---|---|---|
| Age (years) | 47.33 ± 8.38 | 46.04 ±6.47 | 0.062 (N.S) |
| Weight (kg) | 68.19 ± 8.59 | 64.81± 7.08 | <0.001*** |

| | | | |
|---|---|---|---|
| Height (m) | 1.60 ± 0.02 | 1.62± 0.03 | <0.001*** |
| BMI (kg/m$^2$) | 26.38 ± 3.63 | 24.70 ±3.07 | <0.001*** |
| FPG (mg/dl) | 182.61 ± 46.33 | 88.12 ±11.04 | <0.001*** |
| HbA1c (%) | 8.67 ± 2.51 | 5.26 ±0.51 | <0.001*** |

*** p<0.001 Highly significant, p>0.05 Not Significant

- No significant difference was found in the age group between cases and controls; hence individuals were age-matched. (p=0.062)
- Diabetic cases had higher BMI as compared to the Control group (P<0.001). FPG and HbA1c levels were higher in cases as compared to controls(p<0.001)

**Figure 2: Distribution of different demographic variables**

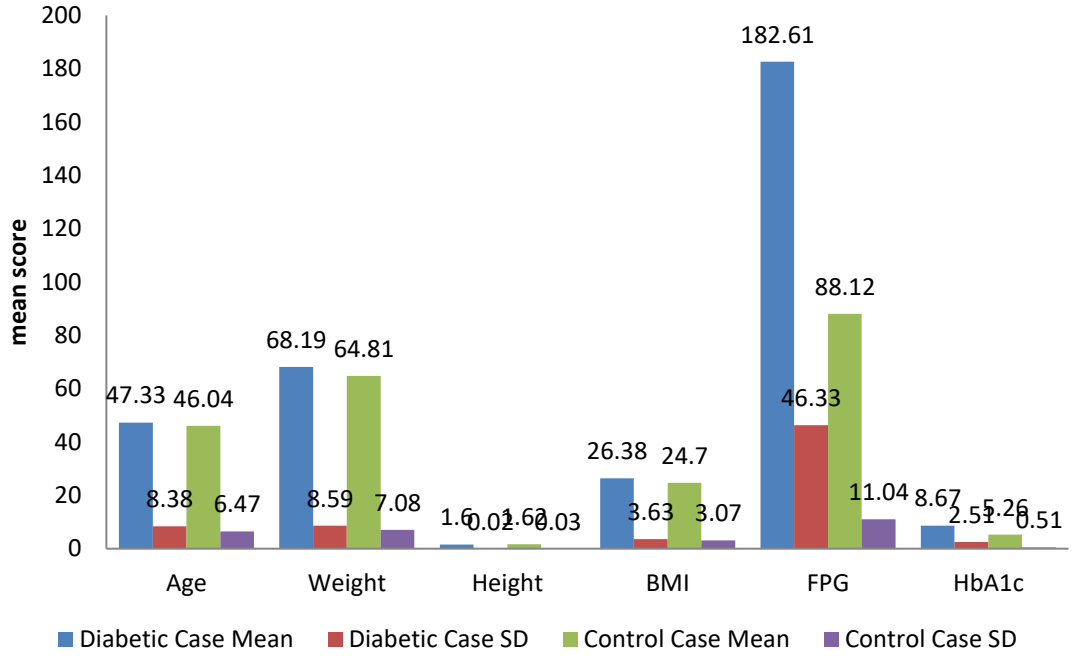



**Table 3**: Comparison of Serum RBP4 levels between Diabetic & Non-Diabetic controls (Independent Student's t-test)

| **Parameter** | **Diabetic Cases (Mean ± SD)** | **Controls (Mean ± SD)** | **P-value** |
|---|---|---|---|
| RBP4(ng/ml) | 76.53±26.96 | 53.77±12.62 | <0.001*** |

*** p<0.001 Highly significant

➢ The concentration of serum RBP4 in the blood plasma of type 2 diabetes mellitus patients(76.53±26.96ng/ml) was found to be significantly higher than the healthy controls(53.77±12.62ng/ml), (P<0.001).

