

Association between anorexia nervosa and other specified eating or feeding disorders and paranoia in adolescents: what factors are involved?

Associazione tra anoressia nervosa e altri disturbi specifici dell'alimentazione o della nutrizione e paranoia negli adolescenti: quali fattori sono coinvolti?

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SUMMARY. Introduction. In eating disorders, the association with other psychiatric symptoms is of particular interest. The association between anorexia nervosa and psychotic symptoms is less studied than that with affective disorders (anxiety/depression). The aim of this study is to describe a psychotic symptom (paranoia) in adolescents with anorexia nervosa looking at several potential explicative associated factors: eating disorder symptoms, body image concerns, depression and social anxiety. Our hypothesis is that paranoia in anorexia nervosa patients is better explained by the concomitant depression and social anxiety symptoms than the core symptoms of the disease (eating disorder symptoms or body image concerns). **Methods.** This is a retrospective study. Consecutive, help-seeking adolescents, admitted to the Eating Disorder Service of the Integrated Pediatric Care Department, Luigi Vanvitelli University Hospital, constituted the sample. Data was obtained through retrospective collection of clinical interviews and self-report questionnaires, used for the routine assessment of these patients, administered by trained and expert child and adolescent psychiatrists, they were the Eating Attitude Test-26 (EAT-26), the Body Uneasiness Test-A (BUT-A), the Children Depression Inventory (CDI), the Liebowitz Social Anxiety Scale-Children and Adolescents (LSAS-CA) and the paranoia subscale of the Specific Psychotic Experiences Questionnaire (SPEQ). **Results.** We obtained data from 92 adolescents with anorexia nervosa and other specified feeding or eating disorder (OSFED). Our regression model explained that paranoia (SPEQ-paranoia subscale) in this population was better explained by depression (CDI) (coefficient= 0.415 SD: 0.210, $p=0.052$) and social anxiety symptoms (LSAS-CA) (coefficient= 0.253 SD: 0.060; $p<0.001$) than eating disorder symptoms (EAT-26) (coefficient= 0.092 SD: 0.107; $p=0.398$) and body image concerns (BUT-A) (coefficient= 1.916 SD: 2.079; $p=0.359$). **Conclusions.** This study has some theoretical, clinical and treatment implications. It is important to carrying out screening for the presence of psychotic symptoms in patients with eating or feeding disorders. These symptoms and associated factors (depression and social anxiety) may complicate the clinical picture of the disease with the need, in certain cases, of psychopharmacological drugs and, among these, anti-psychotics. Finally in the psychotherapy context, paranoid ideas may be subject of treatment for patient with anorexia nervosa.

KEY WORDS: anorexia, paranoia, psychosis, adolescents, youth.

RIASSUNTO. Introduzione. Nei disturbi della nutrizione e dell'alimentazione, l'associazione con altri sintomi psichiatrici è di particolare interesse. L'associazione tra anoressia nervosa e sintomi psicotici è meno studiata rispetto a quella con sintomi affettivi (ansia/depressione). Lo scopo di questo studio è di descrivere un sintomo psicotico (la paranoia) negli adolescenti con anoressia nervosa e di esaminare diversi fattori esplicativi potenzialmente a esso associati; tali fattori sono: sintomi relativi al disturbo del comportamento alimentare, preoccupazioni relative all'immagine corporea, depressione e ansia sociale. La nostra ipotesi è che la paranoia nei pazienti con anoressia nervosa sia in relazione con la presenza di depressione e ansia sociale piuttosto che con i sintomi nucleari della malattia (sintomi del disturbo del comportamento alimentare o preoccupazioni relative all'immagine corporea). **Metodi.** Il disegno dello studio è trasversale retrospettivo. Il campione era costituito da adolescenti accolti presso il Servizio dei disturbi del comportamento alimentare del Dipartimento Integrato di Pediatria dell'Ospedale Universitario "Luigi Vanvitelli". I dati sono stati ottenuti attraverso una raccolta retrospettiva di interviste cliniche e questionari, utilizzati per l'assessment di routine di questi pazienti: Eating Attitude Test-26 (EAT-26), Body Uneasiness Test-A (BUT-A), Children Depression Inventory (CDI), Liebowitz Social Anxiety Scale-Children and Adolescents (LSAS-CA) e la sottoscala della paranoia dello Specific Psychotic Experiences Questionnaire (SPEQ). **Risultati.** I dati erano relativi a 92 adolescenti con anoressia nervosa e altri disturbi della nutrizione e dell'alimentazione. Il nostro modello di regressione ha spiegato che la paranoia (sottoscala della paranoia della SPEQ) in questa popolazione era maggiormente associata alla depressione (CDI) (coefficiente= 0,415 DS: 0,210, $p=0,052$) e ai sintomi di ansia sociale (LSAS-CA) (coefficiente= 0,253 DS: 0,060; $p<0,001$) rispetto ai sintomi del disturbo del comportamento alimentare (EAT-26) (coefficiente= 0,092 DS: 0,107; $p=0,398$) e preoccupazioni relative all'immagine corporea (BUT-A) (coefficiente= 1,916 DS: 2,079; $p=0,359$). **Conclusioni.** Questo studio ha diverse implicazioni teoriche, cliniche e terapeutiche. È importante eseguire lo screening per la presenza di sintomi psicotici nei pazienti con disturbi della nutrizione e dell'alimentazione. Questi sintomi e fattori associati (depressione e ansia sociale) possono complicare il quadro clinico della malattia con la necessità, in alcuni casi, di una terapia psicofarmacologica e, tra questi, l'uso degli antipsicotici. Infine, nel contesto della psicoterapia, la paranoia può essere oggetto di trattamento per i pazienti con anoressia nervosa.

