Original Article

Metabolic Abnormalities, Inflammatory Markers and Endothelial Dysfunction in Hyperprolactinemia due to Prolactinoma before and after Normalization of Serum Prolactin: A Prospective Case Control Study

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Abstract

Background: Hyperprolactinemia is associated with obesity, dyslipidemia, insulin resistance, and low-grade inflammation which may promote endothelial dysfunction (EnD). Limited work has been done on EnD in prolactinomas and we, therefore, studied serum markers of inflammation and EnD in patients with prolactinomas before and after treatment with dopamine agonists. **Methodology:** Fifty-six treatment naïve patients with prolactinomas and fifty-three (apparently healthy age and sex-matched) controls were enrolled in the study and subjected to clinical assessment and laboratory investigations including blood glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, urea, creatinine, uric acid, erythrocyte sedimentation rate (ESR), highly sensitive C-reactive protein (hsCRP) and markers of EnD i.e., intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Patients were treated with a dopamine agonist (cabergoline) and parameters (like ESR, hsCRP, ICAM-1, and VCAM-1) were measured at 12 weeks. **Results:** The majority of the patients (84%) were female, more than half (52%) had metabolic syndrome and over a third (36%) were obese. Blood glucose fasting, HbA1c, lipid fractions, ESR, hsCRP, ICAM-1, and VCAM-1 were significantly higher in patients than in controls. Median ICAM-1 was 1331.95 ng/ml (IQR 803.43-1825.99) in patients vs 753.04 ng/ml (IQR 402.04-871.55) in controls, $P < 0.001$ and median VCAM-1in patients was 971.35 ng/ml (IQR 695.03-1285.23) as against 634.56 ng/ml (IQR 177.49-946.50) in controls, $p0.001$. Serum ICAM-1 and VCAM-1 correlated positively with hsCRP. On multivariate regression analysis, serum hsCRP was the only significant predictor of change in ICAM-1 and VCAM-1. Normalization of serum PRL with CAB resulted in a significant decrease in metabolic parameters, ESR, hsCRP, ICAM-1, and VCAM-1. **Conclusion:** Hyperprolactinemia because of prolactinoma is associated with EnD secondary to systemic inflammation and metabolic abnormalities which improve after treatment with DA.

Keywords: Endothelial dysfunction, inflammation, metabolic syndrome, obesity, prolactinoma

Introduction

Prolactinomas are the commonest functioning tumors of the pituitary gland.[1] Hyperprolactinemia (HPL), besides its effect on the breast and gonads, is associated with systemic effects like weight gain, metabolic syndrome (MS), insulin resistance (IR), and chronic low-grade inflammation.[2-6] The postulated mechanisms for metabolic effects of PRL include decreased dopaminergic tone, defect in lipogenesis, and altered adiponectin and leptin release.[7-11] HPL also promotes vascular stiffness,[12] increases carotid intimal medial thickness (CIMT)[13] and cardiovascular mortality.[14]

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Endothelial dysfunction (EnD) abnormalities are increased in HPL and are characterized by a change of vascular endothelium from vasodilatory and antithrombotic to vasoconstrictors and

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prothrombotic state.[13,15,16] These studies observed decreased flow-mediated dilation (FMD) of the brachial artery on vascular doppler images, which is operator dependent and has inter-observer variation. Another approach to study EnD is to measure serum adhesion molecules like intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, which are increased in patients with preclinical atherosclerosis and can predict cardiovascular events.[17-21] Data on serum markers of EnD in patients with prolactinoma are limited. In one study, levels of E-selectin did not differ between patients with prolactinomas and controls, although levels decreased in the former after treatment with cabergoline (CAB).[22] The present study was designed to assess metabolic abnormalities and markers of inflammation [erythrocyte sedimentation rate (ESR), highly sensitive C-reactive protein (hs-CRP)] and EnD [ICAM-1 and VCAM-1] in patients with HPL because of prolactinomas and change in these parameters after 12 weeks of CAB treatment.