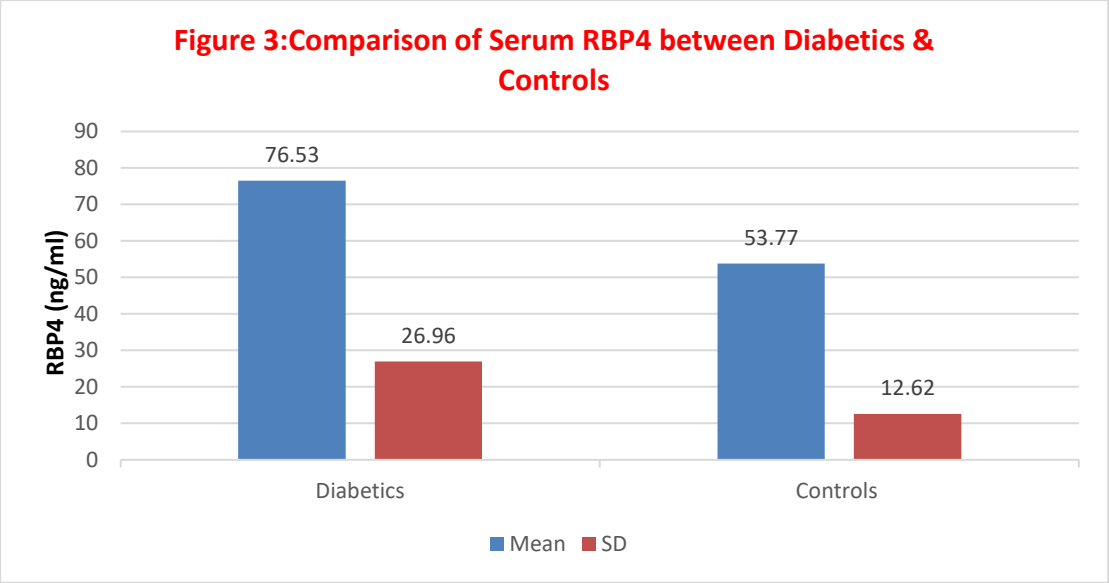



**Table 4**: Comparison between RBP4 levels in males and females in type 2 diabetes mellitus patients and controls (Independent Student's t-test)

| **Parameters** | **Males (Mean ± SD)** | **Females (Mean ± SD)** | **P-value** |
|---|---|---|---|
| **RBP4 (ng/ml) Cases** | 80.54 ± 29.79 | 72.54 ± 23.58 | 0.115(NS) |
| **RBP4 (ng/ml) Controls** | 55.71 ± 13.16 | 51.87 ± 11.88 | 0.106(NS) |

p>0.05 Not Significant (NS)

➢ RBP4 concentration is higher in males as compared to females in both diabetic and control groups, although non significantly (p>0.05)

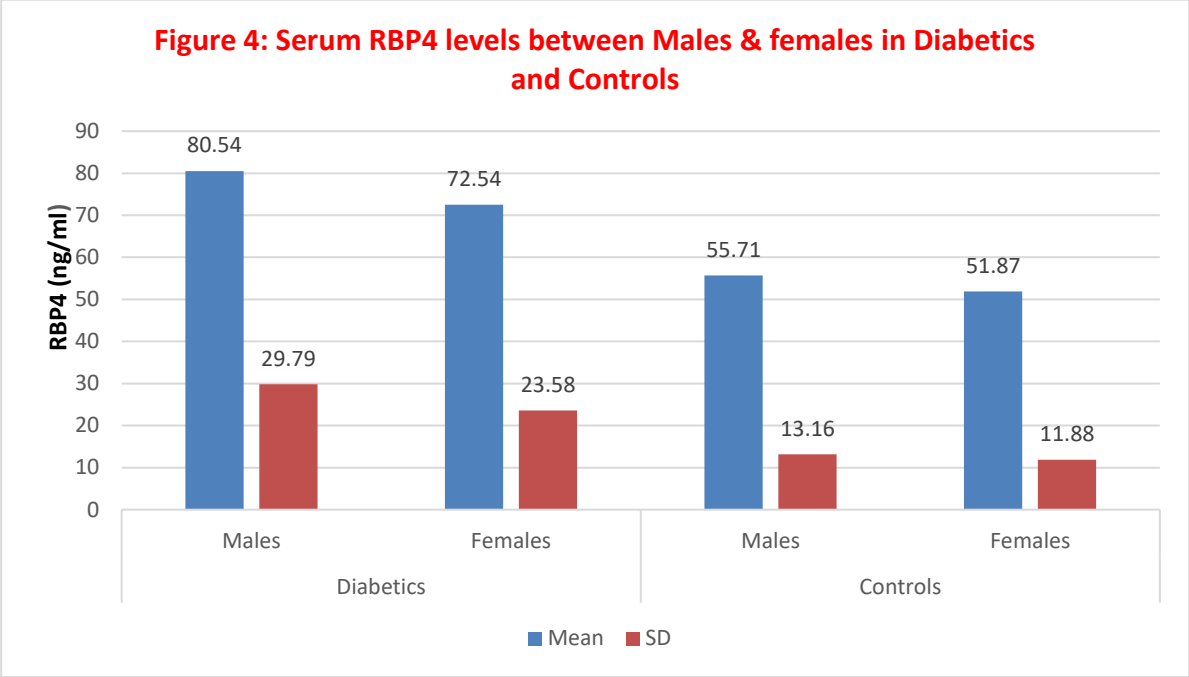



**Table 5**: Correlation of FPG, HbA1c & BMI with Serum RBP4 among Diabetic patients (Pearson's correlation coefficient)

| S.No | Parameters | r-value | P-value |
|---|---|---|---|
| a | Serum RBP4 & FPG | 0.465 | $p<0.001^{***}$ |
| b | Serum RBP4 & HbA1c | 0.673 | $p<0.001^{***}$ |
| c | Serum RBP4 & BMI | 0.863 | $p<0.001^{***}$ |

