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Vitamin D3 as possible diagnostic marker of Eating Disorders

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Abstract

Purpose

Eating Disorders (EDs) refer to a group of psychiatric conditions in which disorderly food intake results in impaired psychological functioning or physical health. Nowadays, these disorders represent an increasing problem in modern society. There are no universally validated clinical parameters to confirm, disprove or simply help to identify EDs except for diagnostic criteria on psychiatric basis. The aim of this study was the assessment of Vitamin D3 level in patients with EDs to understand if it might be a valid clinical biochemistry parameter useful as prognostic marker.

Methods

The sample consists of 28 female patients, who suffer from EDs. Blood samples were examined in terms of blood count, glucose, cholesterol and Vitamin D3 levels. The other clinical biochemistry parameters were analysed to understand if the Vitamin D3 was the only altered parameter.

Results

The parameters that appear altered are glycemia, cholesterol and, in particular, Vitamin D3. Significant results were obtained comparing controls with restrictive-type anorexia nervosa (p value= 0,003) and with purging-type anorexia nervosa (p value= 0,007).

Conclusion

There are currently no universally validated and diagnostic reliable clinical biochemistry parameters for EDs but, in the light of the findings, but our research indicates the potential use of Vitamin D3 as a biomarker for anorexia nervosa.

Level of evidence

Level III: Evidence obtained from a single-center cohort study.

Keywords: Eating Disorders; Anorexia Nervosa; Bulimia Nervosa, Other Specified Feeding or Eating Disorder, Vitamin D3; Glycemia; Cholesterolemia.

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Introduction

Eating Disorders (EDs) refer to a group of psychiatric conditions in which disorderly food intake results in impaired psychological functioning or physical health (1).

Currently, EDs are a seriuos public health problem involving more young women in the more industrialized Western world. In Italy, about three million people suffer from EDs.

EDs are currently classified within the DSM-5, the latest version of the diagnostic and statistical manual of mental disorders, as follows: a) ANOREXIA NERVOSA (AN) – characterised by the alteration of body image, which is fundamental in the diagnosis, development and maintenance of the disorder itself. DSM-5 also categorizes the AN in restrictive type and purging type; b) BULIMIA NERVOSA (BN) – patients affected by this disorder have: recurrent binge eating episodes, feel they lose control during the meal, use several and inappropriate compensatory practices; usually, the patient is normal or overweight; c) BINGE EATING DISORDERS (BED) – defined by the regular and uncontrolled consumption of unusually large quantities of food, without subsequent purging episodes; d) OTHER SPECIFIED FEEDING OR EATING DISORDER (OSFED) – a person must present with feeding or eating behaviours that cause clinically significant

distress and impairment, but do not meet the full criteria for any of the other disorders; the category that was known as eating disorder not otherwise specified (EDNOS), in past edition of DSM, has been removed, and there are two new categories: other specified feeding or eating disorder (OSFED) and unspecified feeding or eating disorder (UFED).

There are also EDs from child classifications: Pica, Rumination disorder, Avoiding/restricting eating disorder.

From an epidemiological point of view, EDs are a widespread health problem, especially among young women in the Western world, even if the percentage of affected boys is abruptly increasing last years. The onset of these disorders mainly occurs during the adolescent period (2). It is an extremely critical period characterised by a high psycho-physical vulnerability (2-3). Usually, females are affected more than men, even though numbers are changing considerably. In fact, around 20 years ago, the male to female incidence ratio of EDs was 1:10 to 1:15, while now, especially in the BED, it went to 3:4 (3).

EDs are pathologies of multifactorial origin, in whose development **individual** (these factors can be different from one patient to another and linked to different aspects of our existence), **socio-cultural** (represented by the idealization of thinness, family homeostasis and all those events that could be perceived as traumatic), **psychological** (modern evolutionary psychology defines the EDs as the loss of the ability to cognitively process and regulate the emotions for which, by missing such psychic structure regulation, the subject would be more strongly influenced by external factors) and **genetic** (twin-based heritability estimates of 50-60%) factors contribute; in order to become ill in a definite way, it is therefore necessary that many events occur in synergy (4).

In this contest the genetic architecture of AN is really strong. Recently important studies have been published in which genetic contribution to the aetiology of EDs was confirmed not only by the association of several single nucleotide polymorphisms (SNPs) but also by genome-wide association studies (GWAS) with this group of disorders. In particular, has been confirmed an association between the SNPs located in serotonin receptor (5-HT2AR) and in brain-derived neurotrophic factor (BDNF) genes and the susceptibility to aberrant eating behaviours (5). Moreover, thanks to GWAS de novo variant have been found in different genes, such as CSMD1, CREB3, PTPRD and GAB1, all belonging to a same signalling pathway involving neuron differentiation and dopamine pathway (6). In contrast the results of GWAS led by Watson and co-workers show a low association between genetic factor and AN predisposition, suggesting a reconceptualization of AN as a metabo-psychiatric disorder (7).

1.1 Vitamin D3 and AN

Low blood Vitamin D3 levels have been reported in patients affected by various diseases (8-10).