Patients and Methods

Subject selection

This prospective case-control study was conducted at the Endocrinology department of a tertiary care hospital from October 2018 to January 2021. The study subjects comprised patients diagnosed with prolactinoma and fulfilling the inclusion criteria (signs and symptoms of HPL, serum PRL above upper limit of normal, presence of adenoma on pituitary magnetic resonance imaging (MRI), and absence of any treatment for HPL). Patients were excluded if they were taking drugs known to cause HPL, had hypertension, diabetes mellitus (DM), coronary artery disease (CAD), polycystic ovary syndrome (PCOS), ischemic stroke, peripheral arterial disease (PAD), rheumatological diseases, chronic liver disease, chronic kidney disease, malignancy, chronic infections or had received radiotherapy. For comparison, seemingly healthy women [controlled for age and gender], were selected as controls from the community and hospital visitors, as part of the departmental outreach program, who were counseled and educated about study protocol. Written informed consent was taken from each study participant and the study was approved by the institution's ethics committee (Protocol number- RP 14/2019).

Study protocol

Both patients and controls had a measurement of serum levels of inflammatory markers like ESR and hsCRP and of EnD markers like ICAM-1 and VCAM-1. Patients were treated with oral CAB (Cabgolin; Sun pharma Pvt Ltd, India) with starting dose of 0.5 mg weekly. Serum PRL measurement was repeated after one and three months and the dose of CAB was increased to 0.5 mg twice weekly if required. Measurement of serum inflammatory and EnD markers was repeated in all patients at 12 weeks. In the case of large prolactinomas with optic nerve compression, suprasellar, or para sellar extension, MRI was repeated at three months to reassess the tumor size and extension. If at 12 weeks, PRL continued to be high or there was no decrease in tumor size, the CAB dose was further up-titrated [Figure 1].

Clinical assessment

All the study participants received a detailed clinical assessment. Relevant history focusing on menstrual disturbances (amenorrhea, oligomenorrhea) in women, premature ejaculation or erectile dysfunction (in men), galactorrhea, infertility, decreased libido, weight gain, headache, visual disturbances, and drug intake was obtained from all participants. Subjects were examined for blood pressure (BP) and anthropometry like height, weight, waist circumference (WC), and hip circumference (HC). Measurements were performed with patients barefoot in light clothing and by a single examiner. Height was measured with a wall-mount Stadiometer (SECA 13, Hamburg, Germany). Body weight (in kg) was measured on a digital scale balance (SECA 13, Hamburg, Germany). WC was measured midway between the lowest rib margin and iliac crest while HC was measured at the widest levels over the greater trochanters. A body mass index (BMI) of 23-27.4 Kg/m^2 was defined as overweight and that of 27.5 Kg/m^2 ormoreasobesity.[23] International Diabetes Federation (IDF) criteria were used to define MS.IDF definition of MS includes the presence of central obesity (WC >90 cm in males and >80 cm in females), with any two of the following: hypertension (≥130/85 mm Hg) or FPG ≥100 mg/dl or TG ≥150 mg/dl or HDL ≤50 mg/dl (females) and <40 mg/dl (males).[24] Central hypogonadism in men was defined as suggestive signs and symptoms with serum total testosterone less than 250 ng/dl and luteinizing hormone (LH) <10 IU/L, and in women as cessation of menstrual cycles for more than three months and follicle-stimulating hormone (FSH) of <5 IU/L.[25]

Laboratory measurements

A baseline early morning fasting blood sample was drawn in all patients and controls for the following investigations: Hemoglobulin (Hb), total leucocyte count (TLC), platelet counts, urea, creatinine, bilirubin, alanine aminotransferase (ALT), alkaline transferase (ALP), total protein, albumin, glucose, glycated hemoglobin (HbA1c), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TG), calcium (Ca), phosphorous (PO4), uric acid (UA), triiodothyronine (T3), tetra Iodothyronine (T4), thyroid stimulating hormone (TSH), PRL, FSH, LH, total testosterone, cortisol, ESR, hsCRP, ICAM-1 and VCAM-1. The blood was allowed to clot at room temperature (15-25 degree Celsius) and centrifuged for 15 minutes to obtain haemolysis-free serum. The serum was collected in separate plastic tubes and some parts were stored at -70 degrees Celsius till further analysis.

Measurements of urea, creatinine, bilirubin, ALT, ALP, total protein, and albumin were carried out on same-day automated chemistry analyzer (HITACHI-912). Plasma glucose was also estimated same day by enzymatic method using glucose oxidase and peroxidase on an automated chemistry analyser (HITACHI-912).