*** p<0.001 Highly significant

- Serum RBP4 is positively correlated with FPG of diabetic patients (r=0.465), (p<0.001). This signifies a significant increase in serum RBP4 levels with an increase in FPG values in the diabetic group.
- Serum RBP4 is positively correlated with HbA1c of diabetic patients (r=0.673), (p<0.001). This signifies a significant increase in serum RBP4 levels with an increase in HbA1c levels in the diabetic group.
- Serum RBP4 is positively correlated with the BMI of diabetic patients (r=0.863), (p<0.001). This signifies a significant increase in serum RBP4 levels with an increase in BMI in the diabetic group

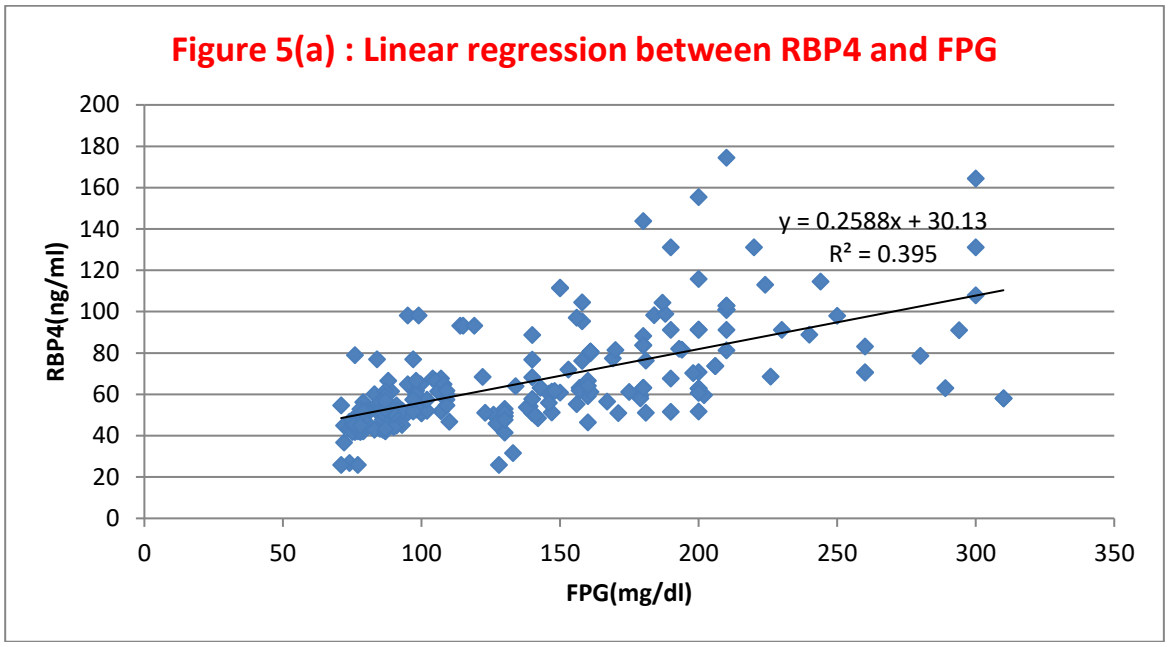


Figure 5(a) : Linear regression between RBP4 and FPG
RBP4(ng/ml)
FPG(mg/dl)
y = 0.2588x + 30.13
R² = 0.395
0
20
40
60
80
100
120
140
160
180
200
0
50
100
150
200
250
300
350

