Assessment of serum CX3CL1/fractalkine level in Han Chinese girls with anorexia nervosa and its correlation with nutritional status: a preliminary cross-sectional study

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ABSTRACT

The chemokine (C-X3-C motif) ligand 1 (CX3CL1), also named fractalkine (FKN), has been implicated in psychiatric disorders and functions as a novel adipocytokine. However, no attention has been paid to the role of FKN in anorexia nervosa (AN). The current study was performed to explore FKN levels in AN to determine its role in the involvement of AN. A total of 96 girls aged 11–18 years with AN (n=34), healthy controls (HC; n=32) and simple obesity (OB, n=30) were enrolled in the cross-sectional study. Blood samples were collected during the fasting state. Serum FKN concentrations were determined using ELISA. The skinfold thickness (TSF) of the biceps and triceps as well as mid-arm muscle circumference (MAMC) were used to determine the nutritional status. Our results showed that serum FKN levels were significantly lower in the AN group than in the control and OB groups. After adjusting for body mass index (BMI), FKN concentrations in the AN group were statistically higher than in the HC and OB groups. Significant correlations between serum FKN and body weight, BMI, Cole index and serum insulin were observed. In addition, serum FKN levels were positively related to TSF and MAMC in all subjects. Serum FKN concentrations are attenuated in girls with AN compared with healthy adolescents and are positively related to nutritional status. The lower FKN levels may be regulated by nutrition status and response to starvation. After adjusting for BMI, higher FKN levels may reflect that persistent inflammation is present in patients with AN.

Significance of this study

What is already known about this subject?

- Inflammation has been involved in the pathogenesis of anorexia nervosa.
- The chemokine (C-X3-C motif) ligand 1 (CX3CL1), also named fractalkine (FKN), has been implicated in psychiatric disorders and functions as a novel adipocytokine.
- FKN could regulate diet-associated neuroinflammation.

What are the new findings?

- Serum FKN levels were significantly lower in the anorexia nervosa (AN) group than in control and single obesity (OB) girls.
- After adjusting for body mass index (BMI), FKN concentrations in the AN group were statistically higher than in the healthy controls and OB groups.
- FKN levels were positively correlated with nutritional status in adolescents.

How might these results change the focus of research or clinical practice?

- The lower FKN levels may be regulated by nutrition status and response to starvation. After adjusting for BMI, higher FKN levels may reflect that persistent inflammation is present in patients with AN.

INTRODUCTION

Anorexia nervosa (AN) is a chronic psychosomatic syndrome occurring mainly among adolescent females with eating disorders and inappropriate nutrition habits who refuse to maintain a minimally normal body weight.[1] AN is often reported to have the highest mortality rate among all psychiatric disorders.[2] Although the pathogenesis remains largely unknown, genetic, neurobiological and environmental factors have been involved in the etiology of AN.[3]

In recent years, an increasing number of investigators embracing a consensus of opinion related to the pathogenesis of AN in their studies have been devoting much attention to the importance of an inflammatory component.[4] Inflammatory cytokines are fundamental regulators of body metabolism; when they become dysregulated, they create physiological chaos leading to the development of a number of autoimmune, metabolic and psychiatric disorders.[5] Cytokines released from immune cells are vital to communication between the central nervous and immune systems.[6] In this sense, cytokines may play an important role both in the etiology of AN and the pathogenesis of related subsequent complications. A growing body of evidence has indicated the presence of

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a long-standing proinflammatory state in AN. Increases in inflammatory mediators such as cytokines were extensively demonstrated in AN, with interleukin (IL)-6 (IL-6), tumor-necrosis factor-α (TNF-α) and IL-1 being the most commonly associated with AN.[7 8] The causes for this proinflammatory state are largely unknown, although both genetic and environmental factors have been proposed as relevant.[9 10]