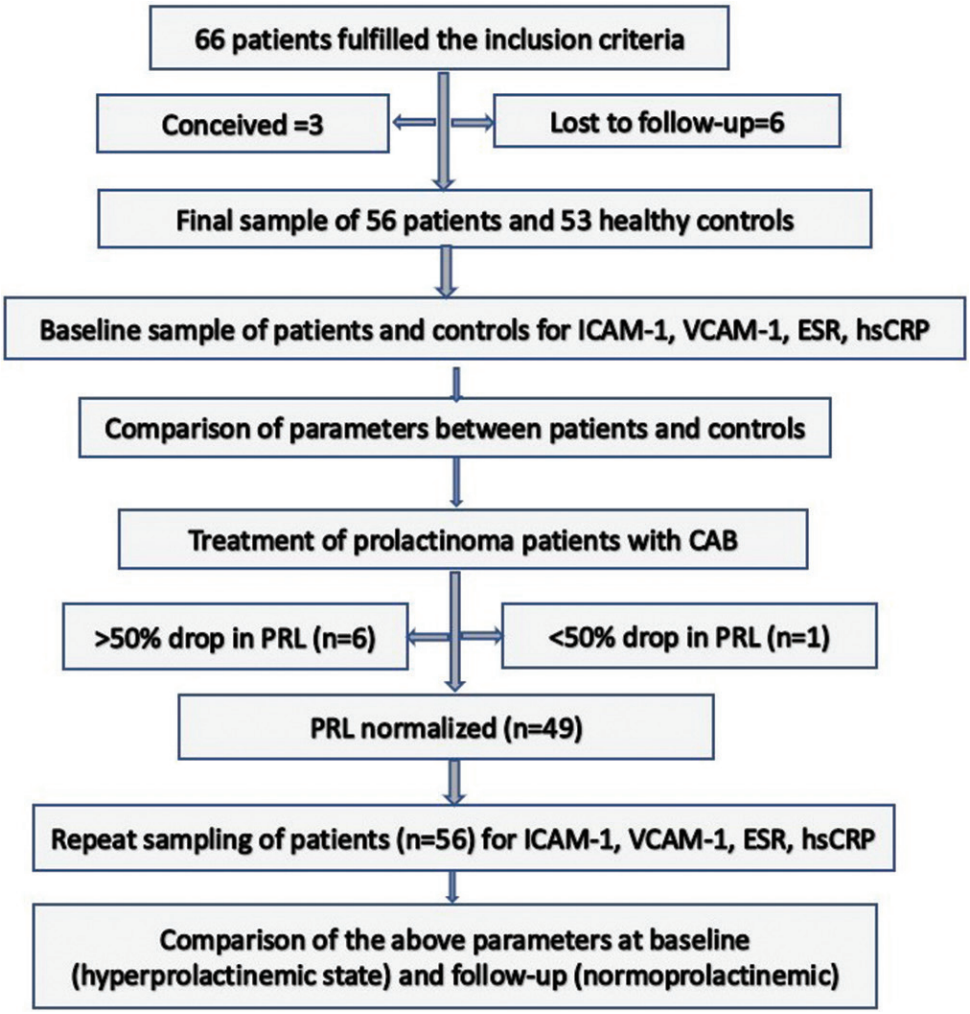

Figure 1: PERT chart showing study design and flow

Lipid parameters were analyzed same day with commercially available enzymatic reagents (Audit Diagnostics, Ireland) adapted to the HITACHI-912autoanalyser. HbA1c was measured using high-performance liquid chromatography on an Avantor A9 HbA1c analyzer with whole blood collected in an ethylene diamine tetra acetic acid (EDTA) tube. Urea, creatinine, bilirubin, ALT, ALP, total protein, albumin, glucose, HbA1c, lipid profile, Ca, PO4, and uric acid were estimated on the same day. Serum PRL [normal range: 1-27 µg/L (women); 1-20 µg/L (men)], TSH, T3, T4, FSH, LH, cortisol, and testosterone were measured by commercial Chemiluminescent Immunoassay (Beckman Coulter Unicel, DXI);

