High circulating fibroblast growth factor-21 levels as a screening marker in fatty pancreas patients

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28 **Abstract**

29 **Background:** The study aimed to detect the serum levels of fibroblast growth factor-21 (FGF-

30 21) in fatty pancreas (FP) patients and to investigate their potential clinical value.

31 **Methods:** We screened patients with FP using transabdominal ultrasound. The anthropometric,

32 biochemical and serum levels of FGF-21 were compared between the FP group and the normal

33 control (NC) group. A receiver operating characteristic (ROC) curve was used to evaluate the

34 predictive value of serum FGF-21 for FP patients.

35 **Results:** Compared with the NC group, body mass index, fasting blood glucose levels, uric acid

36 levels and cholesterol levels of the FP group were significantly higher, while the high-density

37 lipoprotein level was lower. In addition, levels of serum FGF-21, resistin, leptin and tumor necrosis

38 factor-α were significantly higher than those in the NC group, while the serum adiponectin level

39 was lower. Pearson analysis showed serum FGF-21 levels in FP patients were negatively

40 correlated with leptin. The ROC curve showed the best critical value of the serum FGF-21 level in

41 FP patients was 171 pg/mL (AUC 0.744, P=0.002, 95% confidence intervals 0.636-0.852).

42 **Conclusion:** Serum FGF-21 was closely related to fatty pancreas. Detecting serum FGF-21

43 levels may help identify the population susceptible to FP.

44 **Keywords:** Fatty pancreas, Fibroblast growth factor-21, Prognostic marker, Metabolic diseases,

45 Adipocytokine.

46

47 **Introduction**

48 Adipose tissue, as the energy storage of the body, is mainly distributed throughout the body

49 subcutaneously and around the abdominal organs. An imbalance in energy metabolism can lead to

50 fat deposition in abnormal areas, such as the liver and muscle fascia, and these abnormal deposits

51 are called ectopic fat deposits [1]. It has been found that fat can be deposited heterotopically in

52 pancreatic tissue, which is usually referred to as fatty pancreas (FP) and this condition has a

53 definite pathophysiological significance [2]. As early as 1933, Ogilvie [3] found via autopsy that

54 there was a difference in pancreatic fat content between obese and nonobese people. Li et al. noted

55 that the incidence of fatty pancreas varied from 16% to 69.7% in different countries [4]. Previously,

56 our team found that the prevalence of fatty pancreas in the examined population was approximately

57 2.7% in Yangzhou, China [5]. The specific pathogenesis of fatty pancreas remains unclear.

58 Fibroblast growth factor-21 (FGF-21) is a member of the endocrine fibroblast growth factor

59 subfamily [6]. The main physiological function of FGF-21 is to maintain the balance between

60 glucose and lipid metabolism [6]. Animal experiments have shown that excessive expression of

61 FGF-21 could lead to body fat reduction [7]. In addition, exogenous FGF-21 plays a clear role in

62 promoting glucose uptake and reducing blood glucose and liver fat deposition [7]. Clinical studies

63 have shown that the circulating FGF-21 level was closely related to metabolic diseases. Serum

64 FGF-21 levels in patients with fatty liver, obesity, metabolic syndrome and diabetes were

65 significantly higher than those in healthy volunteers [8-11].

66 FGF-21 also plays an important role in pancreatic diseases [12-14]. FGF-21 is highly

67 expressed in pancreatic exocrine cells, and its expression can be significantly increased when acute

68 pancreatitis occurs [15]. Johnson et al. showed that FGF-21 gene knockout mice experienced

69 aggravated acute pancreatitis and that exogenous FGF-21 could significantly reduce the severity

70 of caerulein-induced acute pancreatitis in mice [16]. Recent animal experiments have confirmed

71   that recombinant human FGF-21 can reduce inflammation, pancreatic cysts high-grade

72   intraepithelial neoplasia and pancreatic cancer in high-fat-diet-fed mice, suggesting that FGF-21

73   may be used for the prevention and treatment of pancreatic cancer [17]. Surprisingly, FGF-21-

74   deficient mice exhibit a phenotype of pancreatic fat deposition [18]. However, there are no reports

75   on the correlation between FGF-21 and fatty pancreas. Hence, in the present study, circulating

76   FGF-21 levels in patients with fatty pancreas were detected for the preliminary assessment of their

77   relationship.