Vitamin D3 is a secosteroid hormone synthesized in the skin from 7-dehydrocholesterol and hydroxylated in the liver and kidney. Vitamin D3 has a specific cellular and nuclear receptors (VDRs). In the nucleus it is able to regulate the expression of almost 900 genes. In particular Vitamin D3 is involved in the regulation of calcium and phosphate metabolism, immune response, and brain development. The best biomarkers for Vitamin D3 status are 25-hydroxyVitamin D3 (25(OH)D3) or colecalciferol. Low 25(OH)D3 serum levels in neurological, autoimmune and infectious diseases are a common finding (8-11). It has been proposed to use 25(OH)D3 as a serum biomarker in neurodegenerative disorders together with inflammatory markers (12-20). Substantial evidence shows that Vitamin D3 deficiency is associated with cognitive impairment (20-24), and an association between low 25(OH)D3 serum levels and the risk of developing Alzheimer's Disease (AD) and Parkinson's Disease (PD) has been reported by several authors (9, 25-28).

Vitamin D3 has been hypothesized to be a key parahormone acting in different diseases: obesity, diabetes, cancer, cognitive impairment, and dementia with important regulatory functions in innate immunity. However, there are no studies showing extraskeletal changes associated with hypovitaminosis D3 in EDs and until now, Vitamin D3 deficiency in patients with EDs has been correlated only with the risk of osteoporosis (29, 30).

A recent study by Tasegian et al., provides a unique insight into the association among the very low level of Vitamin D3, the decrease of VDR, leukopenia, and 5-HTTLPR extended to emotional dysfunction. The results induce to hypothesise that the severe hypovitaminosis D3 might be responsible for the lack of inflammatory response and reduction in mood in patients with long-term EDs (31).

The study was aimed to analyse the behaviour of vitamin D3 in EDs in order to establish if it might be used as possible new biomarker in addition to others clinical biochemistry parameters in them.

Material and Methods

2.1. Ethics approval

All participants (or their parent or legal guardian in the case of patients under 16) provided written informed consent prior to inclusion in this project and were treated in accordance with the Declaration of Helsinki. For data protection all participants were assigned a unique research identifier. The study protocol and process were assessed and approved by the Ethics Committee of the Aziende Sanitarie (CEAS) della Regione Umbria, Italy.

2.2 Patients

The sample consists of 28 female patients, who suffer of EDs, admitted to Palazzo Francisci, Todi, Italy. The facility welcomes patients from all over the country and it is the only Italian residence that welcomes patients under 14 years of age. Were recruited all patients with EDs who succeeded each other over the same period of stud, between March-May 2019.

Criteria for exclusion: a) Patients of male sex; b) female patients with EDs from childhood classifications: Pica, rumination disorder, Avoiding/restricting eating disorder.

26 control samples (CTR) with a normal BMI (18,5-24,9) are recruited in the study, between the same period (March-May 2019).

2.3 Materials

Blood samples from patients with an EDs were examined at the time of hospitalisation. Chemistry parameters refer to the report issued by Pantalla hospital (USL Umbria1) and the databases were made available by the facility.

2.4 Clinical Measures

The blood count of each patient has been evaluated by focusing the attention to white blood cells, red blood cells and platelets: the alteration of the white blood cells refers to a marked leukopenia (4,50-10,80*103/mmc) with a particular granulocyte decrease and elevation of the lymphocyte part; the red blood cell alteration refers to an erythrocytopenia (4,20-5,40*10^6/mmc); the platelet alteration refers to a marked platelet dystrophy (130-400*10^3/mmc).

Blood glucose, cholesterol, Vitamin D3 were also selected at the time of hospitalisation:

The glycemic index was divided into ranges such as hypo- (70 mg/dl), hyper- (>110 mg/dl) and normo-glycaemia (70-110 mg/dl); cholesterol was divided into hypo- (130 mg/dl), hyper- (>200 mg/dl) and normal-cholesterolaemia (200 mg/dl); Vitamin D3 values were divided into sufficient (30-100 ng/ml) and insufficient (10-30 ng/ml).

BMI (subdivided from a range of very serious underweight of the value of 12-14, up to a normal weight range of 18.5-24.9) and the main clinical manifestations in the skin, muscle and genitourinary tract, were considered, which might suggest the EDs in the first place.

2.5 Statistical analysis

The statistical analysis was performed with SPSS programme. In particular, statistical differences of the VD3, cholesterol, glucose, erythrocyte, and leukocyte blood level medians of five different groups identified in relation to the diagnosis (CTR, AN-RES, AN-PURG, BN and OSFED) and of eight different groups identified in relation to diagnosis-age (< 14years, > 14 years) were investigated by using non-parametric Kruskal-Wallis test. Then, for significant results of both five and eight groups, Pairwise Comparison was used as post hoc test to compare samples in pairs (i.e. AN-RES against CTR, AN-PUR against CTR, BN against AN and OSFED again CTR).