PAROLE CHIAVE: anoressia, paranoia, psicosi, adolescenti, giovani.

INTRODUCTION

Anorexia nervosa (AN) is characterized by restriction of food intake leading to weight loss or a failure to gain weight and difficulties in maintaining an appropriate body weight for height, age, and stature. Fear of becoming fat or gaining weight, and, in many individuals, distorted body image. Twelve months prevalence of AN in female is 0.4% with female/male ratio of 10:1¹. AN has the highest incidence in the middle and final phase of adolescence and the onset occurs mainly between 18 and 21 years². There are several risk factors for AN, they are genetic factors and puberty and body changes in adolescence. Other specified feeding or eating disorder (OSFED) is a condition in which there are feeding or eating symptoms that causes distress or impairment but the diagnostic criteria are not met¹.

In AN and OSFED, the association with other psychiatric symptoms is of particular interest because they have an impact on the diagnosis, treatment and course of the disorder. Several studies have analysed the presence of psychiatric comorbidities. Hudson et al.², found that a high percentage of participants had positivity for at least one diagnostic criterion of DSM-IV core disorders and in detail AN was associated with mood, anxiety, impulse-control, and substance use disorders. In the same study on 10.123 adolescents supplement (age range 13-18, AN prevalence 0.3%), AN was associated with oppositional defiant disorder and social impairment. There are also studies that investigated comorbidities of AN in adolescent³⁻⁵.

Other studies have evaluated the association with psychiatric symptoms. Reviewing these studies it is evident that the association with affective or classically “neurotic symptoms” has been much more studied than the possible association with the psychotic symptoms⁶. The contributions on the latter topic have taken different directions. First of all, the association between AN and schizophrenia and/or psychotic symptoms; secondly, the use of antipsychotics in the multi-level treatment of AN^{7,8}. On the other hand, looking at the AN psychotic symptoms association, the contributions appear to be poorly systematized and mainly related to case reports or adult studies^{6,9-15}. Miotto et al. found that female patients with AN (n. 61) were more likely to endorse the items “Never feeling close to another person” and “Idea that something is wrong with your mind” at the “paranoid ideation” and “psychoticism” dimensions in the SCL-90R⁶. Hudson et al.¹³ demonstrated psychotic symptoms in 17 of 130 consecutive patients with AN. Grounds et al.¹⁰ described atypical psychotic episode in 7 cases of AN and they hypothesized that psychological stress may contribute to the onset of psychotic symptoms. Therefore this association remains understudied and poorly understood, especially in adolescence, with some critical and methodological problems (cross-sectional studies, small samples, outpatient/inpatient, and self-administered questionnaires versus semi-structured interviews)¹⁶. A non-marginal problem is represented by the temporal direction of the association and if AN symptoms precede the onset of psychotic symptoms or the contrary. McGrath et al.¹⁷ tested the bidirectional association between DSM-IV (Diagnostic and Statistical Manual of Mental Disorder – fourth edition) mental disorders, including AN, and psychotic symptoms in a large survey across 18 countries including 31.261 respondents. Authors found that the associa-

tion between AN and psychotic experiences was not bidirectional with only the temporally relationship between AN and the subsequent onset of psychotic experiences (OR 2.8, 95% CI=1.0-7.8) and not vice versa.