Materials and methods

Study population

80   The study was performed at the Affiliated Hospital of Yangzhou University in Yangzhou, China.

81   We selected 99 fatty pancreas subjects and 16 healthy normal control subjects from the physical

82   examination center of Affiliated Hospital of Yangzhou University from August 2018 to June 2019.

83   Fatty pancreas subjects who met the following criteria were excluded: (1) subjects aged < 18 years

84   or > 65 years; (2) subjects with acute or chronic inflammatory diseases; (3) subjects with a previous

85   diagnosis of chronic pancreatic, liver or kidney disease; (4) subjects with severe immune system

86   disorders or pregnancy; (5) subjects with incomplete information or refusal to provide clinical

87   blood samples. Besides, the inclusion criteria of normal control subjects were as follows: (1)

88   subjects aged 18 - 65 years; (2) subjects without fatty pancreas; (3) subjects without any acute or

89   chronic diseases; (4) subjects without previous histories of acute or chronic diseases; (5) subjects

90   without severe immune system disorders or pregnancy or cancers; (6) subjects without incomplete

91   information or refusal to provide clinical blood samples. The exclusion criteria were those who

92   did not meet the inclusion criteria.

93   This study conformed to the ethical principles of the Declaration of Helsinki. The study was

94   approved by Ethics Committee of Affiliated Hospital of Yangzhou University (Ethical Application Ref:

95   2018-YKL11-27- topic1). All participants agreed to participate in the study, and written informed

96   consent was obtained from each subject.

Diagnosis of fatty pancreas and fatty liver

98   As previously described, all subjects underwent transabdominal ultrasonography to diagnose fatty

99   pancreas and fatty liver [5]. Operations were performed by skilled surgeons with more than 10

100 years of experience using transabdominal ultrasonography (with an abdominal convex array probe,

101 frequency: 3.5-5 MHz, LOGIQ E9, GE, USA). The ultrasound diagnostic criteria for fatty liver

102 were as follows: the anterior echo of the liver was enhanced while the posterior echo was

103 weakened, and the tubular structure of the liver could not be clearly displayed [19]. The

104 characteristics of the ultrasonic image of fatty pancreas were as follows: diffuse strong echoes of

105 pancreatic parenchyma, normal or slightly increased volume, similar or slightly higher

106 echogenicity compared to the adipose tissue in the area of the superior mesenteric artery [5,20].

Anthropometric and biochemical findings

108 Clinical information, including the subject's identity, age, gender, body weight, height, blood

109 pressure, past medical history, drug history, history of smoking and alcohol intake, was recorded

110 using a standardized questionnaire. Height and body weight were assessed using standardized and

111 calibrated scales. Additionally, the body mass index (BMI) was calculated for each subject [BMI

112 = body weight (kg)/square of height (m^2)]. The subjects included "continuous smokers"

113 (continuous or cumulative smoking for 6 months or more in a lifetime) and "nonsmokers"

114 (including those who quit smoking $\geq$1 year ago) [5]. The subjects also included "continuous

115 drinkers" (drinking volume $\geq$20g/d; drinking duration $\geq$2years) and "nondrinkers" (individuals

116 who had not consumed alcohol for $\geq$half a year) [5].

117   All subjects fasted for at least 8 hours the night before the visit, and blood samples were

118   collected with the participants in a quiet state on an empty stomach the next morning. Blood

119   samples were sent to the laboratory for uniform testing (dry chemical method), and the remaining

120   unused serum was frozen in an ultralow temperature freezer for analysis using an enzyme linked

121   immunosorbent assay (ELISA). Diabetes was defined as a fasting blood glucose (FBG) level ≥ 7.0

122   mmol/L or a previous diagnosis by a doctor [21].