Results

In order to understand the significance of biochemical parameters in relation to EDs, patients were analyzed according to the diagnosis. 28 female patients were considered for the study. They were divided in 4 different groups: patients affected by restrictive-type anorexia nervosa (AN-RES), patients affected by purging-type anorexia nervosa (AN-PUR), patients affected by bulimia nervosa (BN), patients affected by other specified feeding or eating disorder (OSFED). The analysis of the frequency of the various pathologies showed that 64% of patients suffered from AN-RES, 18% from AN-PUR, 7% from BN, and 11% from OSFED (Figure 1). In table 1 age, BMI, clinical manifestations in the skin, muscle and genitourinary tract of all patients in according with diagnoses are reported.

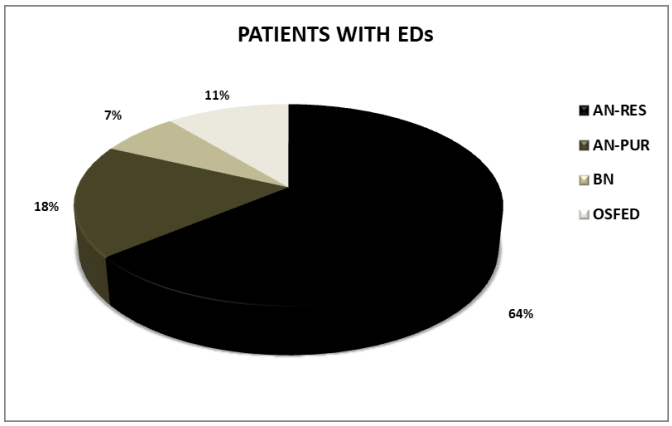

Figure 1. Different diagnoses of Eating Disorders. Percentage distribution of different subtypes of Eating Disorders in a population of 28 female patients. AN-RES (restrictive-type anorexia nervosa) in black, AN-PUR (purging-type anorexia nervosa) in dark grey, BN (bulimia nervosa) in light grey, and OSFED (other specified feeding or eating disorder) in white.

To further explore the how mucwh EDs could affect young subjects, we assessed the age of the patients. Regarding the age of the patients 21% were under 14 years of age, with an average age of 11,6 ± 0,5 and a median of 12, and 79% were over 14 years of age, with an average age of 22,3 ± 8,4 and a median of 19 (Figure 2).

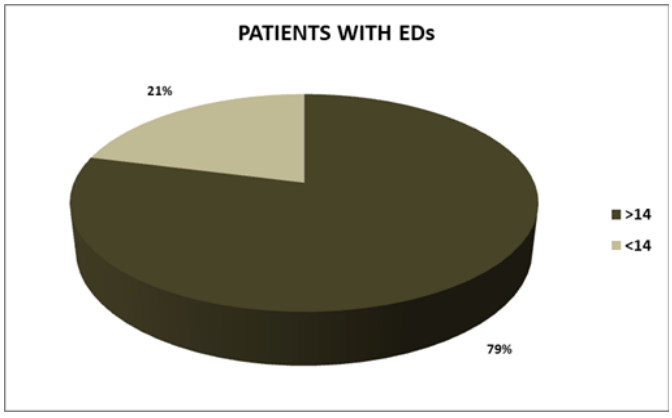

Figure 2. Patients with Eating Disorders divided by age. While 79% were over 14 years old, 21% were under 14 years old.

To get more into the clinical part of the study, the blood count of each patient was analysed, thus obtaining a first distinction between patients with regular blood counts and patients with irregular blood counts (leukocytes and red blood cells) and correlating them to the type of EDs.

Table 1. Sociodemographic characteristics (age) and clinical characteristics (BMI, clinical manifestations in the skin, muscle and genitourinary tract) of all the patients in the study according to type of EDs.