ESR was measured by Westergren's method at one hour. hsCRP was estimated by hsCRP ELISA Kit (Diagnostic Biochem Canada Inc.); the sensitivity of the assay was 10 ng/ml and intra- and inter-assay coefficients of variance were 5% and 9.5%, respectively. ICAM-1 and VCAM-1 were estimated using the ELISA method (Diaclone ELISA kit, France). Sensitivity for ICAM-1 was <0.6 ng/ml, and intra-assay and inter-assay coefficients of variation were 1.03% and 3.93% respectively. For VCAM-1, the sensitivity of the assay was 0.6 ng/ml and intra-assay and inter-assay coefficients of variation were 0.45% and 1.44%, respectively.

Imaging

All patients underwent contrast-enhanced MRI of the sellar, parasellar, and suprasellar region with dynamic contrast studies performed on 1.5 teslas, (Siemens, Magneton Avanto, MR scanner, Germany). Precontrast T1- and T2-weighted spin echo coronal and sagittal sections were acquired using a small field of vision (20 × 25 cm), thin slices (3 mm), and a high-resolution matrix (256 × 512). After a bolus injection of intravenous gadolinium, six consecutive sets of three images were obtained in the coronal plane every 10 seconds to detect small adenomas. All the MRI images were reported by experienced radiologists and cross-checked by pituitary experts (BAL, AIW, MIB). Adenomas were categorized into microprolactinomas if <10 mm or macroprolactinomas, if ≥10 mm in size.[26] Patients were also subjected to perimetry in the case of macroprolactinoma using a Humphrey field analyzer.

Statistical analysis

The statistical software, Statistical package for social sciences (SPSS) version 20 was used to analyze the data. The

Kolmogorov-Smirnov test was applied to test the normality of the sample. The continuous variables have been shown in terms of descriptive statistics i.e., mean and standard deviation for parameters that were normally distributed or by median and interquartile range for non-normally distributed data. Categorical variables were defined in terms of frequency and percentage. The Chi-square test was applied to compare categorical variables between patients and controls. Student's independent t-test and Mann-Whitney test were used to compare the continuous variables between patients and controls for normally and non-normally distributed data respectively. Log transformation of data was done when needed and specified. Adjustment for confounders between patients and controls was carried out with ANCOVA. Partial correlation and regression analysis were also performed and are specified in the text. All results were described on a 5% level of significance i.e., P value < 0.05 considered as significant.

Results

A total of fifty-six consecutive patients with prolactinoma (forty-seven females and nine males) and fifty-three (age and sex-matched) apparently healthy controls (43 females and 10 males) were enrolled. The mean age of patients was 29.26 ± 7.90 years and that of controls was 28.05 ± 7.26 years (p = 0.469). The median duration of symptoms was 12 months (IQR 4-16 months) and there was no difference in symptom duration between micro and macroprolactinomas (p = 0.24). Twenty-nine of forty-seven (62%) females had microadenoma and only 18 had macroadenoma; in contrast, all of the nine male patients had macroadenoma. The median of the maximum prolactinoma diameter was 6.5 mm (IQR 5-8 mm) in microadenomas and 20 mm (IQR 13-32) in macroadenomas. Perimetry was performed in 27 macroadenoma patients and was abnormal in 13 patients (48.1%). One female patient had associated primary hyperparathyroidism and was provisionally diagnosed with multiple endocrine neoplasia type 1 (MEN1) syndromes. The most common symptoms in females were galactorrhoea (75%), menstrual irregularity (71%), headache (55%), and infertility (38%), while in males, 90% had headaches followed by visual field abnormalities in 77%, weight gain in 55% and infertility in 27.9%. Median PRL in patients was 199 ng/ml (range 52-14242 ng/ml, IQR 107-577.50). Overall, twenty-two patients (39.28%) were having central hypogonadism: men 66.6% (n = 6 out of 9), women 34% (n = 16 out of 47).

