An-Najah National University

Faculty of Graduate Studies

Association between Autism and Iron Deficiency in Autistic Children in the Northern West Bank

By

Sajed Faisal Al Ali

Supervisor

Dr. Aidah Abu Elsoud Alkaissi

Co-Supervisor

Dr. Sabrina Russo

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This Thesis was successfully defended on 26/ 6/2013, and approved by:

Defense Committee Members	**Signature**
1. Dr. Aidah Abu Elsoud Alkaissi/ Supervisor	.....
2. Dr. Sabrina Russo / Co-Supervisor	.....
3. Dr. Ameen Thaljy / External Examiner	.....
4. Dr. Walid Basha / Internal Examiner	.....

الإهداء

- إلهي لا يطيب الليل إلا بشكرك ولا يطيب النهار إلا بطاعتك .. ولا تطيب اللحظات إلا بذكرك .. ولا تطيب الآخرة إلا بعفوك .. ولا تطيب الجنة إلا برؤيتك ﷻ..الله جل جلالهﷻ

- إلى من بلغ الرسالة وأدى الأمانة .. ونصح الأمة .. إلى نبي الرحمة ونور العالمين .. ﷺ سيدنا محمد صلى الله عليه وسلمﷺ

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الاقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان :

Association between Autism and Iron Deficiency in Autistic Children in the Northern West Bank

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم لنيل أية درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is there researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

APA	American Psychiatric Association
ASD	Autistic Spectrum Disorders
DSM-IV	The 4th edition of the Diagnostic and Statistical Manual of Mental Disorders
Food Repertoire	How many unique foods (including beverages) each child consumed over a day
HCT	Hematocrit
HGB	Hemoglobin
ICD-10	The 10th revision of the International Classification of Diseases
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
IRB	Institutional Review Board
MCV	Mean Corpuscular Volume
NO	Number
RDW	Red Cell Distribution Width
SF	Serum ferritin
UK	United Kingdom
USA	United State of America
WHO	World Health Organization

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Abstract

Background: Iron has an important role in cognitive, behavioral, and motor development. A high prevalence of iron deficiency (ID) has been reported in people with autism. Children with autism are at risk for ID and this condition may increase the severity of psychomotor and behavioral problems, some of which already inherently exist in these children.

Aim: The aim of this study is to investigate the association between autism and iron deficiency in autistic children in the northern West Bank and to identify food selectivity and compare indices of food selectivity among children with autism, children with mental disorders and typically developing children (normal children).

Participant and Methods: 90 children with an age range of 3 to 13 years participated in a case control study distributed into study group and two control groups. Thirty children diagnosed with autism according to DSM-IV and ICD-10criteria served as a study group, 30 children with mental disorders other than autism served as a control group, and 30 typically developing children taken from the public functioned as a second control group. The three groups were matched for age, gender and geographical

area. Serum ferritin, hemoglobin, hematocrit, mean corpuscular volume, and red cell distribution width values were measured and analyzed with food habit survey and demographic data.

Results: ID was detected in 20% (N = 6/30) of autistic children based on Serum ferritin level (SF< 10µ/l), compared with 0% for the two control groups (p= 0.0001). Anemia was defined as hemoglobin <110g/l for children under the age of 6 years and hemoglobin <120g/l for children between 6 and 13 years of age .When analysis done for HGB to these six children (children who have low serum ferritin); it was found that 66.6% (4/6) of the children two were pre-school male children (HGB is less than 110g/1), and the other two were one male and one female of school children (HGB is less than 120g/l) have iron deficiency anemia, and the iron deficiency anemia was 13.3% (4\30) for all autistic group. The results indicated that these differences were for males. It was found also that the frequency of low iron intake in these children was associated with feeding difficulties and food selectivity; there was a significant difference between children in the autistic group who chose foods with a red color as a favorite 23% (7/30) compared to the other two control groups: 0%, respectively (p= 0.0001). The results demonstrated also a significant difference in the frequency of snacks per day (≥ 4) in autistic children 40% (12/30) compared to both mental disorder 16.7 % (n = 5/30) (p = 0.006) and typically developing children 6.7% (n = 2/30) groups (p = 0.001).