N°	Diagnoses	Age	BMI	Skin	Muscle	Genitourinary
1	AN-RES	11	14,2	pale-dehydrated	hypotrophic	no menarche
2	OSFED	11	14,1	hypotrophic	normotrophic-normotonic	secondary amenorrhea
3	AN-RES	12	15,1	hypertrophic-lanugo	hypotrophic	no menarche
4	AN-RES	12	15,4	pale-lanugo	hypotrophic-hypotonic	secondary amenorrhea
5	AN-RES	12	15,6	hypertrophic-lanugo	hypotrophic	secondary amenorrhea
6	AN-RES	12	17,2	normal	normotrophic-normotonic	no menarche
7	AN-RES	15	13,8	pale-normohydrated	normotrophic-normotonic	secondary amenorrhea
8	AN-RES	15	14,4	pale-lanugo	hypotonic	primary amenorrhea
9	AN-PUR	16	14,3	normal	hypotonic	regular menstrual cycle
10	AN-RES	16	12,8	pale-dehydrated	hypotrophic	secondary amenorrhea
11	AN-RES	16	13,2	pale-dehydrated	hypotrophic-hypotonic	secondary amenorrhea
12	AN-RES	16	15,1	hypotrophic	hypotrophic	primary amenorrhea
13	AN-RES	16	15,2	hypotrophic	hypotrophic	secondary amenorrhea
14	AN-PUR	17	16,2	pale-lanugo	hypotrophic	secondary amenorrhea
15	AN-RES	19	13,5	pale-dehydrated	hypotrophic-hypotonic	secondary amenorrhea
16	AN-RES	19	13,6	normal	normotrophic-normotonic	secondary amenorrhea
17	AN-RES	19	13,7	hypotrophic-lanugo	hypotrophic-hypotonic	secondary amenorrhea
18	AN-RES	19	15,2	hypotrophic-lanugo	hypotrophic	regular menstrual cycle
19	AN-RES	20	14,7	normal	hypotrophic	secondary amenorrhea
20	AN-RES	20	15,1	pale-lanugo	hypotrophic-hypotonic	secondary amenorrhea
21	OSFED	23	17,1	normal	normotrophic-normotonic	regular menstrual cycle
22	AN-PUR	24	14,2	hypotrophic-lanugo	hypotrophic	secondary amenorrhea
23	AN-RES	29	15,1	cyanosis	hypotrophic-hypotonic	secondary amenorrhea
24	BN	29	15,7	pale-dehydrated	hypotrophic-hypotonic	secondary amenorrhea
25	BN	29	23,3	normal	hypotonic (Bechet syndrome)	secondary amenorrhea
26	AN-PUR	32	14,6	normal	hypotrophic	secondary amenorrhea
27	AN-PUR	32	16,0	pale-dehydrated	normotrophic-normotonic	secondary amenorrhea
28	OSFED	50	23,3	normal	normotrophic-normotonic	regular menstrual cycle

Results showed that of the total number of patients analysed (28), 71% had irregular blood counts (20 patients), of which 65% were patients with AN-RES (13), 10% with AN-PUR (2), 10% with BN (2) and 15% (3) with OSFED (Figure 3).

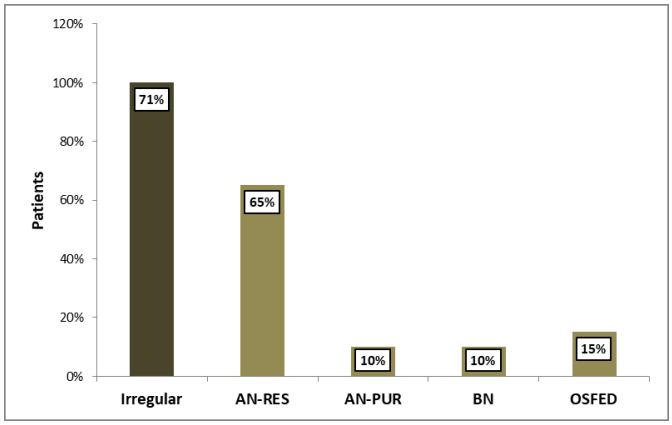

Figure 3. Percentage of patients with irregular blood count and procedure divided by type of EDs. AN-RES, restrictive-type anorexia nervosa, AN-PUR, purging-type anorexia nervosa, BN, bulimia nervosa, OSFED, other specified feeding or eating disorder.

The normal values for leukocytes are 4,50 – 10,80*10^3/mmc, but patients showed a lower level than normal with an average of 2,93 ± 0,72*10^3/mmc in AN-RES patients, 3,75 ±0,07*10^3/mmc in AN-PUR patients and 3,59 ±0,44*10^3/mmc in OSFED patients. The Kruskal-Wallis test confirmed the result with a statistical significance between CTR and AN-RES group with a p value = 0,01(Figure 4a, c). As well as leukocytes, erythrocytes in the control are between 4,20 – 5,40*10^6/mmc, but the average was 3,35 ± 0,25*10^6/mmc in AN-RES patients and 3,93 ± 0,18*10^6/mmc in OSFED patients. Only 2 patients with BN exhibited a higher level of red blood cells than normal with an average of 5,45 ± 0,07*10^6/mmc. The Kruskal-Wallis test showed a strong statistical significance between CTR and AN-RES with a p value = 0,001 (Figure 4b, d).

In addition, at a later stage in the study, the blood glucose, cholesterol and Vitamin D3 values were determined and always correlate with the type of EDs. Results showed that 25% had hypoglycaemia (7), of which 71% corresponded to patients