123   Dyslipidemia was defined as meeting any of the following: high serum total cholesterol

124   (CHO) ($\geqslant$5.17 mmol/L), high triglyceride (TG) levels ($\geqslant$1.7 mmol/L), decreased high density

125   lipoprotein (HDL) levels (<1.03 mmol/L), high low density lipoprotein (LDL) levels ($\geqslant$4.1

126   mmol/L), or a previous diagnosis of dyslipidemia by a doctor[5].

127   **Measurement of FGF-21 and adipocytokine levels in human serum**

128   Levels of serum FGF-21, adiponectin, leptin, resistin and tumor necrosis factor-alpha (TNF-α)

129   were quantified using ELISA kits (USCN KIT INC, Wuhan, China). All operations were carried

130   out in strict accordance with the kit instructions. The quantification of the results was calculated

131   according to the instructions. Averaged the readings for standards, controls, and samples and

132   subtracted the average zero standard relative light unit. Created the standard curve on log-log graph

133   paper, with FGF-21 or adipocytokines concentration on the y-axis and the relative light unit value

134   on the x-axis. Drawn the best fit straight line through the standard points and it could be determined

135   by regression analysis. If samples had been diluted, the concentration read from the standard curve

136   must be multiplied by the dilution factor.

137   **Statistical analysis**

138   Statistical analysis was performed with IBM SPSS 19.0 software. Normality and homogeneity of

139   variance tests were performed for each group of data by Shapiro-Wilk and Levene's test.

140   Continuous measurement data that were normally distributed are presented as the mean ± standard

141   deviation (Mean±SD). Continuous variables that were not normally distributed are presented as

142   medians (25th and 75th percentiles), and categorical variables are presented as percentages (n%).

143   The differences between the fatty pancreas (FP group) and normal control (NC group) groups were

144   determined using Student's t tests and Mann-Whitney U tests. The qualitative data were compared

145   using the chi-square test. Pearson analysis was used to evaluate the correlation between FGF-21

146   and other factors. GraphPad Prism 7 was used to generate receiver operating characteristic (ROC)

147   curve. A two-sided p value <0.05 was used to indicate statistically significant differences.

148   **Results**

149   **Basic and clinical characteristics of the study population**

150   In this study, 99 fatty pancreas patients (FP group) were enrolled, and 16 healthy subjects were

151   enrolled as the normal control group (NC group). As shown in Table 1, the proportion of males in

152   the FP group and NC group was 58.6% (58/99) and 50% (8/16), respectively (P = 0.524). The

153   average age and BMI in the FP group were significantly higher than those in the NC group (P =

154   0.003, P < 0.001). There was no difference in the proportion of smokers or drinkers between the

155   two groups. Besides, the proportion of fatty liver, diabetes, hypertension, obesity, dyslipidemia in

156   FP group were significantly increase than that in NC group (All P < 0.001). This also suggested

157   that fatty pancreas may have an inseparable relationship with metabolic diseases.

158   Then, clinical characteristics were compared between the FP and NC groups. As shown in

159   Table 2, it is self-evident that some key metabolic indicators, including serum uric acid (UA),

160   glucose, CHO and TG were significantly higher in the FP group than in the NC group (All P <

161   0.05), with the corresponding serum HDL were decreased (P < 0.001).

Comparison of serum FGF-21 and adipocytokine levels

We measured the levels of serum FGF-21 and adipocytokines (adiponectin, resistin, leptin and TNF-α) in the two groups. As shown in Figure 1, the serum levels of FGF-21 and resistin in the FP group were significantly higher than that in the NC group, and adiponectin showed the opposite trend (All P<0.05). In addition, the serum leptin and TNF-α levels in the FP group showed the upward trend in the FP group without a significant difference. Interestingly, in Supplementary Table 1, we found that the levels of serum FGF-21 in male of FP group were significantly higher than that in female of FP group (P=0.004). However, the levels of serum leptin and TNF-α in female of FP group were significantly higher than that in male (All P<0.01). There was no significant difference between the two groups in serum resistin and adiponectin levels (Supplementary Table 1).