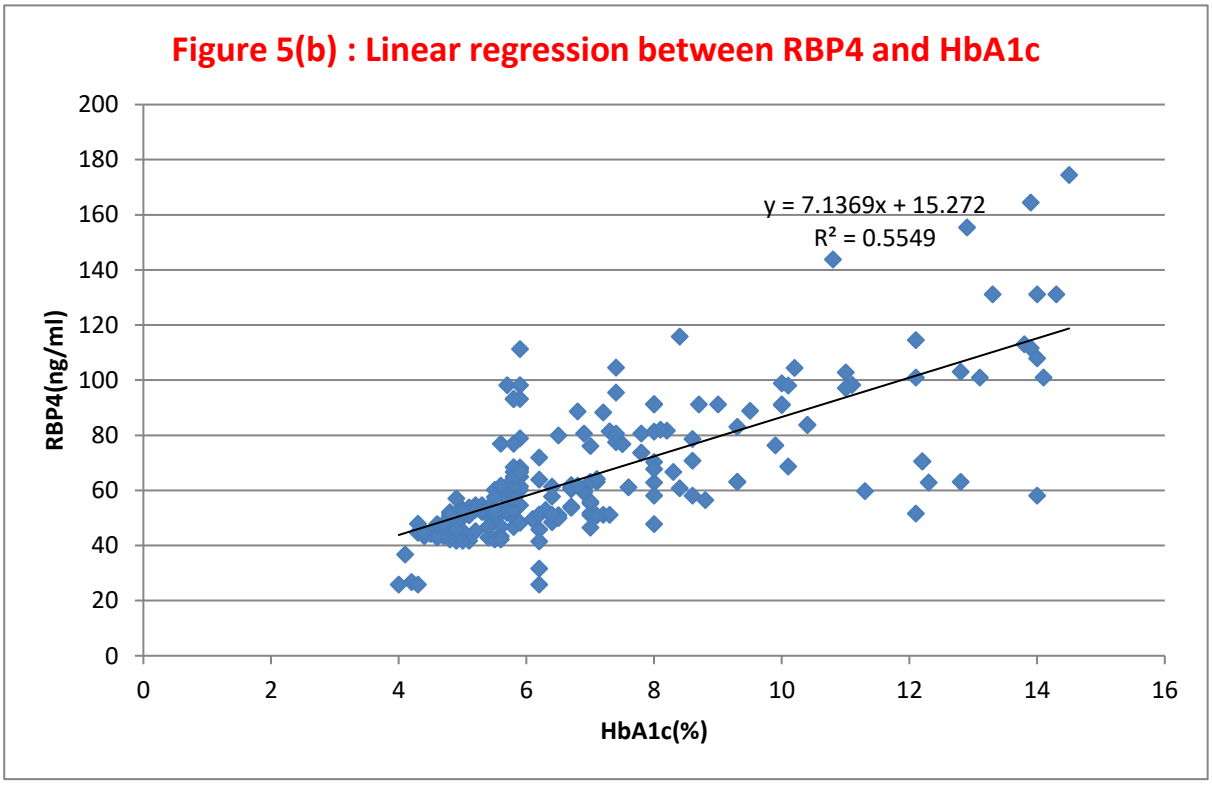


Figure 5(b) : Linear regression between RBP4 and HbA1c
RBP4(ng/ml)
HbA1c(%)
y = 7.1369x + 15.272
R² = 0.5549
0
20
40
60
80
100
120
140
160
180
200
0
2
4
6
8
10
12
14
16