The mean BMI was significantly higher in patients than controls; 26.49 ± 5.23 (kg/m^2) in patients vs 22.58 ± 3.97 (kg/m^2) in controls, P < 0.001 [Table 1]. Twenty patients with HPL were obese as against four controls. Around 52% (n = 29) patients had MS as against 13.2% (n = 7) controls. Hypertension was detected in five (9%) patients and two (4%) controls, while DM was diagnosed in four (7%) prolactinoma patients and one (1.88%) control. Among non-obese prolactinoma patients (n = 36), MS was present in 38.8% (n = 14) as against 8.1% (n = 4) non-obese controls (p = 0.001). Baseline hematological and biochemical parameters of patients and controls were comparable. HPL patients had significantly higher levels of BGF, HbA1c, UA, TC, TG, and LDL-C. Mean serum cortisol was higher in patients than in controls (12.25 ± 3.31 in patients vs 10.30 ± 2.42 ug/dl in controls, P = 0.001) [Table 1].

Table 1: Anthropometric, metabolic, inflammatory and EnD parameters in prolactinoma patients and controls

Parameter	Cases (n=56)	Controls (n=53)	P
Age (years)	29.26±7.90	28.05±7.26	0.469
Weight (kg)	70.428±14.93	59.84±11.37	<0.001
BMI (kg/m^2)	26.49±5.23	22.58±3.97	<0.001
WC (cms)	89.89±13.51	79.45±9.52	<0.001
WHR	0.97±0.08	0.91±0.08	0.001
HbA1c (%)	5.45±0.99	4.84±0.47	0.001
BGF (mg/dl)	96.87±29.09	85.94±10.51	0.011
UA (mg/dl)	4.83±1.31	3.67±1.39	0.002
TC (mg/dl)	172.91±39.70	149.01±39.87	0.002
TG (mg/dl)	159.10±57.67	132.73±53.90	0.015
LDL-C (mg/dl)	112.19±28.04	96.64±24.81	0.003
HDL-C (mg/dl)	41.01±6.69	41.67±9.50	0.674
PRL$^{\#}$(ng/ml)	199 (107-577.50)	11.4 (9.3-15)	0.006
T3 (ng/ml)	1.28±0.41	1.27±0.37	0.886
T4 (ug/dl)	8.25±1.60	8.31±1.04	0.883
TSH (uIU/ml)	3.45±2.18	3.60±2.05	0.721
FSH (IU/L)	6.46±9.14	6.04±8.35	0.803
LH (IU/L)	4.65±4.08	4.38±1.32	0.649
Cortisol (ug/dl)	12.25±3.31	10.30±2.42	0.001
Testosterone^{+}(ng/dl)	339.50±214.22	619.52±232.83	0.016
ICAM-1$^{\#}$ (ng/ml)	1331.95 (803.43-1825.99	753.04 (402.04-871.55)	<0.001*
VCAM-1$^{\#}$ (ng/ml)	971.35 (695.03-1285.23)	634.56 (177.49-946.50)	0.001*
ESR (mm Ist hour)	23.61±8.67	14.49±6.97	<0.001*
hsCRP$^{\#}$ (mg/L)	3.61 (1.69-9.01)	1.45 (0.46-2.60)	0.001*

$^{\#}$Expressed as median and IQR, Other parameters as mean±SD, $^{+}$males only, *After adjusting for BMI

Mean ESR in patients was higher than in controls [23.61 ± 8.67 (mm Ist hour) in patients as against 14.49 ± 6.97 (mm Ist hour)] in controls, P = 0.001. The median hsCRP was significantly higher in patients than controls [3.61 mg/L (IQR 1.69-9.01) in patients and 1.45 mg/L (IQR 0.46-2.60) in controls], P = 0.001. The median ICAM-1 was 1331.95 ng/ml (IQR 803.43-1825.99) in patients and 753.04 ng/ml (IQR 402.04-871.55) in controls, P < 0.001, while as the median VCAM-1 in patients was 971.35 ng/ml (IQR 695.03-1285.23) as against 634.56 ng/ml (IQR 177.49-946.50) in controls, P < 0.001) [Table 1]. On subgroup analysis, even non-obese and eugonadal patients had significantly higher HbA1c, BGF, LDL-C, UA, ESR, hsCRP, ICAM-1 and VCAM-1 than normal BMI and eugonadal controls respectively [Tables 2 and 3]. After adjusting for BMI, there was no significant difference in mean WC, BGF, TC, TG, LDL-C, and HDL-C

Table 2: Anthropometric, metabolic, inflammatory and EnD parameters of non-obese prolactinoma patients and controls