Chemokines are a superfamily of small, cytokine-like proteins that induce cytoskeletal rearrangement, adhesion and directional migration in various cells through their interaction with G-protein-coupled receptors.[11] In the central nervous system, chemokine signaling plays a pivotal role in neuropathological processes, in addition to the relevant role played under physiological processes, such as neuronal migration and modulation of synaptic transmission.[12] According to the differences of conserved cysteine motifs, chemokines are classified structurally into four basic subfamilies: C, CC, CXC and CX3C.[13] Fractalkine (FKN) (CX3CL1), the sole CX3C chemokine that signals through a single known receptor (CX3CR1), is implicated in recruitment and adhesion of both monocytes and T cells in various diseases,[14] existing in soluble and transmembrane forms.[15]

It has been identified that severe malnutrition in AN is also related to changed glucose and lipid metabolism and multiple endocrine perturbations.[16] Some of these abnormalities may be linked to altered adipocytokine production.[17] CX3CL1-CX3CR1 axis functions a novel inflammatory adipose chemokine system that modulates monocyte adhesion to adipocytes.[18 19] It is expressed in obese adipose, upregulated in adipose and adipocytes under inflammation status.[18] It could also modulate monocyte–adipocyte interactions and correlate with metabolic syndrome.[18 20] Recent studies have shown that FKN could be induced in white adipose tissue and hypothalamus neurons in mice by a high-fat diet.[21 22] Inhibiting hypothalamic FKN or CX3CR1 deficient mice is resistant to diet-induced inflammatory activity,[23] implicating its role in the regulating of diet-associated neuroinflammation.

Understanding the involvement of FKN in AN may potentially open new treatment possibilities. However, so far, no reports available have described the relationships between FKN and AN. Therefore, in this study, we measured the serum FKN levels in patients with AN to determine its role in AN.

SUBJECTS AND METHODS

A total of 34 girls diagnosed with the restrictive form of AN according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) classification[24] were enrolled in the cross-sectional study. All the girls experienced psychological evaluation and psychiatric consultation by two experts in our hospital. The control group included 32 healthy, regularly menstruating female volunteers with BMI-SD score (SDS) between −2.0 and +2.0 who were recruited from high schools. The Cole index was also calculated to assess nutritional status of enrolled participants defined by the following: wasting <75%; undernourished 75–85%; mildly undernourished 85–90%; adequately nourished 90–100%; overnourished >110%.[25] We also drafted 30 girls with simple obesity (OB) defined as BMI-SDS >2.0 with normal glucose tolerance for the black control. This study was approved by the Ethical Committee in our hospital and written informed consent was obtained from all examined subjects and their parents prior to participation.

Simulation study for evaluating the sample size

We also ran a simulation study to evaluate the sample size enrolled in our study by using Power Analysis and Sample Size (PASS) 2008 Statistical Software (Utah, USA). All the obtained parameters and conditions corresponding to the study design were taken into account. The acceptable statistic power was set at 0.80.

Definition of nutritional status

The skinfold thickness (TSF) of the biceps and triceps was identified with a conventional skinfold caliper using standard techniques.[26] The TSF was computed by using the average of two skinfolds (on both arms). The mid-arm circumference was measured with a tape measure, and the mid-arm muscle circumference (MAMC) was calculated as follows:[27]

$$\text{MAMC (cm)} = \text{mid-armcircumference (cm)} - 3.142 \times \text{TSF (cm)}$$

Laboratory methods

Blood was drawn between 07:00 and 08:00 after at least a 12 hours fast. In the control and OB groups, blood samples were obtained in the follicular phase of the menstrual cycle. Serum FKN levels were measured using specific ELISA kits (R&D Systems, Minneapolis, Minnesota, USA), according to the manufacturer's protocol. Each sample was tested in duplicate and reflected the mean of the two measurements. The concentrations were determined based on the standard curve for a series of dilutions using the standards available in the kit (recombinant human FKN). The intra-assay and interassay coefficients of variation in the FKN ELISA kit were 4.2% and 6%, respectively. The examination of insulin level was performed by immunoradiometric assay, using the kit by Immunotech (Germany).