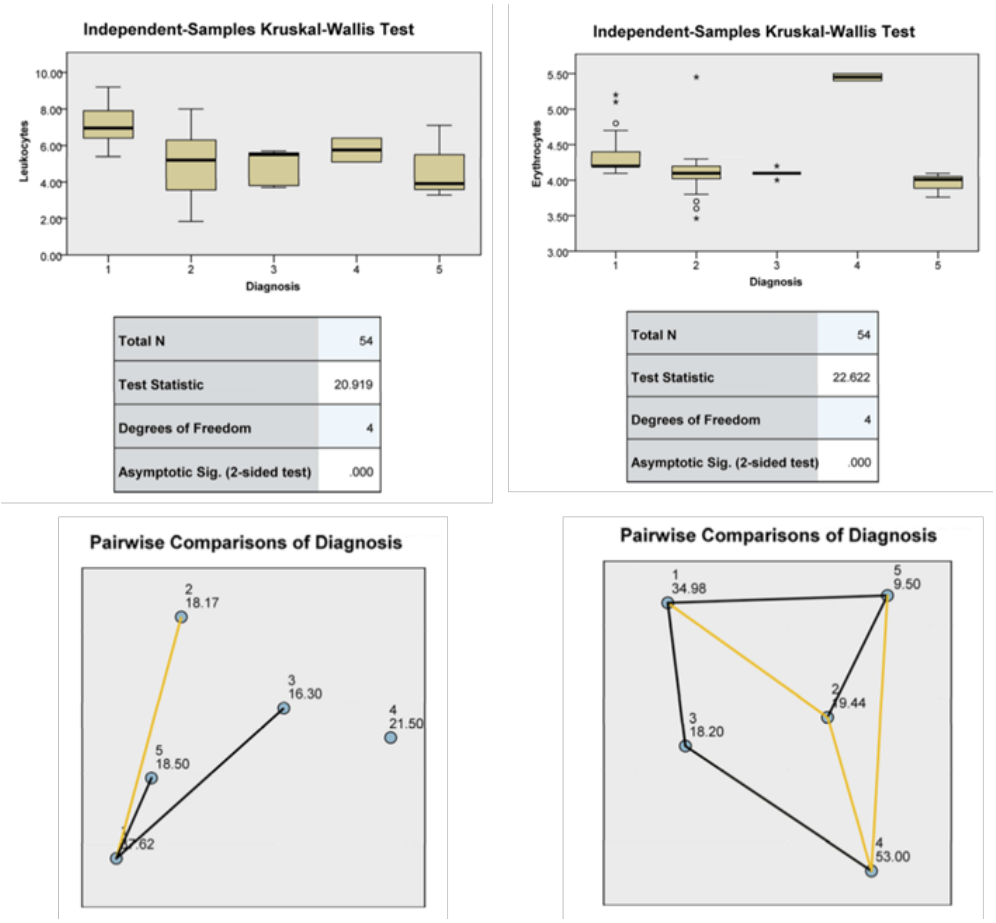

Figure 4. Statistical analysis using non-parametric Kruskal-Wallis: a) leukocytes levels and EDs types (number 1 CTR, number 2 AN-RES, number 3 AN-PUR, number 4 BN and number 5 OSFED) b) erythrocytes levels and EDs types (number 1 CTR, number 2 AN-RES, number 3 AN-PUR, number 4 BN and number 5 OSFED), c) Pairwise Comparison to compare leukocytes levels and EDs types in pairs (significant correlation in yellow lines) d) Pairwise Comparison to compare erythrocytes levels and EDs types in pairs (significant correlation in yellow lines).

with AN-RES (5) and 29% to patients with OSFED (2) (Figure 5). The normal range for glycemia is 70 – 110 mg/dl. Patients

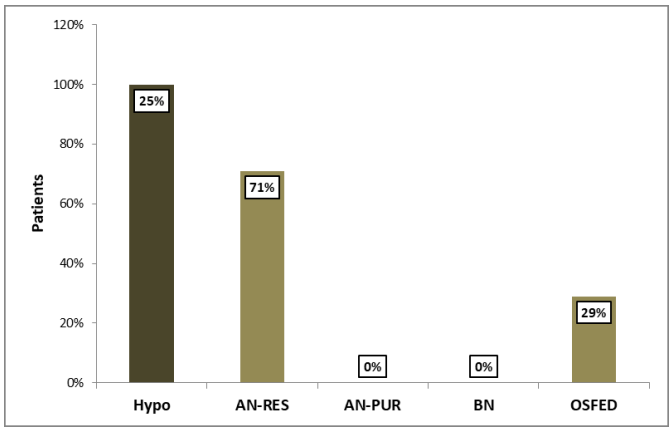

Figure 5. Percentage of patients with hypoglycemia and divided by type of EDs. AN-RES, restrictive-type anorexia nervosa, AN-PUR, purging-type anorexia nervosa, BN, bulimia nervosa, OSFED, other specified feeding or eating disorder.

with hypoglycaemia showed an average of 61,14 ± 8,67, but the Kruskal-Wallis test didn't provide significant results (data not shown).