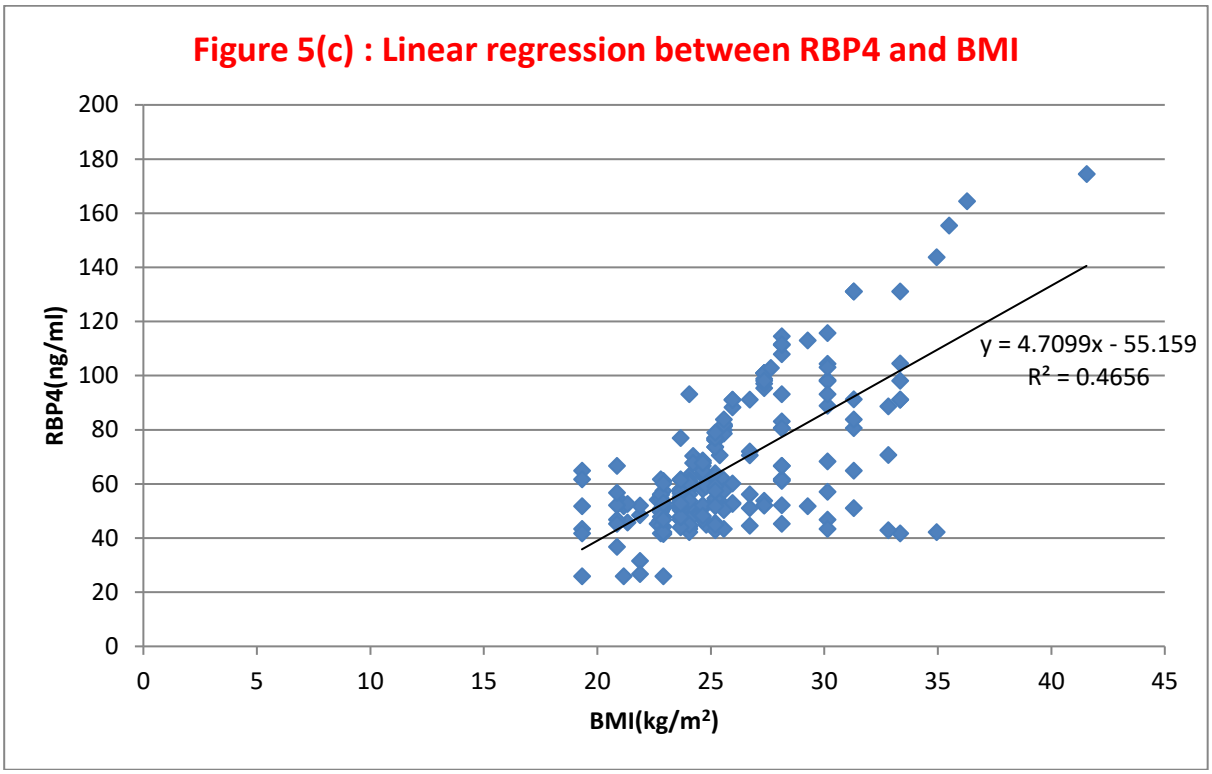
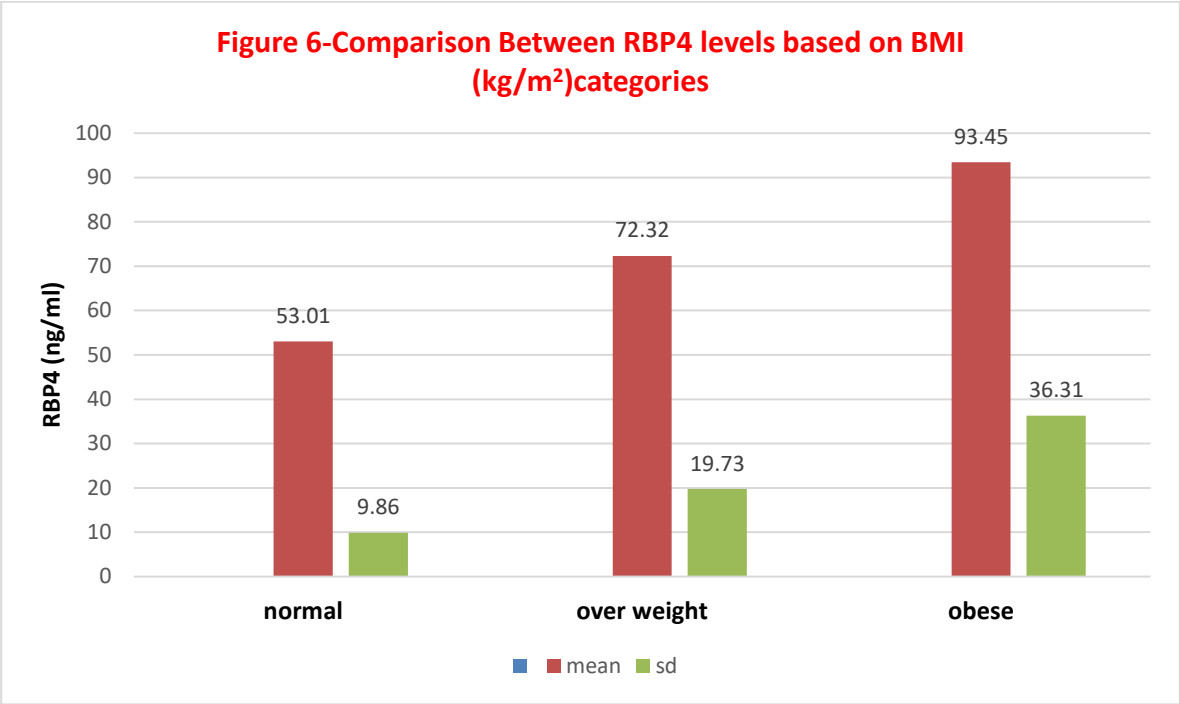



**Table 6**: Comparison between RBP4 based on BMI categories as normal weight, overweight and obese individuals (ANOVA Test)

| | BMI | | | |
|---|---|---|---|---|
| **Parameter** | **Normal (n=119) (Mean ± SD)** | **Overweight (n=75) (Mean ± SD)** | **Obese (n=32) (Mean ± SD)** | **P-value** |
| RBP4(ng/ml) | 53.01 ± 9.86 | 72.32 ± 19.73 | 93.45 ± 36.31 | <0.001*** |

*** p<0.001 Highly significant

➢ A higher RBP4 concentration was found in Obese individuals than in overweight and normal-weight participants(P<0.001)




## DISCUSSION

Retinol binding protein 4 (RBP4), a specific carrier for retinol in circulation, is produced and released mainly by hepatocytes (80%) and adipocytes (20%). Previous studies have reported that the elevation of adipocyte-secreted RBP4 contributes to the development of insulin resistance in mice.[13] However, these associations are not supported by all studies in humans, as several studies found no correlation between circulating RBP4 levels and insulin resistance, impaired glucose tolerance, or type 2 diabetes.[14] Therefore, the present study was carried out to evaluate serum levels of RBP4 in diabetic patients and compare them with healthy controls.