Furthermore, in the last years the conceptual framework of psychosis has taken a new direction in order to evaluate psychotic symptoms in the general population also called “psychotic like experiences” (PLEs)¹⁸. Freeman et al. pointed out that emotional disorders frequently develop prior to and accompany psychotic symptoms^{19,20}. On the other hand, PLEs are common among adolescents¹⁸ and among these paranoia, an exaggerated or unrealistic belief that others wish to harm us²¹, is one of the most frequent in terms of occurrence²². Moreover, paranoia is increasingly conceptualized as a symptomatic dimension not necessarily linked to the narrow concept of psychosis²³. Freeman introduced the concept of a paranoia spectrum and pointed out that paranoid thinking may be a reliable experience in a general population. He demonstrated that paranoia was associated with a range of other psychiatric symptoms such as: anxiety, worry, phobias, post-traumatic stress and insomnia as well as other demographic, economic and social correlates²⁴. Garety et al.²⁵ described a cognitive psychological model in which the interplay between biological, psychological and social factors contribute to the development of positive psychotic symptoms and more in detail paranoia may build upon emotional concerns such as social anxiety and interpersonal worry themes²¹.

Therefore the aim of this study is to describe paranoia in an adolescent AN population looking at several potential associated and explicative factors: eating disorder symptoms, body image concerns, depression and social anxiety. Our hypothesis, in line with cognitive model of positive symptoms of psychosis, is that paranoia in AN patients is more explained by the concomitant depression and social anxiety symptoms than core features of the disease (eating disorder symptoms or body image concerns). We assumed that AN preceded paranoia in line with the literature¹⁷.

MATERIAL AND METHODS

Sample and design

This is a retrospective study. We retrieved data through retrospective collection of clinical interviews administered by trained and expert child and adolescent psychiatrists and self-report questionnaires from clinical records of the patients admitted to the Eating Disorder Service of the Integrated Pediatric Care Department, “Luigi Vanvitelli” University Hospital. These tools were part of the routine assessment for these patients. Inclusion criteria were Diagnostic And Statistical Manual Of Mental Disorder 5th edition (DSM-5) based clinical diagnosis of AN or OSFED (participants who did not meet strict diagnostic criteria for anorexia nervosa). Exclusion criteria were intellectual disability and other neurodevelopmental disorders. The sample (72 patients) had previously been included in study on a psychiatric comorbidity in AN (during this time, the sample increased by 20 subjects)⁵. The study was performed in accordance with the 1964 Helsinki declaration and its later amendments. Patients or their legal representatives gave consent to the clinical routine assessment.

Measures

- **Eating problems:** we used the Eating Attitude Test-26 (EAT-26) for symptoms and concerns about eating disorders. The EAT-26 included 26 items with Likert scale response options (never, rarely, occasionally, frequently, always). A score at or above 20 represents a cut-off for problems about dieting, body weight or concerns on eating behaviors. EAT-26 demonstrated good psychometric profile²⁶.
- **Body image:** for the body image problems we used the Body Uneasiness Test-A (BUT-A) that is a 34-item self-report questionnaire with questions about weight phobia, body image concerns, avoidance, compulsive self-monitoring, detachment and estrangement feelings towards one's own body (depersonalization). BUT-A demonstrated good psychometric properties²⁷.
- **Depression:** for detection of depressive symptoms we used the Children Depression Inventory (CDI) that was constituted by 27 self-report items with a 3-point likert scale (0, 1, or 2). The score range from 0 to 54. A score of 19 is considered a cut-off for depressive symptoms. CDI demonstrated good psychometric properties²⁸.
- **Social-Anxiety:** the Liebowitz social anxiety scale-children and adolescents (LSAS-CA) is a 24 items interview (12 items refer to social interaction situations, 12 items refer to performance situations). Each item assesses the fear level and the avoidance level on a Likert type scale: Fear level (0= none, 1= mild, 2= moderate, 3= severe) and avoidance level (0= never, 1= occasionally, 2= often, 3= usually). It provides seven scores: anxiety related to social interaction; performance anxiety; total anxiety; avoidance of social interaction; avoidance of performance situations; total avoidance; total score. LSAS-CA demonstrated good psychometric profile²⁹.
- **Paranoia:** we used the paranoia subscale of the Specific Psychotic Experiences Questionnaire (SPEQ). It was constituted by 15 items with a 6-point Likert scale (0= not at all, 1= rarely, 2= once a month, 3= once a week, 4= several times a week, 5= daily) SPEQ demonstrated good psychometric properties³⁰. We already used this scale for other study²².
- **Other variables:** we collected demographical and personal data from patient's clinical records.