It is remarkable that serum FGF-21 levels were negatively correlated with leptin (r = -0.261, P = 0.005) in the Pearson correlation analysis. In addition, there was no clear correlation with adipocytokines (adiponectin, resistin and TNF-α) or key metabolic clinical indicators (Glucose, CHO, LDL, TG and HDL) (Table 3).

Fatty pancreas prediction analysis

Serum FGF-21 levels showed significantly greater discriminative ability for FP. Area under ROC curves (AUC) of FGF-21 were remarkably larger than adipocytokines (adiponectin, resistin, leptin and TNF-α) (Figure 2). To determine the critical value of FGF-21 for predicting FP, ROC curves were assessed and are presented in Table 4. The results showed that 171 pg/mL was the best critical value (AUC 0.744, P=0.002, 95% Confidence Interval (95% CI) 0.636-0.852). The corresponding sensitivity was 80.8%, and the specificity was 68.8% (Table 4).

Discussion

185 Although FP was discovered many years ago, its pathophysiology was still unclear. Most of the

186 existing studies focused on clinical morbidity and its related risk factors [22,23]. Previous studies

187 showed that FP was closely related to metabolic diseases such as dyslipidemia, diabetes and fatty

188 liver [24-26]. Increased age, central obesity, and fatty liver were independent risk factors for FP

189 [5]. To date, there are no clinical molecular indicators for predicting or diagnosing FP.

190   FGF-21 is a recently discovered cytokine closely related to glycolipid metabolism and is the

191 only protein in the fibroblast growth factor family that has no mitogenic activity [27]. The

192 physiological function of FGF-21 is mainly involved in glycolipid metabolism and the metabolic

193 regulation of insulin, body weight reduction and insulin resistance improvement [28-30]. High-

194 fat-diet-fed FGF-21-deficient mice exhibited liver fat accumulation and obvious lipid metabolic

195 disorders; in contrast, injection of FGF-21 protein into diet-induced diabetic mice reversed the

196 steatosis of the liver and restored the normal structure of the liver [31,32]. Additionally, serum

197 FGF-21 levels in fatty liver or diabetic patients were higher than those in the participants in the

198 NC group, with a negative correlation with serum HDL and a positive correlation with BMI and

199 serum TG [8]. These results suggest that FGF-21 is closely related to metabolic diseases.

200   The levels of serum FGF-21 in patients with FP, as a common metabolic disease, have not

201 been reported. Johnson et al. showed that FGF-21-deficient mice had fat deposition in pancreatic

202 tissue, which suggests that FGF-21 may be involved in the development of FP [18]. In this study,

203 serum FGF-21 levels in the FP group were significantly higher than those in healthy controls,

204 which is consistent with the previously reported changes in serum FGF-21 levels observed in other

205 metabolic diseases, such as fatty liver, obesity, and diabetes [8,9,10,11]. However, this finding

206 does not seem to match the animal experiment of Johnson et al. [18]. The exact reasons are not yet

207 clear. We made possible speculations based on leptin. It is well known that leptin deficiency in

208 mice leads to obesity and insulin resistance [33]; however, the current discordant finding is that

209 almost all obese subjects had increased serum leptin levels [34]. A relatively reasonable

210 explanation is "leptin resistance". It is not clear whether there is "FGF-21 resistance" in

211 populations with metabolic diseases, which requires further study.