The present study showed that an equal distribution of gender-matched individuals was taken into the study. Also, no significant difference was found in the age group between cases and control, hence individuals were age-matched. (p=0.062). **(Table 1).** The main findings of various measured parameters showed that the mean values of FPG, HbA1c, and BMI were 182.61±46.33mg/dl, 8.67±2.51%and 26.38±3.63kg/m2 respectively in Type 2 diabetes mellitus patients, which was found to be statistically higher than the mean values of their respective controls 88.12±11.04 mg/dl, 5.26±0.51 % and 24.70±3.07 kg/m2 (p<0.001) **(Table 2).**

The main perspective of our study was to compare serum levels of RBP4 in diabetic patients and we found that the concentration of serum RBP4 in type 2 diabetes mellitus patients was (76.53±26.96ng/ml) which was significantly higher than the healthy controls(53.77±12.62ng/ml), (P<0.001) **(Table 3).** This result is consistent with previous research, carried out by **Cho et al.**[15]**, Takebayshi K et al.**[16], **and Tan MI et al.**[17]

Free RBP4 in blood plasma is synthesized and secreted from adipocytes and majorly from the liver. This high concentration of RBP4 in the blood plasma of diabetic participants is probably correlated with its function in maintaining metabolic control. **Ma et al**. stated that the important role of hepatic RBP4 is to maintain metabolic control.[17]

Several mechanisms linking RBP4 to insulin resistance and type 2 diabetes have been investigated. It may be demonstrated that the RBP-4 gene is located on chromosome 10 (10q23-q24) in humans in a region that contains at least 1 gene i.e., hexokinase 1, the gene encoding a key enzyme in the initial step of glucose metabolism.[3] Furthermore, increased serum RBP4 levels are known to stimulate hepatic gluconeogenesis through the stimulation of phosphoenolpyruvate carboxykinase and the attenuated insulin signaling in skeletal muscle. Insulin signaling in primary human adipocytes was affected by RBP4 by blocking the insulin-stimulated phosphorylation of insulin receptor substrate-1 at serine in position 307.[4] Further, the link between RBP4 and type T2DM could also be 2 mediated through impaired insulin secretion, insulin levels which was not measured in the present study.

In contrast, **Erickstrup et al.** stated that when compared to people with normal glucose tolerance, the plasma concentration of RBP4 was lower in T2DM patients ($p<0.05$).[14] **Comucci EB et al** found that higher RBP4 levels (104.8 ± 76.8 ng/mL) were observed in lean-control individuals when compared to obese having normal glucose tolerance (87.9 ± 38 ng/mL) and obese with type 2 diabetic individuals (72.2 ± 15.6 ng/mL).[3]

Somehow, there seems to be a number of factors interfering with correlations that should be considered when interpreting results. Furthermore, genetic differences in the profile of studied populations, relationships among gender, and age, have an important role in the interpretation and comparison of data obtained. The inconsistency in the results might be due to the small number of samples in this study, therefore, further research in a larger area is suggested.

Interestingly, in our study RBP4 concentration was found to be higher in males (80.54 ± 29.79ng/ml) than in females (72.54 ± 23.58ng/ml) in the diabetic group along with the control group 55.71 ± 13.16ng/ml and 51.87 ± 11.88ng/ml respectively, although it is not statistically significant ($p>0.05$) (**Table 4).** On the other hand, investigations with statistically significant higher RBP4 levels in males have been observed by other authors like **Cho et al.**[15], **Ulgen F et al**[18], **Khalil H et al**. [19], **and Yeli Wang et al.**[20]. While, **Rhie YJ et al.** suggested no statistically significant difference in serum RBP4 between males and females (54.94 ± 20.18 vs 49.67 ± 16.66 mg/L, $P = 0.166$). [21]

Plasma RBP4 concentrations were found to be sexually dimorphic, which had previously been reported for adipokines, such as leptin and adiponectin, and explained on the basis of different fat amounts and the influences of sex hormones. No difference was found in plasma RBP4 levels with respect to fat amounts or body fat percentages in the present study. Thus, it appears that differences in sex hormone status might affect RBP4 plasma levels.