Parameter	Cases ($n=36$)	Controls ($n=49$)	*P*
Age (yrs.)	29.26±8.45	27.77±7.13	0.877
Weight (kg)	62.83±10.4	57.93±9.31	0.026
BMI (kg/m^2)	23.57±3.06	21.80±3.02	0.011
WC (cm)	84.52±10.57	78.36±9.01	0.005
WHR	0.94±0.07	0.91±0.08	0.173
HbA1c (%)	5.35±0.76	4.8±0.46	<0.001
BGF (mg/dl)	93.16±21.65	85.51±10.39	0.034
TC (mg/dl)	165.41±35.80	148.89±41.27	0.057
TG (mg/dl)	144.80±47.34	130.44±55.04	0.209
LDL-C (mg/dl)	107.80±24.44	96.5±25.69	0.045
HDL-C (mg/dl)	41.72±7.50	42.10±9.7	0.845
UA (mg/dl)	4.61±1.25	3.62±1.39	0.001
Testosterone$^+$(ng/dl)	334.66±102.03	681.50±115.43	0.009
Cortisol (ug/dl)	12.69±3.80	10.39±2.42	0.001
PRL$^\#$ (ng/ml)	164.01 (110.02-458.03)	11.60 (9.75-15.25)	<0.001
ESR (mm Ist hr)	22.60±13.71	13.71±5.89	<0.001*
hsCRP$^\#$ (mg/L)	2.70 (1.4-6.2)	1.38 (0.45-2.40)	<0.001*
ICAM-1$^\#$ (ng/ml)	1362.56 (789.34-1840.17)	728.20 (328.34-863.12)	<0.001*
VCAM-1$^\#$ (ng/ml)	969.23 (751.10-1195.26)	636.45 (176.12-910.36)	0.001*

$^\#$Expressed as median and IQR, Other parameters as mean±SD, $^+$males only, *After adjusting for BMI

Table 3: Anthropometric, metabolic, inflammatory and EnD parameters of eugonadal prolactinoma patients and controls

Parameter	Cases ($n=34$)	Controls ($n=53$)	*P*
Age (yrs.)	30.05±8.97	28.05±7.26	0.279
Weight (kg)	68.73±14.77	59.84±11.37	0.004
BMI (kg/m^2)	26.31±5.71	22.58±3.97	0.002
WC (cm)	88.85±13.24	79.45±9.52	0.001
WHR	0.96±0.09	0.91±0.08	0.015
HbA1c (%)	5.42±0.91	4.84±0.47	<0.001
BGF (mg/dl)	98.08±29.90	85.94±10.51	0.014
TC (mg/dl)	167.76±38.35	149.01±39.87	0.018
TG (mg/dl)	147.94±48.78	132.73±53.90	0.084
LDL-C (mg/dl)	107.97±25.52	96.64±24.81	0.012
HDL-C (mg/dl)	40.91±6.49	42.67±9.50	0.720
UA (mg/dl)	4.81±1.38	3.68±1.36	0.001
Testosterone$^+$ (ng/dl)	520.50±118.02	681.50±115.42	0.37
Cortisol (ug/dl)	12.49±2.76	10.30±2.42	<0.01
PRL$^\#$ (ng/ml)	144.02 (90.43-244)	12.45 (9.98-15.50)	<0.001
ESR (mm Ist hr)	22.91±8.87	14±5.76	<0.001*
hsCRP$^\#$ (mg/L)	2.17 (1.31-5.38)	1.31 (0.44-2.56)	0.002*
ICAM-1$^\#$ (ng/ml)	1433.75 (687.34-1874.28)	754.04 (403.18-1842.73)	<0.001*
VCAM-1$^\#$ (ng/ml)	969.28 (647.23-1222.30)	638.70 (216.23-929.68)	0.001*

$^\#$Expressed as median and IQR, Other parameters as mean±SD, $^+$males only,*After adjusting for BMI

between patients and controls. However mean HbA1c, UA, cortisol, testosterone, ESR, hsCRP, ICAM-1, and VCAM-1 were significantly higher in patients than in controls, even after adjusting for BMI [Table 1].