Statistical analysis

We used the Statistical Package for the Social Sciences SPSS (version 20.0) and STATA (version 14). First of all a set of descriptive analyses (frequencies, percentage, means and standard deviations, medians and interquartile ranges) have been carried out to describe the sample. A Spearman correlation analysis was performed between paranoia and eating disorder symptoms, body image concerns, depression and social anxiety. A correlation analyses were made also between eating disorder symptoms and body image concerns and between depression and social anxiety. Subsequently, in order to be more explicative about our explicative factors and their interaction in the relationship with paranoia, a hierarchical linear regression has been performed between paranoia (our Dependent Variable - DV) and our Independent Variables (IVs) (eating disorder symptoms, body image concerns, depression and social anxiety). In the first step we entered confounders (sex: 0= male, 1= female; diagnosis: 0= OSFED, 1= AN and BMI), then we entered eating disorder symptoms (EAT-26), next body image concerns (BUT-A), then we entered depression (CDI) and finally social anxiety (LSAS-CA). In this way we investigated more in detail the role of depression and social anxiety symptoms controlling for eating disorder symptoms and body image concerns.

RESULTS

Demographic and clinical data

The sample included 92 patients and female were 79 (85.9%). Mean age was 172.4 months (14 years and 3 months) (SD 22.3, range 132-214) (SD 1.9 years, range 11-17 years e 8 months). Mean body mass index (BMI) was 16.3 kg/m² (SD 2.3, range 11-23.6). Regarding BMI, 53 (57.6%) subjects were under the 5° percentile (pc), 19 (20.7%) between the 10° and the 25° pc, 14 (15.2%) between the 25° and 50° pc, 4 (4.3%) between the 50° and the 75° pc and 2 (2.2%) between the 75° and the 100° pc. Weaning problems (i.e selectivity, food refusal) were manifested in 13 subjects (14.1%). 66 subjects (71.7%) have received a diagnosis of AN (10 males, 15.1%) and 26 (28.3%) had a diagnosis of OSFED (3 males, 11.5%). 79 (85.9%) and 13 (14.1%) participants had respectively a restrictive and binge/purging subtype. The presence of psychiatric comorbidities was exposed in another study⁵. There were no demographics or clinical significant differences between AN and OSFED groups. 16 patients had started drug therapy (SSRI: 3, aripiprazole: 9, SSRI+aripiprazole: 3, lithium: 1). Table 1 showed descriptive statistics of paranoia, eating disorder symptoms, depression and social anxiety; 20 (21.7%) subjects had score on paranoia scale ≥ 38 (75° pc).

Correlation analyses data

Correlation analysis between EAT-26 and BUT was high and significant (0.811, $p < 0.001$). Correlation analysis between CDI and LSAS-CA was high and significant (0.658, $p < 0.001$). Table 2 presented the correlations between paranoia score and eating disorder symptoms (0.623 $p < 0.001$), body image concerns (0.662 $p < 0.001$), depression (0.656 $p < 0.001$) and social anxiety (0.714 $p < 0.001$). In Figures 1-4 there are scatter plot graphs of the correlation analyses.

Regression analysis data

Table 3 indicated results of the hierarchical linear multiple regression analysis between paranoia (DV) and eating disorder symptoms, body image, depression and social anxiety.

Table 1. Descriptive of symptoms.