Conclusion: Results of this study indicated that there is an association between autism, iron deficiency and anemia. Low levels of serum ferritin in autistic children might be a sign of iron deficiency and an early precursor of iron deficiency anemia. These findings suggest that food selectivity is more common in children with autism than in typically developing children. These findings suggest that ferritin levels should be measured in children with autism as a part of routine investigation.

Keywords: Child, Autism, Mental disorder, typically developing children, Iron deficiency, Iron deficiency anemia, Ferritin.

Chapter 1

Introduction

The number of children known to have autism has increased dramatically since the 1980's (Newschaffer, 2007). The reviews of epidemiology in estimating the global prevalence is that one to two cases of autism exist per 2,000 people, and about six per 1,000 people have Autistic Spectrum Disorders (ASD) (Newschaffer, 2007). ASD averages a 4.3:1 male to female ratio (Newschaffer, 2007). In Palestine there is no epidemiological data collected for autism at a national level.

The diagnostic criteria for autism are identified in ICD-10 (World Health Organization, 1993) and in DSM-IV (American Psychiatric Association, 1994). Autism is accompanied by mental retardation in three out of four patients. The underlying etiology of autism it is not known clearly however, it is suggested that autism most likely results from the interaction of multiple genetic and environmental factors (Steyaert & De la Marche, 2008).

Autism is a neurodevelopment disorder characterized by qualitative impairments in social interaction, and verbal and non-verbal communication, along with restricted, stereotyped interests and behaviors (APA, 1994). Autism is a severe, life-long developmental disorder that compromises functioning across multiple domains including social behavior, language, sensory function, and ritualistic/repetitive behaviors and interests. While the etiology of autism is complex and not fully understood, strong evidence from twin and family studies suggests a large genetic contribution (Freitag, 2007; Gupta & State, 2007).

Iron deficiency (ID) is the most common and persevering nutritional disorder and continues to be an important public health problem worldwide (WHO, 2007). Specifically in children in the first years of life, hemoglobin (HGB) levels below 11 g/dL have been related to negative cognitive, social, and emotional effects that may lead to irreversible behavioral squeals, even after appropriate treatment (Carter, 2010; Lozoff, 2007).

There is considerable evidence about the important role of iron in cognitive, behavioral, and motor development (Beard, 2001). It is a component of many enzymes involved in neurotransmitter synthesis, and in iron deficiency, due to decreased activity of associated enzymes, monoamine neurotransmitter systems may be affected (McCann & Ames 2007). A decrease in brain iron concentration is accompanied by changes in serotonergic and dopaminergic systems, in cortical fiber conduction, and myelogenesis (Erikson et al., 2001).

Intellectual impairment in children with autism seems to be related not to anemia itself but to the decrease in iron storage mainly in the central nervous system. This is probably due to the role of iron in intra neural enzymatic activity and in neurotransmitter packaging, reuptake and degradation, as well as in the relationship between iron and D2 dopamine receptors (Nelson et al., 1997; Youdim et al., 1983).

Iron was also found to have a role in myelogenesis and cortical fiber conduction (Ahmed et al., 1995). These finding suggest that iron is necessary for normal development and functioning of the nervous system.

Furthermore, iron depletion may also act as a marker for other nutritional deficiencies, especially in children who are already selective with their diet.

Viteri (1998) stated that the tragedy of iron deficiency is that unless it is severe and prolonged enough to cause anemia, it is silent, not being evident by clinical signs, and therefore iron deficiency must be prevented before anemia is detected. This is especially important in children with autism who already suffer from severe communication, behavioral and intellectual impairment.

Serum ferritin (SF) is the most widely used marker of iron stores in body tissues, including the brain. Low levels are a sign of iron deficiency and an early precursor of iron deficiency anemia. It declines before serum iron when iron stores are depleted and exhibits less variability than serum iron (Hallberg, 2002). Inadequate dietary iron intake was considered a cause of iron deficiency, and low iron intake was thought to be associated with food selectivity which is commonly seen in children with an autistic disorder (AD) (Cermak et al., 2010; Herndon et al., 2009; Johnson, 2008; Xia et al., 2010).