212   We had firstly reported that serum FGF-21 levels were significantly increased in patients with

213 FP and better screened FP patients. In our study, we found that serum FGF-21 levels could better

214 predict FP than leptin, adiponectin, resistin and TNF-α. First of all, as shown in Figure 1, we

215 intuitively found that FGF-21 had the most obvious increase trend and the statistical difference

216 was also the most significant. Secondly, we also found that the area under the ROC curve of FGF-

217 21 was more significant and its P value was the lowest by observing the prediction of FGF-21 and

218 the other four cytokines on FP patients. ROC curve analysis showed that 171 pg/mL was the best

219 critical value of FGF-21 for predicting FP, and the corresponding sensitivity and specificity are

220 80.8% and 68.8%, respectively. Hence, serum FGF-21 could better screen patients with FP and be

221 used as a suitable marker for routine clinical use of FP. We preliminarily screened FP patients by

222 detecting the expression levels of serum FGF-21, which would provide some help for clinical

223 prediction of FP. However, whether serum FGF-21 is the outcome indicator of patients with FP

224 needs further follow-up study by our team.

225   There are several limitations of our study. First, it is difficult to find FP patients without

226 common metabolic syndrome in the clinic. Therefore, the overall sample size of this study was

227 small, and the results may be biased. Second, transabdominal ultrasonography can only be used to

228 identify whether there is fat infiltration in the pancreas, but it cannot quantify the fat content.

229 Therefore, it is difficult to study the correlation between serum FGF-21 and the degree of fat

230 infiltration in the pancreas. Finally, all the serum samples in this study were frozen in a -80℃

231 refrigerator. The results may deviate from the results obtained from fresh serum samples, but the

232 results are still of reference value.

Conclusion

234 To sum up, the present study is the first to present the closely relationship between circulating

235 FGF-21 levels and FP patients. Serum FGF-21 levels may be the specific biomarker of FP, which

236 can help identify the population susceptible to FP. The serum FGF-21 lays a clinical foundation

237 for the diagnosis of FP in the future.

Abbreviations

239 FGF-21: Fibroblast growth factor-21; FP: Fatty pancreas; NC: Normal control; ROC: Receiver

240 operating characteristic; AUC: Area under curve; BMI: Body mass index; ELISA: Enzyme

241 linked immunosorbent; FBG: Fasting blood glucose; CHO: Cholesterol; TG: Triglyceride; HDL:

242 High density lipoprotein; LDL: Low density lipoprotein; TNF-α: Tumor necrosis factor-alpha; SD:

243 Standard deviation; UA: Uric acid.

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Authors' contributions

247 FH: Formal analysis, Writing-original draft preparation, Visualization. LY: Investigation, Data

248 curation. XPY: Conceptualization, Investigation. RYX: Resources, Investigation. MXT:

249 Validation, Supervision. XNL: Validation. LZ: Data curation, Validation. LHH: Writing-review

250 and editing, Investigation. WJG: Writing-review and editing. WMX: Project administration,

251 Writing-review and editing. GTL: Methodology, Writing-review and editing. YBD and GHY:

252 Conceptualization, Writing-review and editing, Funding acquisition. All authors read and

253 approved the final manuscript.

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259 **Data Availability**

260 The data used to support the findings of this study are available from the corresponding author

261 upon request.

262 **Ethics approval and consent to participate**

263 The study was approved by Ethics Committee of Affiliated Hospital of Yangzhou University (Ethical

264 Application Ref: 2018-YKL11-27- topic1). All participants agreed to participate in the study, and

265 written informed consent was obtained from each subject.

266 **Consent for publication**

267 Not applicable.

268 **Competing interests**

269 The authors declare that they have no competing interests.

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371   **Figure Legends**

372   **Figure 1** Comparisons of the levels of serum FGF-21 and adipocytokines (adiponectin, resistin,
373   leptin and TNF-α) in NC and FP group. FGF-21, fibroblast growth factor-21; FP, fatty pancreas;

374 NC, normal control; TNF-α, tumor necrosis factor-alpha.

375

376 **Figure 2** FGF-21 and adipocytokines (adiponectin, resistin, leptin and TNF-α) for prediction in
377 patients with fatty pancreas: ROC analysis. FGF-21, fibroblast growth factor-21; TNF-α, tumor
378 necrosis factor-alpha; ROC, receiver operating characteristic.