On partial correlation analysis (controlled for age, gender, BMI, and presence of hypogonadism), serum PRL concentration (log10) correlated positively with maximum adenoma diameter (r = 0.698, $P < 0.001$) and hsCRP ($r = 0.377$, $P = 0.014$), while as ICAM-1 and VCAM-1 correlated positively with hsCRP ($r = 0.332$, $P = 0.017$ and $r = 0.359$, $P = 0.01$ respectively). On multivariate regression analysis, only hsCRP was found to predict change in ICAM-1 and VCAM-1 ($p < 0.001$, R^2 0.352).

After treatment with CAB, at 12 weeks, serum PRL levels normalized in 49 patients, fell by more than half in six, and decreased by less than 50% in one patient. In addition, there was a significant decrease in weight, BMI, WC, HbA1c, BGF, UA, TC, TG, and LDL-C while HDL-C did not change significantly. Mean ESR decreased from 23.57 ± 8.89 to 19.31 ± 6.60 mm Ist hour ($p < 0.001$), while median hsCRP decreased from 3.48 mg/L (IQR 1.63-8.21) to 2.13 mg/L (IQR 0.42-6.09), $P < 0.001$. Median ICAM-1 and VCAM-1 decreased significantly after CAB treatment [Table 4]. At 12 weeks, MRI was repeated in 20 patients with macroprolactinoma, out of which prolactinoma size decreased by more than 50% in 12 patients and less than 50% in seven patients. One patient had no change in size with progressive worsening of visual symptoms and was subjected to surgery.

Discussion

The effect of PRL on metabolic abnormalities is variable. Physiological levels of PRL are associated with improved insulin sensitivity and decreased risk of T2DM while HPL promotes fat deposition with its consequences.[27] In this study, the prevalence of metabolic abnormalities like obesity, dyslipidemia, hypertension, DM, and MS were higher in untreated prolactinomas as compared to age and sex-matched healthy controls.

Though weight gain/obesity is an important component of HPL,[4,7,28,29] metabolic parameters like BGF, HbA1c, UA, LDL, and TG were higher in non-obese patients than non-obese controls in this study, as has been described previously.[5,6,9,13,30] In addition, patients with prolactinomas had higher inflammatory markers than controls, as has been documented by previous studies. This increased inflammation in HPL could be attributed to adiposity, hypogonadism, or direct stimulatory effects of PRL on immune cells.[16,22,31,32] Nonetheless non-obese and eugonadal patients in this study had worse inflammatory markers than non-obese and eugonadal controls, indicative of pro-inflammatory effects of HPL.[32] In addition, we observed serum PRL (after controlling for age, sex, BMI, and hypogonadism) correlated positively with hsCRP as was documented in previous studies.[15,22] Contrary to this, few authors could not find a significant correlation between serum inflammatory markers and serum PRL or to hypogonadism, likely because of the

Table 4: Baseline and follow-up parameters of patients after 12 weeks of treatment (n=56)

Parameter	Baseline	Follow up	P
Weight (kg)	70.42±14.93	67.28±13.51	<0.001
BMI (kg/m^2)	26.49±5.23	25.7±5.11	0.001
WC (cms)	89.89±13.51	87.06±12.1	<0.001
WHR	0.97±0.08	0.95±0.08	0.006
HbA1c (%)	5.45±0.99	5.08±0.49	<0.001
BGF (mg/dl)	96.87±29.09	86.3±9.73	0.003
UA (mg/dl)	4.83±1.31	3.90±1.11	0.011
TC (mg/dl)	172.91±39.70	147.89±42.71	<0.001
TG (mg/dl)	159.10±57.67	141.36±51.48	0.025
LDL-C (mg/dl)	112.19±28.04	101.81±26.1	0.008
HDL-C (mg/dl)	41.01±6.69	41.34±5.03	0.333
ESR (mm Ist hour)	23.61±8.67	19.31±6.60	<0.001
hsCRP$^{\#}$ (mg/L)	3.61 (1.69-9.01)	2.13 (0.42-6.09)	<0.001
ICAM-1$^{\#}$ (ng/ml)	1331.95 (803.43-1825.99)	1101.35 (568.45-1667.58)	<0.001
VCAM-1$^{\#}$ (ng/ml)	971.35 (695.03-1285.23)	805.01 (453.95-1058.02)	0.001