	Mean (SD)	Median (interquartile range-25/75)	Range
Paranoia	22 (17.7)	18 (7/36)	0-62
EAT-26	27.3 (19.5)	22 (10/22)	0-73
BUT	1.87 (1.28)	1.75 (0.72/2.84)	0-4.5
CDI	16.4 (10.3)	15.5 (8/24)	0-51
LSAS total score	37.7 (29.7)	30.5 (13.2/61)	0-132

Legend: EAT-26= Eating Attitude Test-26; BUT= Body Uneasiness Test; CDI= Children Depression Inventory; LSAS total score= Liebowitz Social Anxiety Scale-Children and Adolescents

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Table 2. Spearman correlation analysis between paranoia, eating disorder symptoms, body image concerns, depression and social anxiety.

	EAT26	BUT	CDI	LSAS total score
Paranoia	0.623 (p<0.001)	0.662 (p<0.001)	0.656 (p<0.001)	0.714 (p<0.001)

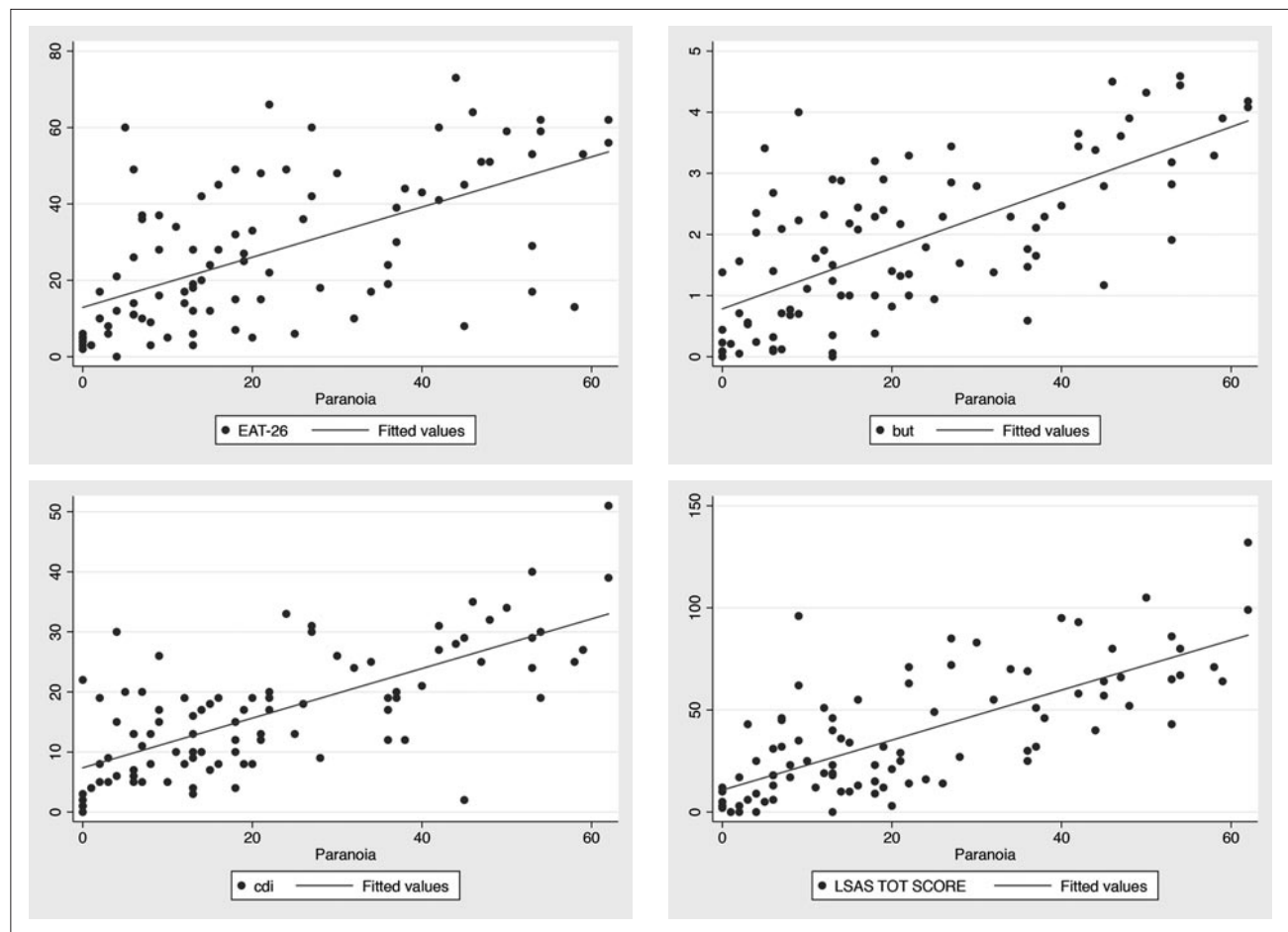
Legend: EAT-26= Eating Attitude Test-26; BUT= Body Uneasiness Test; CDI= Children Depression Inventory; LSAS total score= Liebowitz Social Anxiety Scale-Children and Adolescents.