The presence of ID without anemia is sufficient for occurrence of functional disturbances (Akman et al., 2004; Otero et al., 1999). Besides, it is possible that children with anemia explore the environment less and move less compared from their healthy counterparts and that may preclude them to receive sufficient stimulus and develop new skills (Abbott, 1998).

ID can no longer be considered a simple problem easily reversed by iron therapy. Although ID is one of the few treatable causes of psychomotor retardation if commenced early, there is evidence that these adverse consequences may not be fully reversible with treatment. This may be linked with a critical point in early childhood where lack of iron may have a permanent deleterious effect on the brain (Idjradinata & Pollit, 1993).

Feeding difficulties and selectivity with food are a major concern in the majority of children on the spectrum, in which the child rejection of variety causes problems. There may be selectivity regarding the shape, color, smell and consistency of the food and the way it is presented to the child. Disruptive mealtime behavior and the consumption of large amounts of milk may cause further nutritional difficulties (Baron-Cohen and Bolton, 1993).

The aim of this study is to explore the association between autism and iron deficiency in autistic children in the Northern West Bank and to define food selectivity and compare indices of food selectivity among children with autism, children with other mental disorders and typically developing children.

1.1 Background

1.1.1 Definition of Autism

Autism is a developmental disorder characterized by qualitative abnormalities of social interaction, impairments in communication, and

unusual forms of repetitive behavior (Rapin, 1997). A disturbance of emotion, attention, activity, and thought, and associated behavioral problems occur in children with autism of all ages (Lainhart, 1999). Autism is also defined by the presence of marked social deficits, specific language abnormalities and stereotyped, repetitive behaviors (American Psychiatric Association, 1994).

1.1.2 Diagnostic Age

Onset must be prior to age three years, with delays or abnormal functioning in either social interaction, language as used in social communication, or symbolic or imaginative play (APA, 2000).

1.1.3 Etiology of Autism

The etiology of autism has been debated, with various proponents favoring behavioral, environmental, dietary, viral/immunologic, autoimmune, or genetic theories (Smalley et al., 1988). Inheritance and environmental risk factors contribute to autism as well (Newschaffer et al., 2002; Rodier & Hyman, 1998).

The molecular origin of autism is not known; however, studies of families and twins revealed that inheritance contributes significantly to autism (Bailey, et al., 1995; Spiker, et al., 1994). The genetics of autism consist of numerous loci and interaction of various genes (Risch et al., 1999). The most promising regions among the detected lico were found on chromosome 7q (Ashley-Koch et al., 1999; Buxbaum et al., 2001; Shao et

al., 2002). Environmental factors identified as contributors to autism include intrauterine rubella (Chess, 1997), thalidomide taken during pregnancy (Zwaigenbau et al., 2002), and cytomegalovirus infection (Stubbs et al., 1984). The measles, mumps, and rubella vaccines have been thought to cause autism; however, research has not determined any connection between the vaccines and autism (Dales et al, 2001; Taylor et al., 2002).

1.1.4 Diagnosis of Autism

When sample was recruited the DSM fifth edition wasn't existed yet; so the participants were diagnosed on DSM-IV or ICD-10 by qualified psychiatrist.

[The following is from **Diagnostic and Statistical Manual of Mental Disorders: DSM IV**] (I) A total of six (or more) items from (A), (B), and (C), with at least two from (A), and one each from (B) and (C).

(A) Qualitative impairment in social interaction, as manifested by at least two of the following:

1. Marked impairments in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body posture, and gestures to regulate social interaction.

2. Failure to develop peer relationships appropriate to developmental level.

3. A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people, (e.g., by a lack of showing, bringing, or pointing out objects of interest to other people).

4. Lack of social or emotional reciprocity (note: in the description, it gives the following as examples: not actively participating in simple social play or games, preferring solitary activities, or involving others in activities only as tools or "mechanical" aids).

(B) Qualitative impairments in communication as manifested by at least one of the following:

1. Delay in or total lack of the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime).

2. In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others.

3. Stereotyped and repetitive use of language or idiosyncratic language.
4. Lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level.

(C) Restricted repetitive and stereotyped patterns of behavior, interests and activities, as manifested by at least two of the following:

1. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus.