Values expressed as mean±SD unless specified, $^{\#}$Expressed as median and IQR,

small sample size and with comparatively lesser mean serum PRL.[16,33,34]

The EnD is the precursor event in atherosclerosis where dysfunctional endothelial cells promote vasoconstriction and migration of macrophages to the vessel walls leading to atheroma formation.[18] Data on serum markers of EnD in prolactinomas is limited to a few studies where serum E-selectin and heart-type fatty acid binding protein (H-FAPB) did not differ different between patients and controls.[13,22] These studies were based on a small patient population, including patients with lower levels of obesity and lower levels of mean hsCRP. However, when EnD was measured as FMD of the brachial artery, untreated prolactinoma patients had lower vasodilatory response than controls.[15,16] Likewise in the current study, EnD markers were higher in prolactinomas and positively correlated to hsCRP, which concurs with earlier studies that low-grade inflammation is associated with EnD.[35,36] Though studies have documented a direct correlation between serum PRL and decreased FMD,[15,16] no significant correlation was observed between serum PRL and EnD markers in the current study. This discrepancy is akin to differing actions of HPL and presumed mechanisms of EnD. PRL directly modulates nitric oxide synthesis by endothelial cells,[37,38] causes increased platelet aggregation[33], and promotes vascular stiffness.[12,39] Secondly HPL promotes inflammation which in turn leads to an increase in serum ICAM-1 and VCAM-1.[32,40] Theoretically hypogonadism may promote EnD in HPL[41] as was reported by Yavuz *et al*.[16] However, we observed that eugonadal patients also had higher ICAM-1 and VCAM-1 as is reported earlier that EnD is not dependent on gonadal function.[15] Another significant finding in the present study was the presence of higher serum cortisol in patients than in controls which correlated with BMI. PRL stimulates the growth of adrenal cortical cells and serum PRL is found to correlate with serum cortisol and dehydroepiandrosterone sulfate concentrations in patients with PCOS and prolactinomas suggestive of adrenotrophic action of PRL.[42-44]

After 12 weeks of CAB treatment, serum PRL normalized at 87.5% with improvement in metabolic parameters which is consistent with previous studies, varying in duration from 2-12 months, indicating that metabolic improvement may or may not be associated with a decrease in BMI.[3-6,30,45-47] The decrease in IM (ESR, hsCRP) after 12 weeks of DA treatment follows the trajectory of improvement in metabolic parameters as documented earlier.[5,16,22,46] Similarly, ICAM-1 and VCAM-1 decreased after 12 weeks of CAB treatment. Though data is limited on EnD in prolactinomas, E-Selectin (a marker of EnD) decreased after treatment of prolactinoma with DA and similarly, FMD of the brachial artery which is also a surrogate of EnD improved after PRL normalization and the improvement in EnD may or may not correlate to decrease in serum PRL.[16,22]

To conclude, PRL promotes EnD by inducing inflammation which then upregulates adhesion molecules on endothelial cells and thereby may lead to atherosclerosis. Furthermore, treatment with DA and normalization of serum PRL significantly improves BMI, metabolic abnormalities, inflammatory markers, and EnD markers.

Strengths and limitations

The main strength of this study is its sample size and this is probably the largest study on EnD in patients with prolactinoma. However, we could have included all HPL patients to conclude if ED is because of HPL per se, regardless of etiology or specific to prolactinomas. Also, the confounding effects of weight loss and decrease in inflammatory markers on ICAM-1 and ICAM-1 could not be ascertained. The effects of change in sex steroids on metabolic parameters could not be fully studied.

Conclusion

A higher prevalence of obesity, metabolic abnormalities, and low-grade inflammation (characterized by increased ESR and hs-CRP) and EnD (evidenced by raised levels of ICAM-1 and VCAM-1) is seen in untreated HPL because of prolactinoma. The EnD in a hyperprolactinemic state is directly related to the level of inflammatory markers.

Ethics approval

The study was conducted according to the 1975 Helsinki declaration and was approved by the institutional ethics committee vide protocol number- RP 14/2019.

Informed consent to participate

Written informed consent was obtained from all participants of the study.

Informed consent to publish

All participants provided written informed consent to publish the data.

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Conflicts of interest

There are no conflicts of interest.

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