Statistical analyses

Statistical analysis was carried out with SPSS V.18.0 software (SPSS Statistics, Chicago, Illinois, USA). Data normality was analyzed using the Kolmogorov-Smirnov test. Normally distributed continuous variables were expressed as the mean value±SD, and non-normally distributed continuous variables were expressed as the median (IQR). Values for patients with anorexia, controls and OB participants were compared by means of one way analysis of variance. Bartlett's test was used to examine the homogeneity of group variances, followed with Tukey or Tamhane post hoc tests, where appropriate. BMI was considered a confounding factor and introduced as a covariable. Pearson's or Spearman's correlation was employed to measure the strength association between two variables. All the tests used a CI of 95%, and the differences were considered significant when $p<0.05$.

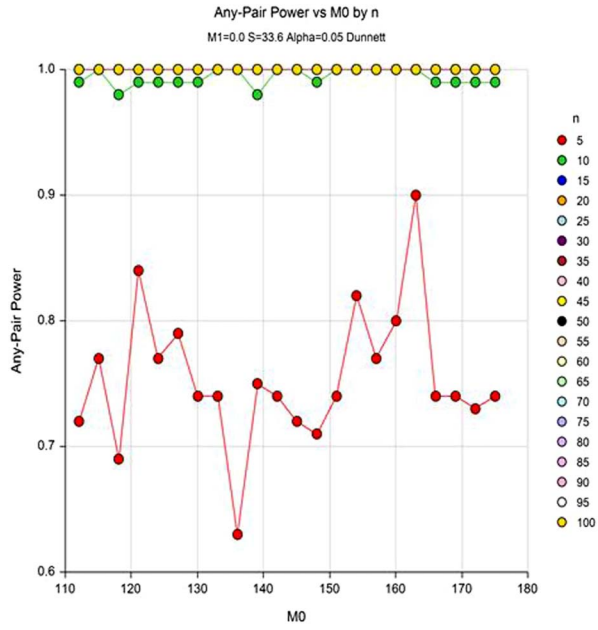

Figure 1 Statistic power analysis for the enrolled sample size according to our obtained results.

RESULTS

There were no significant differences in age of girls among the AN, HC and OB groups. The body weight and BMI in the AN group were significantly lower compared with the healthy control (38.73±1.52 vs 51.71±3.84 kg, p<0.001) and OB (38.73±1.52 vs 84.73±7.97 kg, p<0001) groups. The BMI-SDS in girls with AN was significantly lower compared with healthy control (−2.67±0.15 vs 0.19±0.16, p<0.001) and OB participants (−2.67±0.15 vs 7.14 ±0.45, p<0.001). According to the results of the simulation study, the sample size of each group over 30 is adequate to gain an acceptable power of 0.80 (figure 1). Therefore, the sample size in our study is large enough as the sample size of the three groups are 34, 32 and 30, respectively.

Serum FKN levels in the AN group were significantly lower than in the healthy control (112.06±20.09 vs 138.84±17.49 pg/mL, p<0.001) and OB groups (112.06 ±20.09 vs 176.29±25.06 pg/mL, p<0.001). We also observed a significant difference between OB and healthy subjects (138.84±17.49 vs 176.29±25.06 pg/mL, p<0.001) (figure 2).

However, when FKN was calculated per BMI (FKN/BMI), serum FKN values in girls with AN were significantly higher compared with levels obtained in the healthy control (7.50±1.39 vs 6.88±0.91, p<0.05) and OB girls (7.50±1.39 vs 5.55±0.78, p<0.0001) (figure 3).

Significant associations between serum FKN levels and BMI (r=0.747, p<0.0001), Cole index (r=0.744, p<0.0001), insulin levels (r=0.661, p<0.0001) and MAMC (r=0.601, p<0.0001) in all examined subjects were observed (AN, H, OB) (figure 4). Conversely, there were no significant associations between these parameters in each group.

DISCUSSION

This study investigated the possible involvement of FKN in girls with AN. To the best of our knowledge, this is the first published report on serum FKN concentrations in AN. We found that serum FKN levels were significantly lower in girls with AN than in age-matched controls and OB female teenagers. After adjusting for BMI, FKN concentrations in the AN group were statistically higher than in the HC and OB groups. In addition, FKN levels were positively related to body weight, BMI, insulin levels and nutritional status.