2. Apparently inflexible adherence to specific, nonfunctional routines or rituals.

3. Stereotyped and repetitive motor mannerisms (e.g; hand or finger flapping or twisting, or complex whole-body movements).

4. Persistent preoccupation with parts of objects.

(II) Delays or abnormal functioning in at least one of the following areas, with onset prior to 3 years of age:

(A) Social interaction .

(B) language as used in social communication.

(C) symbolic or imaginative play.

(III) The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder (American Psychiatric Association1994).

ICD-10 Criteria for "Childhood Autism"

A. Abnormal or impaired development is evident before the age of 3 years in at least one of the following areas: receptive or expressive language as used in social communication; the development of selective social attachments or of reciprocal social interaction; functional or symbolic play.

B. A total of at least six symptoms from (1), (2) and (3) must be present, with at least two from (1) and at least one from each of (2) and (3):

1. Qualitative impairment in social interactions is manifest in at least two of the following areas:

a. Failure adequately to use eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction.
b. Failure to develop (in a manner appropriate to mental age, and despite ample opportunities) peer relationships that involve a mutual sharing of interests, activities and emotions.
c. Lack of socio-emotional reciprocity as shown by an impaired or deviant response to other people's emotions; or lack of modulation of behavior according to social context; or a weak integration of social, emotional, and communicative behaviors.
d. Lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g. a lack of showing, bringing, or pointing out to other people objects of interest to the individual).

2. Qualitative abnormalities in communication as manifest in at least one of the following areas:

a. Delay in or total lack of, development of spoken language that is not accompanied by an attempt to compensate through the use of gestures or mime as an alternative mode of communication (often preceded by a lack of communicative babbling).
b. relative failure to initiate or sustain conversational interchange (at whatever level of language skill is present), in which there is reciprocal responsiveness to the communications of the other person;

c. Stereotyped and repetitive use of language or idiosyncratic use of words or phrases.
d. Lack of varied spontaneous make-believe play or (when young) social imitative play.

3. Restricted, repetitive, and stereotyped patterns of behavior, interests, and activities are manifested in at least one of the following:

a. An encompassing preoccupation with one or more stereotyped and restricted patterns of interest that are abnormal in content or focus; or one or more interests that are abnormal in their intensity and circumscribed nature though not in their content or focus.

b. Apparently compulsive adherence to specific, nonfunctional routines or rituals.

c. Stereotyped and repetitive motor mannerisms that involve either hand or finger flapping or twisting or complex whole body movements.

d. Preoccupations with part-objects of non-functional elements of play materials (such as their odor, the feel of their surface, or the noise or vibration they generate).

C. The clinical picture is not attributable to the other varieties of pervasive developmental disorders; specific development disorder of receptive language (F80.2) with secondary socio-emotional problems, reactive attachment disorder (F94.1) or disinhibited attachment disorder (F94.2);

mental retardation (F70-F72) with some associated emotional or behavioral disorders; schizophrenia (F20.-) of unusually early onset; and Rett's Syndrome (F84.12) (World Health Organization. 1992).

1.1.5 Management & Mental Health Nurse Implication for Autism

The primary goals of treatment of autism are to minimize the core features and associated deficits, maximize functional independence and quality of life, and alleviate family distress (Scott et al, 2007). Facilitating development and learning, promoting socialization, reducing maladaptive behaviors, and educating and supporting families can help accomplish these goals. Educational interventions, including behavioral strategies and rehabilitative therapies, are the cornerstones of management of ASDs. These interventions address communication, social skills, daily-living skills, play and leisure skills, academic achievement, and maladaptive behaviors. Optimization of medical care is also likely to have a positive impact on rehabilitative progress and quality of life (Scott et al, 2007). In addition to routine preventive care and treatment of acute illnesses, management of sleep dysfunction, coexisting challenging behaviors or psychiatric conditions, and associated medical problems, such as seizures, may be particularly important. Medications have not been proven to correct the core deficits of ASDs and are not the primary treatment (Scott et al, 2007). However, one kind of medicine (Risperidone) is used for treating some cases of Autism. It is used to treat behavior problems such as aggression, self-injury, and sudden mood changes in teenagers and children

5-16 years old who are affected by autism (The American Society of Health-System Pharmacists 2011).