As for the cholesterolaemia, in the total number of patients analysed, 18% showed hypercholesterolaemia (5), of which 80% corresponded to patients with AN-RES (4) and 20% to

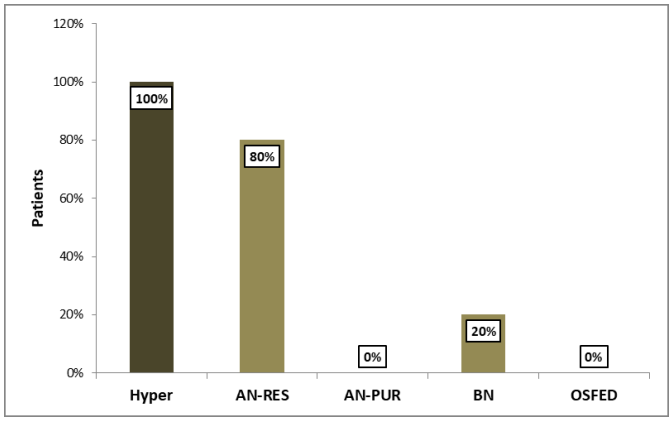

Figure 6. Percentage of patients with hypercholesterolemia divided by type of EDs. AN-RES, restrictive-type anorexia nervosa, AN-PUR, purging-type anorexia nervosa, BN, bulimia nervosa, OSFED, other specified feeding or eating disorder.

patients with BN (1) (Figure 6). The average was 244,2 ± 11,45 mg/dl, over the threshold value of 200 mg/dl, but not statistically significative using Kruskal-Wallis test (data not shown).

Significant results were found to the Vitamin D3 values. In this case two graphs have been generated: one for the sample "adults" (patients over 14 years of age) and one for the sample "girls" (patients under 14 years of age), always correlating them to the type of EDs. Figure 7 shows that patients with AN-RES, 46% of them had sufficient values of Vitamin D3 (6) and 54% of them were Vitamin D3 deficiency (7); patients with AN-PUR, only 20% showed sufficient values of Vitamin D3 (1) while 80% were Vitamin D3 deficiency (4); patients with BN and with OSFED were equal divided: 50% have sufficient values of Vitamin D3 (1) and the other 50% show Vitamin D3 deficiency (1). For the sample "girls" (Figure 8), only AN-RES and OSFED have been reported, the classes in which patients under 14 years of age appeared. Similar results if we compare "adults" and "girls" groups were obtained for AN-RES class: 40% of patients with sufficient values of Vitamin D3 (2) and 60% with Vitamin D3

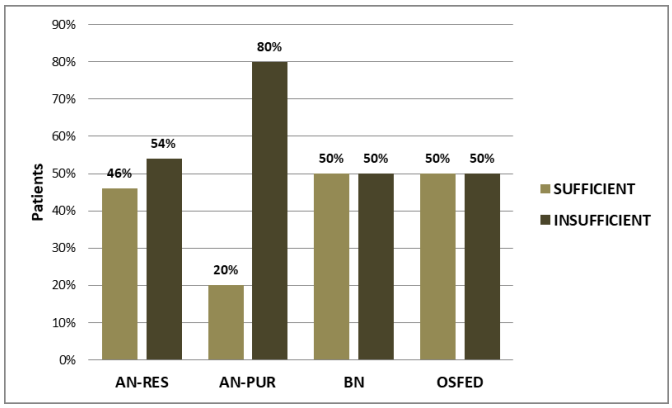

Figure 7. Vitamin D3 values in adults patients divided by type of EDs. AN-RES, restrictive-type anorexia nervosa, AN-PUR, purging-type anorexia nervosa, BN, bulimia nervosa, OSFED, other specified feeding or eating disorder.

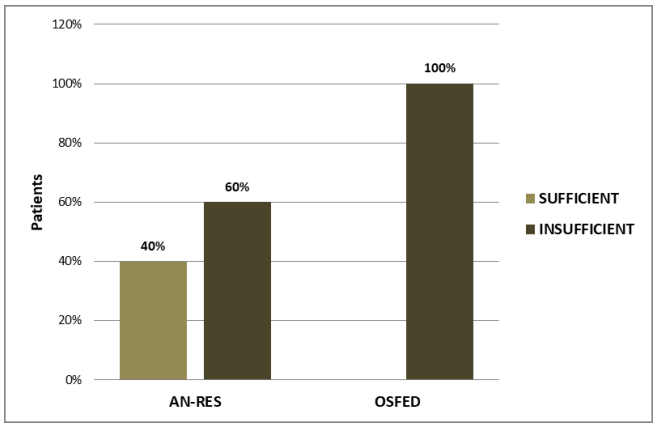

Figure 8. Vitamin D3 values in "girls" patients divided by type of Eating Disorders Only AN-RES, restrictive-type anorexia nervosa and OSFED, other specified feeding or eating disorder.

deficiency (3). Different results were achieved for OSFED subtype because 100% of "girls" showed Vitamin D3 deficiency (only 1 sample) (Figure 8).