First, we observed that FKN levels were significantly lower than healthy control and OB subjects. This phenomenon can be shown from the aspect that FKN serves as an adipokine and changes with the alternation of lipid metabolism,[27] which is shown to be increased in OB, positively associated with insulin resistance and predicts metabolic syndrome in adults.[20 28] Upregulation of FKN has been reported as a pivotal mechanism in processes of leucocyte recruitment, early adipose inflammation, insulin resistance, etc.[29] After adjusting for BMI, FKN concentrations in the AN group were statistically higher than in the HC and OB groups, implicating that persistent inflammation responses are present in AN. The role of inflammation in AN is supported by animal experiments and clinical findings. In the rats model, inflammation could contribute to early satiety through interaction with hypothalamic neuropeptides.[30 31]

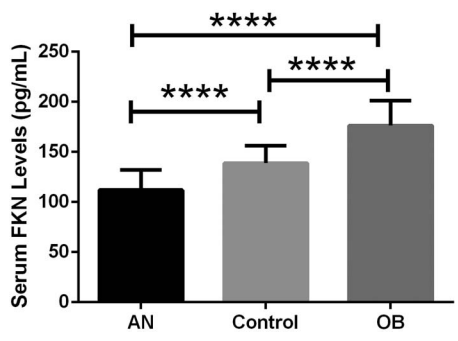

Figure 2 Comparison of serum FKN levels (pg/mL) among the AN, healthy control and OB groups. ****p<0.0001 vs Control and OB groups. Data were expressed as mean±SD.

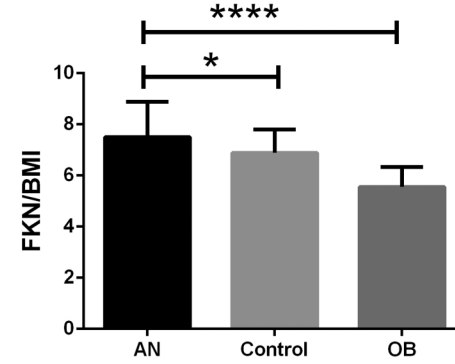

Figure 3 Comparison of serum FKN/BMI (pg/mL/kg/m^2) values among the AN, healthy control and OB groups. *p<0.05 vs HC group; ****p<0.0001 vs OB group. Data were expressed as mean ±SD.