Chapter 2

Literature review

2.1.1 Iron deficiency

Lozoff et al. (1992) reported that nervous system development and function appear to be permanently damaged from early iron deficiency, resulting in impaired cognition, diminished learning capacity and attention span, and neuromotor dysfunction. In young iron deficient children, iron supplementation has been shown to improve growth and psychomotor development as well as behavior (Moffatt et al., 1994).

Dallman et al. (1996) used cutoff reference value for SF of 10 Ug/l for children 1 to 5 years and 12 Ug/l for children 6 to 11 years. Oski et al (1983) stated that iron depletion means that erythropoiesis is normal but iron stores are reduced (serum ferritin < 12 Ug/l) indicating a reduction of iron in the bone marrow, liver and other parts of reticuloendethelial system.

2.1.2 Iron deficiency and Autism

Researches show that a high prevalence of iron deficiency has also been reported in children with autism spectrum disorders (Bilgiç, 2010; Dosman, 2006; Latif, 2002). Inadequate dietary iron intake was considered as a cause of iron deficiency, and low iron intake was thought to be associated with food selectivity which is commonly seen in children with autistic disorders (Cermak, 2010; Herndon, 2009; Johnson, 2008; Xia, 2010).

However, it was shown that serum ferritin concentration return to a normal level with iron supplementation (Dosman et al., 2007) and this finding supports the notion that ID is associated with low iron intake in children

with ASDs. The high frequency of low iron intake in these children is thought to be associated with feeding difficulties and food selectivity (Cornish, 1998).

Children of the autistic spectrum are at high risk of developing iron deficiency and/or iron deficiency anemia which might further compromise their learning and behavioral impairments. Serum ferritin is the most widely used marker of iron stores in body tissues, including the brain. Low levels are a sign of iron deficiency and an early precursor of iron deficiency anemia. It declines before serum iron when iron stores are depleted and exhibits less variability than serum iron (Hallberg 2002).

2.1.3 Diagnostic criteria for Autism

The diagnostic criteria for autism identified in ICD-10 (World Health Organization, 1993) and in DSM-IV (American Psychiatric Association, 1994) are that children which autism characteristically present with impairments in social interaction, communication and imagination. In addition, repetitive behavioral patterns, obsessions with objects and resistance to change in routines are notable features (DSM; ICD-10).

Using DSM-IV, patients could be diagnosed with four separate disorders: autistic disorder, Asperger's disorder, childhood disintegrative disorder, or the catch-all diagnosis of pervasive developmental disorder not otherwise specified. Researchers found that these separate diagnoses were not consistently applied across different clinics and treatment centers. Anyone

diagnosed with one of the four pervasive developmental disorders (PDD) from DSM-IV should still meet the criteria for ASD in DSM-5 or another, more accurate DSM-5 diagnosis. While DSM does not outline recommended treatment and services for mental disorders, determining an accurate diagnosis is a first step for a clinician in defining a treatment plan for a patient (APA, 2013).

The Neurodevelopmental Work Group, led by Susan Swedo, MD, senior investigator at the National Institute of Mental Health, recommended the DSM-5 criteria for ASD to be a better reflection of the state of knowledge about autism. The Work Group believes a single umbrella disorder will improve the diagnosis of ASD without limiting the sensitivity of the criteria, or substantially changing the number of children being diagnosed (APA, 2013).

2.1.4 Repetitive behavior in Autism

In terms of autistic repetitive and ritualistic behavior, the presence of restricted and repetitive interests and behavior is one of the defining characteristics of ASD (American Psychiatric Association, 2000). Repetitive behaviors (RBs) have been addressed extensively in the research literature, with considerable study focused on factors related to the presence of each of two types of RBs: restricted interests/cognitive inflexibility (higher order) and repetitive motor behavior/stereotypes (lower order), a dichotomy suggested by Turner (1999). Research has suggested that higher order RBs may be more indicative of ASD and less related to

the developmental level of the child (Carcani-Rathwell et al., 2006; Szatmari et al., 2005).