Demographic characteristics of participants

1   **Table 1** Demographic characteristics of participants

Characteristic	FP(N=99)	NC(N=16)	*P* value
Males, (n%)	58(58.6%)	9(50%)	0.524
Age, y	48.0±11.4	38.6±12.4	0.003**
BMI, kg/m^2	25.4±3.2	21.7±1.7	<0.001***
Smoking	18(18.2%)	3(18.8%)	0.957
Drinking	21(21.2%)	4(25%)	0.736
Fatty liver	25(25.3%)	0	<0.001***
Diabetes	24(24.2%)	0	<0.001***
Hypertension	28(28.3%)	0	<0.001***
Obesity	14(14.1%)	0	<0.001***
Dyslipidemia	50(50.5%)	0	<0.001***

3   Table 1: Values are expressed in the mean±SD or n%. FP, fatty pancreas; NC, normal control; BMI, body mass
4   index; SD, standard deviation. **P<0.01,***P<0.001.

Biochemistry characteristics of participants

1 **Table 2** Biochemistry characteristics of participants

Characteristic	FP(N=99)	NC(N=16)	*P* value
TP,g/L	73.2±4.7	72.5±3.5	0.556
ALB,g/L	44.3±2.1	44.6±2.9	0.638
TB,umol/L	13.3(10.9,16.3)	16.0(12.4,18.9)	0.056
DB,umol/L	3.7±1.2	4.2±1.8	0.282
ALT,U/L	21.0(15.2,33.0)	24.5(15.3,33.8)	0.982
AST,U/L	20.4(17.0,25.2)	20.6(16.1,25.7)	0.332
γ-GGT,U/L	27.0(18.6,39.6)	15.3(12.6,22.7)	0.190
Cr,umol/L	70.8±16.2	70.0±12.6	0. 847
BUN,mmol/L	5.2±1.3	5.0±1.5	0.608
UA, umol/L	337.0(286.0,404.3)	288.0(250.8,319.0)	0.004**
GLU,mmol/L	5.5(5.1,6.1)	5.0(4.7,5.1)	<0.001***
TG,mmol/L	2.6±2.4	1.3±0.4	<0.001***
CHO,mmol/L	4.8±1.0	4.3±0.6	0.034*
HDL,mmol/L	1.2±0.3	1.4±0.3	<0.001***
LDL,mmol/L	2.6±0.8	2.4±0.4	0.254

3 Table 2: Values are expressed in the mean±SD or the medians (25th and 75th percentiles). FP, fatty pancreas;

4 NC, normal control; TP, total protein; ALB, Albumin; TB, total bilirubin; DB, direct bilirubin; ALT, alanine

5 aminotransferase; AST, aspartate aminotransferase; γ-GGT, gamma-glutamyl transpeptidase; Cr, creatinine;

6 BUN, blood urea nitrogen; UA, uric acid; GLU, glucose; TG, triglyceride; CHO, cholesterol; HDL, high density

7 lipoprotein; LDL, low density lipoprotein; SD, standard deviation.

8 *P<0.05,**P<0.01,***P<0.001.