We performed non-parametric Kruskal-Wallis for Vitamin D3 levels and EDs types (Figure 9a) and significant results were obtained comparing CTR with AN-RES (p value= 0,003) and

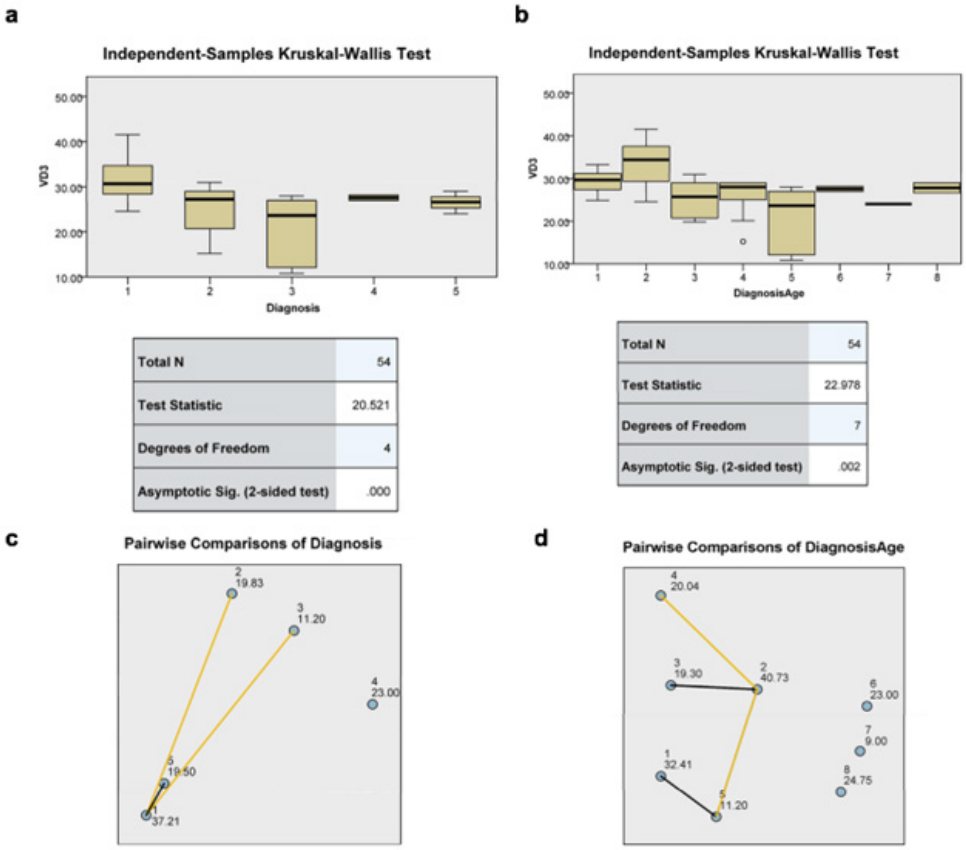

Figure 9. Statistical analysis using non-parametric Kruskal-Wallis: a) Vitamin D3 levels and EDs types (number 1 CTR, number 2 AN-RES, number 3 AN-PUR, number 4 BN and number 5 OSFED) b) Vitamin D3 levels and EDs types in different age groups (number 1 CTR<14, number 2 CTR>14, number 3 AN-RES<14, number 4 AN-RES>14, number 5 AN-PUR, number 6 BN and number 7 OSFED<14, number 8 OSFED>14), c) Pairwise Comparison to compare EDs types in pairs (significant correlation in yellow lines) d) Pairwise Comparison to compare EDs types in different age groups in pairs (significant correlation in yellow lines).

CTR with AN-PUR (p value= 0,007) (Figure 9c). The same statistical analysis for Vitamin D3 and EDs types in different age groups (< 14 years, > 14 years) was carried out (Figure 9b) and statistical differences have been found between CTR > 14 and AN-RES > 14 (p value= 0,014) and CTR >14 and AN-PUR>14 (p value=0,008) (Figure 9d).

Discussion

EDs represent a genuine social epidemic, which is increasingly impacting the pre-adolescent age range; in recent decades, there has been a progressive lowering of the age of onset, up to cases of girls aged 8-9 years (notebooks of the Ministry of Health).

Certainly the Statistical Diagnostic Manual of Mental Disorders (DSM-5) defines and distinguishes, on a psychiatric basis, the different pictures of EDs, but the difficulty in recognizing exactly the EDs compared to other diseases, mental or not, seems to be related to the tendency of people to conceal their disorder and discomfort by avoiding, at least for a long initial period, the help of professionals and the possibility of a rehabilitation. If we add to this that the organic decay, induced by the slimming, makes modifications about the physical and psychic functioning, we understand better how much can be conditioned the possibility of improvement and healing. Therefore there is the need to trace clinical/biochemical parameters in order to establish the patient's condition and thus facilitate the possibility of a timely, targeted therapy, especially for pre-pubescent children, for whom a tardive diagnosis could be fatal, with far more serious consequences on the body and mind. Early onset of EDs may result in increased chances of developing permanent damage, mainly to bones and central nervous system, which have not yet fully matured. Altogether, the parameters that appear strongly altered and with greater frequency refer to the blood exams, to the glycaemia, in part to the cholesterol and, almost always, to the values of vitamin D.