ety (IVs). In the first step we demonstrated that confounders did not have effect on paranoia. In the second step eating disorder symptoms (EAT-26) predicted paranoia with positive and significant coefficient (0.549 SD: 0.084; p<0.001); in the third step body image concerns (BUT-A) predicted paranoia with positive and significant coefficient (8.641 SD: 1.868; p<0.001) and eating disorder symptoms (EAT-26)

were no more significant (0.097 SD: 0.123; p=0.433). In the fourth step, depression (CDI) predicted paranoia with positive and significant coefficient (0.723 SD: 0.216; p<0.001;) with slight change in significance for body image concerns (BUT-A - 4.268 SD: 2.196; p=0.055). In the fifth step, social anxiety (LSAS-CA) predicted paranoia with positive and significant coefficient (0.253 SD: 0.060; p<0.001) with the depression (CDI) coefficient that remains substantially significant (0.415 SD: 0.210, p=0.052) and body image concerns (BUT-A) coefficient, which does not maintain its significance (1.916 SD: 2.079; 0.359). The effects of our all IVs (and confounders) accounted for about 60% (adjusted $R^2=0.599$) of the model with change in R^2_{diff} in the consecutive steps exposed in Table 3.

DISCUSSION

This study focused on the association between AN, OS-FED and paranoia in a sample of adolescents. Results demonstrated that paranoia was dimensionally distributed in



Figures 1-4: Scatter-plots with paranoia as “y” and respectively EAT-26, but, cdi and LSAS-totscore ad “x”.

Table 3. Hierarchical linear regression analysis between paranoia DV and eating disorder symptoms, body image concerns, depression and social anxiety IVs (confounders: sex, BMI, diagnosis).

Paranoia	B (SD)	Beta	T	Sig (p)	R ² _{diff} (sig)
Step 1					
Sex	5.522 (5.3)	0.109	1.039	0.302	0.043 (p=0.274)
BMI	0.623 (0.8)	0.082	0.699	0.487	
Diagnosis	7.950 (4.6)	0.200	1.717	0.090	
Step 1 Previous plus EAT 26	0.549 (0.084)	0.603	6.563	<0.001	0.319 (p<0.001)
Step 3 previous plus EAT26	0.097 (0.123)	0.107	0.787	0.433	0.128 (p<0.001)
BUT	8.641 (1.868)	0.626	4.626	<0.001	
Step 4 previous plus EAT26	0.071 (0.117)	0.078	0.610	0.543	0.060 (p=0.001)
BUT	4.268 (2.196)	0.309	1.943	0.055	
CDI	0.723 (0.216)	0.422	3.346	0.001	
Step 5 previous plus EAT26	0.092 (0.107)	0.102	0.867	0.389	0.080 (p<0.001)
BUT	1.916 (2.079)	0.139	0.922	0.359	
CDI	0.415 (0.210)	0.242	1.972	0.052	
LSAS total score	0.253 (0.060)	0.422	4.236	<0.001	

Legend: EAT-26= Eating Attitude Test-26; BUT= Body Uneasiness Test; CDI= Children Depression Inventory; LSAS total score= Liebowitz Social Anxiety Scale-Children and Adolescents.

adolescents with AN and OSFED. In comparison with other studies that have examined the dimensional distribution of paranoia in different samples, our descriptive of paranoia were higher than those of Ronald et al. (mean 12.14 and median 10.00) in the general population but lower than in adolescent help seeking screened positive for PLEs (mean 34.64 and median 38)^{22,30}.