Correlation between FGF-21 and other clinical indicators

1 **Table 3** Correlation between FGF-21 and other clinical indicators

Characteristic	R	95% CI	*P* value
BMI,kg/m^2	0.024	-0.138,0.197	0.797
Leptin,ng/mL	-0.261	-0.388, -0.121	0.005[**]
Adiponectin,ug/mL	-0.016	-0.171,0.149	0.864
Resistin,ng/mL	-0.018	-0.206,0.161	0.850
TNF-α,pg/mL	0.010	-0.172,0.185	0.916
TG, mmol/L	-0.098	-0.243,0.098	0.297
CHO, mmol/L	-0.136	-0.277,0.027	0.148
HDL, mmol/L	-0.144	-0.336,0.090	0.125
LDL, mmol/L	-0.069	-0.222,0.108	0.463
Glucose,mmol/L	0.011	-0.109,0.189	0.908

3 Table 3: FGF-21, fibroblast growth factor 21; 95% CI, 95% confidence interval; BMI, body mass index; TNF-
4 α, tumor Necrosis Factor-alpha; TG, triglyceride; CHO, cholesterol; HDL, high density lipoprotein; LDL, low
5 density lipoprotein. [**]P<0.01.
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Performance of FGF-21 and adipocytokines in predicting fatty pancreas patients

1   **Table 4** Performance of FGF-21 and adipocytokines in predicting fatty pancreas patients

Variables	AUC (95% CI)	Cut-off value	P	Sen,%	Sepc,%
FGF-21	0.744(0.636,0.852)	171.0	0.002**	80.8	68.8
Leptin	0.643(0.478,0.808)	0.75	0.068	92.9	37.5
Resistin	0.632(0.518,0.746)	8.02	0.091	49.5	87.5
Adiponectin	0.667(0.540,0.793)	6.72	0.033*	49.5	81.3
TNF-α	0.681(0.518,0.844)	54.33	0.020*	67.7	75.0

3   Table 4: FGF-21, fibroblast growth factor 21; AUC, area under the curve; Sen, sensitivity; Spec, specificity; 95

4   % CI, 95% confidence intervals; TNF-α, tumor necrosis factor-alpha. *P<0.05,**P<0.01.

Figure 1

Comparisons of the levels of serum FGF-21 and adipocytokines (adiponectin, resistin, leptin and TNF-α) in NC and FP group.
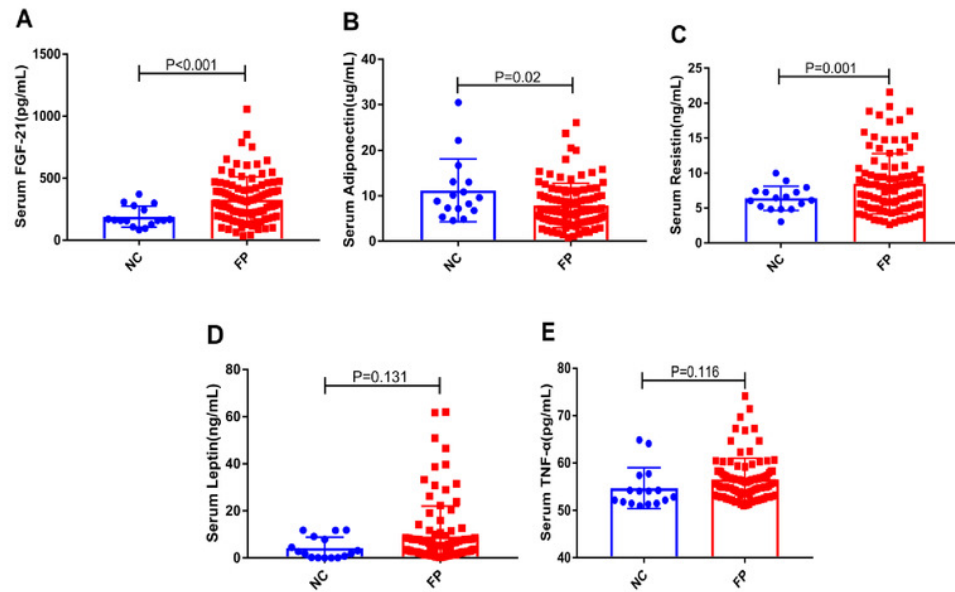
Figure 2

FGF-21 and adipocytokines (adiponectin, resistin, leptin and TNF-α) for prediction in patients with fatty pancreas: ROC analysis.