Osteoporosis and osteopenia are common in particular in AN, where low circulating levels of Vitamin D3 have been confirmed. Now the question is: the low level of this vitamin is due to the restricted diet or there is a genetic alteration in Vitamin D3 receptor (VDR) that may influence the internalization of Vitamin D3. Few studies investigated the correlation between Bone Mineral Density (BMD) and genetic predisposition in girls affected by AN. Recently strong effects were observed in two different SNPs located in VDR (Bsml, Fokl), in particular bb genotype of BsmI was related with femur Z-scores (p = 0.103) and of the Ff genotype of FokI with verte-

bra Z-scores (p = 0.097) (32). Moreover, a study analysed SNPs of genes encoding Vitamin D3 receptor, estrogen receptor alpha (ESR1), collagen type I and calcitonin receptor (CTR) with positive correlations (33).

Finally, in this regard, a recent study describe the low level of Vitamin D3, the reduction of VDR and the presence of 5-hydroxytryptamine transporter (5-HTTLPR) variant short allele (S) in patients with a very long history of AN and BN. In particular, it was hypothesized the possibility that very low level of Vitamin D3 and the reduction of VDR in blood cells might be responsible for the S allele of the 5-HTT polymorphism that was described to be related to EDs (31).

A recent study with 20 female AN patients from Sweden demonstrated no differences between AN and CTR in the level of Vitamin D3 (34). In contrast our study confirms the presence of hypovitaminosis D in patients with EDs, in particular we found a strong correlation between CTR and AN-RES and between CTR and AN-PUR.

Again, according to the values specified in the last document of the Ministry of Health, there is a considerable leukopenia and erythrocytopenia, for the majority of patients; an appreciable hypoglycaemia and hypercholesterolaemia, although not statistically significant, mostly among "adults" sample; a marked hypovitaminosis, in all patients studied. There would still be to discuss briefly about the clinical signs regarding skin, muscle and genitourinary tract.

The results obtained from this research bring into clear relief the frequency with which hypotrophy, pallor, dehydration and lanugo skin, hypotrophy and reduced muscle tone, but especially the amenorrhea (one of the most indicative signs of endocrine disruption) appears as common markers among young women being studied and are therefore more likely to occur in patients with EDs, making the diagnosis a little more simplistic. Unfortunately, few studies have explored the association between Vitamin D3 and EDs, in particular AN, but it is already known the involvement of osteoporosis, impaired bone density and metabolism and moreover increased fracture rate as joint causes in AN (35, 36). Exactly the mechanism behind these complications is still not fully understood and for this reason we believe that Vitamin D3 level and metabolism have a crucial role in the diagnosis and prognosis of AN. Recently it has been confirmed (37) a reduction in Vitamin D3 and parathyroid hormone levels in about 35% of patients with AN compared to healthy controls (38).

Still nowadays the exams for EDs diagnosis are divided in four major group: **Physical exams** (include measuring height and weight, vital signs, such as pressure and temperature, skin and nails aspect and examining the organs and abdomen), **Blood tests** (complete blood count, electrolytes and proteins, thyroid, liver and kidney functionality), **Psychological evaluation** (feelings and eating habits) and **other studies** (such as X-rays to look for bone density, electrocardiograms to check heart irregularities, nuclear magnetic resonance, etc.).

This study adds for the first time a strong correlation between patients with AN-RES and AN-PURG and low levels of Vitamin D3. In the cohort with more than 14 years old we found a deficiency in Vitamin D3 in 54% of patients with AN-RES and 80% of patients with AN-PUR. While in the patients with less than 14 years old the 60% of AN-RES exhibit a low level of Vitamin D3. All together these results demonstrate that Vitamin D3 is an important parameter and we must include it in the blood tests when there is a suspect of EDs.

One of the limitations of this study is the small sample size, but it is an interesting stimulus to continue the research in this field considering an age-specific ranges (for example less or more than 14 years old) for improve the accuracy and the reliability of the results.

Conclusions

In conclusion, although EDs are psychiatric diseases and mainly characterized by a multifactorial aetiology, by patient reports (inspected at the time of hospitalisation) it is possible to trace the most common and significant clinical signs that could confirm the presence of EDs or otherwise be helpful in completing an optimal diagnosis of EDs.

Limit: however, it should be considered that an alteration of the body water and the adaptive responses mediated by our body as a result of malnutrition can, sometimes, distort the blood count tests.

In addition, from the analysis of our data, but also after comparison with the previous literature and, specifically, through a careful comparison with the document of the Ministry of Health (see above), there is a need for further study.

Unfortunately, there are currently no universally validated and diagnostic reliable clinical biochemistry and molecular parameters (39), but in the light of the findings, our research could be an interesting stimulus to continue the survey, perhaps with a larger sample, linking it to other important evaluations.

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Author Contributions

Conceptualization, M.R.C. and E.A.; Methodology, V.L.B.; Formal analysis G.G.; Software, F.F.P.; Investigation, V.L.B. and M.C..; Data Curation, M.C. and T.B.; Writing – Original Draft Preparation, M.R.C. and V.L.B.; Writing – Review & Editing, M.R.C. and G.G.; Supervision, E.A. and L.D.R; Project Administration, T.B and L.D.R.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Ethical approval

The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent

All participants (or their parent or legal guardian in the case of patients under 16) provided written informed consent prior to inclusion in this project.

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