Adolescents with AN could potential be at risk for psychotic symptoms and this association could influence the nature and course of the disorder⁶. Kouidrat et al.¹⁶ argued that paranoid ideas could lead patients to think that food is poisoned or contaminated. Mavroggiorgou et al.¹² indicated that psychotic symptoms could occur after a fasting period. In a psychopathological perspective Lyon et al. suggested that AN is distributed along a continuum between the presence of neurotic and psychotic symptoms¹⁴. Moreover beliefs in AN may have the similar conviction than delusions and the potential to produce high distress and preoccupation¹⁵. Also paranoid personality disorder or dissociation/paranoid symptoms of borderline personality disorder could be potentially associated with feeding and eating disorders^{31,32}. Recently two case reports reported the association between AN and psychotic symptoms^{33,34}; in detail one study identified paranoid belief in a 12 years old male with AN³³. A recent review pointed out that psychotic symptoms may present in the course of AN. Interestingly when the two conditions co-occur, psychotic symptoms were associated with eating themes³⁵.

In the correlation analysis all our predictive variables (eating disorder symptoms, body image concerns, depression and social anxiety) were significantly associated with para-

noia. On the other hand in the regression analysis eating disorder symptoms and body image concerns were initially predictive for paranoia but when depression and social anxiety entered in the model they were no more significant. We assumed, in light of our results, that in this population (AN), paranoia might be better explained by the greater presence of depression and especially social anxiety in presence of equal level of eating disorder symptoms and body image concerns. So the account of the presence of paranoia in this population could be not primarily explained by the nuclear psychopathology of AN (eating disorder symptoms and body image concerns). Other studies have produced results that can be read in this direction. Steinglass et al.¹¹ found that the presence of psychotic symptoms was not correlated with the overall measure of AN severity (BMI, duration of illness, lowest BMI, total EDI score) but with the EDI (Eating Disorder Inventory) sub-scale *drive for thinness* in 25 subject with EDs. Hudson et al.¹³ in their sample found that 16 out of 17 patients with psychotic symptoms had major affective disorders. Furthermore our results were still significant after controlling for BMI and this is important just because many studies described a link between psychotic symptoms and malnourishment/starvation^{12,36,37}.

Otherwise our results may be supported with recent cognitive models of psychotic symptoms that underline the role of emotional factors in the development and maintenance of paranoia^{21,25}. Several studies demonstrated that paranoia is more likely to develop in subjects with high depression and social anxiety^{38,39}. Depression could directly contribute to the persistence of psychotic symptoms and some cognitive processes are implicated, they are: negative schematic beliefs

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about the self, worry and problem solving difficulties⁴⁰. Cognitive model also proposed a hierarchical structure of paranoia extending from social concerns to suspiciousness of threats, with social phobic content and emotional concerns being the core feature of the entire structure²¹.

CONCLUSIONS

This study has some potential theoretical, clinical and treatment implications, which however need to be confirmed by other studies. It is important to carrying out screening and assessment procedures for the presence of psychotic symptoms and paranoia in patients with AN. In detail, paranoia can shape the clinical presentation of the AN especially in the aspect of mistrust towards doctors and family members about food-related issues, and this has a significant impact on the patient's collaboration in treatment and the therapeutic alliance. Therefore paranoia and indirectly higher depression and social anxiety might be considered factors which make the patient more refractory to treatment with the need, in certain cases, of psychopharmacological drugs and, among these, antipsychotics. Finally in the psychotherapy context, paranoid idea may be subject of treatment for patient with AN.

This study has some limitations. First of all, the retrospective nature of the sample that prevents us from establishing the directionality of the relationship between the factors studied. We have assumed, in line with the literature¹⁷, that paranoia follows the diagnosis of EDs. Despite this, cross sectional and longitudinal studies are needed to confirm these results and better clarify the presence of this relationship and the factors involved. Furthermore the retrospective nature of the design study offers poor data quality control because the observers collected data for other aims.

The lack of a control group is an important limitation as well as the assessment with self-report questionnaire that may have a tendency to overestimate the observed phenomena; with respect to this, the questionnaires used are all validated and well studied in the international scientific panorama. Finally, the potentially mixed sample, 66% AN and 26% OSFED, can be considered a limitation.

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