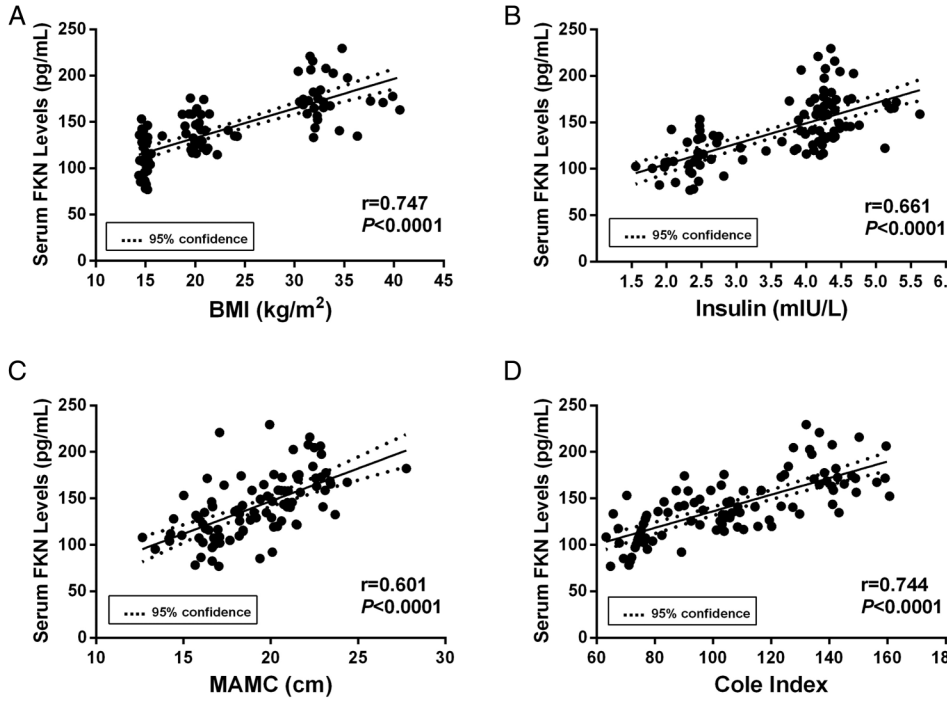

Figure 4 Diagram showing the positive association of serum FKN levels (pg/mL) in all subjects with BMI (A), serum insulin levels (B), MAMC (C) and Cole index (D). AN, anorexia nervosa; BMI, body mass index; FKN, fractalkine; MAMC, mid-arm muscle circumference; OB, obesity.

Inflammation cytokines including IL-6 and IL-1β could interact with leptin, a key regulator of ingestion behaviors involved in AN, to perform anorexigenic effects, while TNF-α could promote the production of anorexigenic peptides.[32 33] On the other hand, in many chronic human diseases, inflammation is found to partially account for the decreased appetite and reduced hunger in patients with anorexia.[32 34] In some cases of AN, significant weight gain and mental improvement are observed after using immunosuppressive therapies.[35 36] One previous study demonstrated that serum FKN levels are elevated in patients with moderate–severe depression,[37] a disorder that shared similar genetic and environmental factors with AN,[38] suggesting that an increase in FKN may aggravate a psychopathological vicious cycle.

There are several important limitations in our work. First, this cross-sectional study was conducted as a single-center trial in our hospital among Han Chinese girls. Therefore, random studies in multiple centers are necessary. Second, we did not examine the FKN messenger RNA or protein expressions in subcutaneous adipose tissues due to ethical reasons, although it may provide much more valuable information for the potential role of FKN in the AN process. Third, we did not examine other inflammatory cytokines such as TNF-α or IL-6, etc, failing to obtain the information of their associations with the serum FKN levels.

Collectively, we found that compared with healthy girls, serum FKN levels are attenuated in girls with AN. Conversely, obese girls have elevated FKN levels. When calculated per BMI (FKN/BMI), the results in the control and OB groups were lower than in the AN group. Our findings support the possible role of FKN in accordance with body weight and regulation of appetite in adolescent females. Meanwhile, FKN may also exert as a cytokine to participate the inflammation process in addition with other systemic investigation of FKN secretion to different metabolic status deserves further study in the future.

Table 1 Clinical data of the girls in all groups

	AN (n=34)	HC (n=32)	OB (n=30)
Serum FKN levels (pg/mL)	112.06±20.09*	138.84±17.49	176.29±25.06
Age (years)	15.71±1.23	16.04±0.92	15.85±1.13
Body weight (kg)	38.73±1.52*	51.71±3.84	84.73±7.97
Height (cm)	162.53±4.12	163.16±3.72	162.65±3.94
BMI (kg/m^2)	15.01±0.42*	20.57±1.31	33.40±2.74
BMI-SDS	−2.67±0.15*	0.19±0.16	7.14±0.45
Cole index (%)	75.52±6.56*	104.56±9.04	140.42±10.42
Insulin (mIU/L)	2.38±0.33*	4.20±0.32	4.47±0.43
TSF (mm)	11.82±1.35*	13.81±2.02	15.91±2.12
MAMC (cm)	16.6±1.8*	19.7±2.5	22.2±2.4

Cole index, patient's BMI expressed as a percentage of the median BMI for age and sex of children: <75%=emaciation, 75–85%=undernutrition, 85–90% =mild undernutrition, 90–100%=normal range; >110%=overnutrition.
*p<0.0001, AN versus HC and AN versus OB.
AN, anorexia nervosa; BMI, body mass index; FKN, fractalkine; HC, healthy control; MAMC, mid-arm muscle circumference; OB, obesity; SDS, SD score; TSF, skinfold thickness.

Contributors SZ and JC researched the literature and conceived the study. All authors were involved in protocol development, gaining ethical approval, patient recruitment and data analysis. They also approved the final version of the manuscript.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethical Commitee of the second Xiangya Hospital of Central South University.

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