Moreover, in our study, correlations were done between serum RBP4 levels with inclusive criteria of type 2 diabetes mellitus (FPG & HbA1c) using Pearson's correlation coefficient. **(In Table:5, Figure 5a, b&c).** It was observed that serum RBP4 is positively correlated with FPG of diabetic patients ($r=0.465$), ($p<0.001$). This signifies a significant increase in serum RBP4 levels with an increase in FPG values in the diabetic group. Similar correlations were given by **Cho et al. [15]**, **Park et al.** [22], **Khalil H et al.** [19], **Grosjean F et al.**[23]

**Cho et al.** stated that fasting plasma glucose levels were found to increase with plasma RBP4 quartile and were found to be an independent factor determining plasma RBP4 levels other than obesity (Yang et al.) The possible mechanism underlying increased fasting plasma glucose levels in subjects with higher plasma RBP4 levels probably concerns increased hepatic glucose output, as RBP4 has been reported to upregulate the expression of PEPCK, a key enzyme in hepatic gluconeogenesis, in the liver.[15]

Also, **Khalil et al**. concluded that over secretion of RBP4 may negatively affect beta-cell function and included that RBP4 could be one signal from insulin-resistant tissues that beta-cell secretion, and this could be behind the mechanism of increased rbp4 and type 2 diabetes mellitus.[19] In contrast, **Lewis et al**. suggested RBP4 does not show a correlation with fasting plasma glucose.

In our study findings, serum RBP4 is positively correlated with HbA1c of diabetic patients ($r=0.673$), ($p<0.001$). This signifies a significant increase in serum RBP4 levels with an increase in HbA1c levels in the diabetic group. Same findings have been shown by **Comucci EB et al.**[3]**, Khalil H et al. [19]and Jia-Ying li et al.**[24]

**Khalil et al.** proposed several mechanisms linking RBP4 to insulin resistance and type 2 diabetes that has been investigated. The RBP-4 gene is located on chromosome 10 (10q23-q24) in humans in a region that contains at least 1 interesting gene, hexokinase 1, the gene encoding a key enzyme in the initial step of glucose metabolism. Insulin signaling in primary human adipocytes was affected by RBP4 by blocking the insulin-stimulated phosphorylation of insulin receptor substrate-1 at serine in position 307.[19] The link between RBP4 and type T2DM could also be mediated through impaired insulin secretion, insulin levels were not measured in the present study.

Lastly in our study, serum RBP4 is positively correlated with the BMI of diabetic patients ($r=0.863$), ($p<0.001$). This signifies a significant increase in serum RBP4 levels with an increase in BMI in the diabetic group**.** Same observations were also found by **Rhie YJ et al. [21] and Jia-Ying li et al**.[24]

Additionally, in our study, a higher RBP4 concentration was found in obese individuals than in overweight and normal-weight participants($P<0.001$) (**Table 6 & Figure 6**). **Timothy E Graham et al.** [25] **and Rhie YJ et al.**[21] also conducted such group investigations and found the highest serum RBP4 levels in the Obese group. However, reverse levels have been observed by

**Comucci EB et al.**[3] The difference in results may be attributed to a difference in ethnicity or degree of obesity.

## CONCLUSION

The concentrations of serum RBP4 were found to be increased in Type 2 diabetes mellitus patients and related to various clinical parameters of diabetes, suggesting a role in its pathogenesis. Serum RBP4 levels are found to be higher in males than females, although non-significantly, and found to be related to the degree of adiposity as its highest levels are found in obese individuals as compared to overweight and lean. The concentration of serum RBP4 was observed as increased and co-related with various clinical parameters of diabetes i.e., FPG, HbA1c & BMI. Thus, lowering RBP4 levels could be a new strategy for treating type 2 diabetes mellitus, especially in obese individuals.

Taken together, the significant relationship between RBP4, obesity, and different parameters of T2DM like FPG and HbA1c supports the role of RBP4 as a driver, modulator, and biomarker of insulin resistance. This highlights the importance to understand the mechanism regulating the synthesis and secretion of RBP4.

Findings from a few other clinical studies show discrepancies in the association with RBP4, possibly arising due to differences in studied populations, age, and gender, as well as the different methodological evaluations of RBP4 circulating. Therefore, more mechanistic studies are required to understand the role of RBP4 in the onset and progression of "obesity diseases".

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## CONFLICT OF INTERESTS

No conflict of interest was declared.

## REFERENCES

1. Danaei G, Finucane MM, Lu Y, Singh G, Cowan M, Paciorek C et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet. 2011Jul;378(9785):31–40
2. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM et al. Serum retinol-binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature.2015;436(7049):356–62.
3. Comucci EB, Vasques AJ, Geloneze B, Calixto AR, Pareja JC, Tambascia MA. Serum levels of retinol-binding protein 4 in women with different levels of adiposity and glucose tolerance. Arq Bras Endocrinol Metab.2014 Oct;58(7):709-14.
4. Ge Li, Issy C. Esangbedo, Lu Xu, Junling Fu, Lujiao Li, Dan Feng et al,Childhood retinol-binding protein 4 (RBP4) levels predicting the 10-year Risk of insulin resistance and metabolic syndrome: the BCAMS study. Cardiovas diabetol.2018 May;17(1):69.
5. Whiting Dr, Guariguata L, Weil C, Shawj.IDF Diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract.2011; 94:311–21
6. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes-estimates for the year 2000 and projections for 2030. Diabetes Care.2004; 27(3):1047–53.
7. Y sowjanya, v siva prabodh, D Vidya, Sripad. Role of Iron Metabolic indices in type 2 Diabetes Mellitus. IOSR Journal of Dental and Medical Sciences.2017;16(12):1-5.
8. Marjan Tabesh, Atsa Noroozi, Masood Amini, Awat Feizi, Sahar Saraf-Bank, and Maryam Zare Association of retinol-binding protein 4 with metabolic syndrome in first-degree relatives of type 2 diabetic patients. J Res Med Sci. 2017; 22:28.
9. Singh PS, Sharma H, Zafar KS, Singh PK, Yadav SK,Gautam RK et al. Prevalence of type 2 diabetes mellitus in rural population of India-a study from Western Uttar Pradesh. Int J Res Med Sci..2017;5(4):1363-7.
10. Huang, J. and A. Vieira (2006). Human Quantikine®ELISA RBP4 Immunoassay. J. Mol. Med. 17:627.
11. Braham D, Trinder P. An improved color reagent for the determination of blood glucose by the oxidase system. Analyst.1972; 97(151):142-5.
12. 17) Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, et al. The IFCC Reference Measurement System for HbA1c: a 6-year progress report. Clin Chem. 2008;54(2):240-8.
13. Rocchi M, Covone A, Romeo G, Faraonio R, Colantuoni V. Regional mapping of RBP4 to 10q23----q24 and RBP1 to 3q21-q22 in man. Somatic Cell

and Molecular Genetics.1989;15(2):185–90
14. Erikstrup C, Mortensen OH, Nielsen AR, Fischer CP, Plomgaard P, Petersen AM, Krogh-Madsen R, Lindegaard B, Erhardt JG, Ullum H, et al. RBP-to-retinol ratio, but not total RBP, is elevated in patients with type 2 diabetes. Diabetes Obes Metab 2009;11(3):204–12
15. Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, Lee HK, Park KS. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care. 2006 Nov;29(11):2457-61.
16. Takebayashi K, Suetsugu M, W Sadao,A Yoshimasa,I Toshihiko. Retinol binding protein-4 levels and clinical features of type 2 diabetes patients. J Clin Endocrinol Metab.2007 Jul;92(7):2712–9.
17. Tan MI, Hisana AN, Ridwan A, Fitri LL, Fajar PA. Diabetes and Retinol-Binding Protein (RBP4): The Study of Free and Exosomal RBP4 in Blood Plasma and Urine of Patients. Biomed. & Pharmacol. J.2020;13(2), 693-9.
18. Ulgen F, Herder C, Kuhn MC, Willenberg HS, Schott M, Scherbaum WA, Schinner S. Association of serum levels of retinol-binding protein 4 with male sex but not with insulin resistance in obese patients. Arch Physiol Biochem 2010;116(2):57–62
19. Khalil HM, Abdalla AE, Shrif SM, Hasani IW. Retinol Binding Protien-4 Levels in Early Diabetic Nephropathy in Egyptian Patients with Type 2 Diabetes. Global J. Biotech. & Biochem.2015;10 (1): 39-46.
20. Wang Y, Sun L, Lin X, Yuan JM, Koh WP, Pan A. Retinol binding protein 4 and risk of type 2 diabetes in Singapore Chinese men and women: a nested case-control study. Nutrition & Metabolism.2019;16:3.
21. Rhie YJ, Choi BM, Eun SH,Son CS, Park SH, Lee KH. Association of Serum Retinol Binding Protein 4 with Adiposity and Pubertal Development in Korean Children and Adolescent. Korean Med Sci.2011; 26:797-802.
22. Park SE, Lee NS, Park JW, Rhee EJ, Lee WY, Oh KW et al. Association of urinary RBP4 with insulin resistance, inflammation, and microalbuminuria. European Journal of Endocrinology. 2014;171: 443–9
23. Grosjean F, Esposito P, Maccarrone R, Libetta C, Canton AD, Rampino T. RBP4: A Culprit for Insulin Resistance in End Stage Renal Disease That Can Be Cleared by Hemodiafiltration. BioMed Research International.2017; Article ID 7270595, 1-8.
24. Li JY, Chen XX, Lu XH, Zhang CB, Shi QP, Feng L. Elevated RBP4 plasma levels were associated with diabetic retinopathy in type 2 diabetes. Bioscience Reports. 2018; 38: BSR20181100.
25. Graham TE, Yang Q, Bluher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med. 2006; 354:2552–63.