2.1.5 Feeding difficulties and selectivity

The feeding rituals that children with ASD often demand extend to other aspects of mealtime, including insistence on specific methods of preparation, food types, and mealtime rules (Raiten & Massaro, 1986; Schreck & Williams, 2006; Williams et al., 2000). These rituals, as well as the patterns of food selectivity described in the research literature, most frequently exemplify higher order RBs (Ahearn et al., 2001; Raiten & Massaro, 1986; Schreck et al., 2004; Williams et al., 2005; Williams et al., 2000). This indicates that the presence of rituals at mealtime is more likely to be related to autistic symptomatology and its neurological bases than to behavioral noncompliance or purely developmental factors. Many anecdotal reports have documented specific feeding rules, including insistence that all foods on a plate should be the same color, eating the same food at each meal, requiring that foods be presented in a particular order, or requiring that foods not touch each other on a plate (e.g., Ernsperger & Stegen-Hanson, 2004; Legge, 2002). Lending credence to this contention is the inclusion by Bodfish, Symons, and Lewis (1999) of an item relating to “eating/mealtime” behaviors in the Repetitive Behavior Scale—Revised (RBS–R; Bodfish et al., 1999); one of six items within the ritualistic behavior subscale, this item lists as examples “strongly

prefers/insists on eating/drinking only certain things; eats or drinks items in a set order; insists that meal-related items are arranged in a certain way"

A study examining food acceptance assessed the feeding behaviors of 30 autistic children (Ahearn et al., 2001). This study excluded children who had previously been referred for treatment of aberrant feeding behavior. Half of the children were found to exhibit certain patterns of food acceptance, including selectivity by food category or texture. What this finding means is unclear, as noted in the article, because there was no comparison group to assess feeding patterns of normally developing children. With regard to feeding, available evidence indicates that autistic children tend to have increased food selectivity (Ahearn et al., 2001; Field et al., 2003).

2.1.6 Gastrointestinal dysfunction

Many parents report gastrointestinal symptoms in their autistic child; however, until recently, gastrointestinal symptoms of these children received little attention. D'Eufemia et al. (1996) reported increased intestinal permeability in 9 of 21 (43%) patients with autistic disorder. The report of Wakefield et al. (1998) represents the first effort to evaluate the gastrointestinal tract in children with autism. In a recent case report it was described 3 children with autistic spectrum disorder and chronic diarrhea that had an increased pancreatico-biliary secretory response after secretin injection, suggesting that gastrointestinal dysfunction might be associated with autism (Horvath, 1998).

Horvath (1999) reported that “twenty-two” of “twenty-five” autistic children (88%) had symptoms such as nighttime awakening with irritability, signs of abdominal discomfort, or pushing on the abdomen.

Few studies have addressed gastrointestinal problems in children with autistic disorder. Goodwin et al (1971) studied 15 randomly selected children with autism and found that 6 had either bulky, odorous, or loose stools or intermittent diarrhea; one had celiac disease. In a study, 43% of the autistic patients without symptoms or evidence of any gastrointestinal disease had altered intestinal permeability (Goodwin et al 1971). Low concentrations of serum α1 -antitrypsin were reported in children with typical autism (Walker-Smith & Andrews 1972). A finding that is indicative of intestinal protein loss. In a case report gastrointestinal and behavioral observations on 3 children with autistic spectrum disorder were reported (Horvath et al., 1998), although gastrointestinal symptoms frequently accompany the manifestations of autism, little attention has been paid to this aspect of this developmental behavioral disorder, and a gastrointestinal workup has not been part of the regular medical evaluations. Sudden unexplained irritability or aggressive behavior, mood change, discomfort, and nighttime awakenings in these children were considered to be part of the brain dysfunction and not manifestations of organic problems. A significant percentage of children with autistic disorder are reported to be low functioning and have only prelinquistic communicative behavior. A plausible reason for the paucity of gastrointestinal evaluation of these children may be their inability to

verbalize and describe their abdominal pain or discomfort and a lack of cooperation (Horvath et al., 1998). The upper gastrointestinal evaluations of children with autistic disorder support the presence of a chronic inflammatory process in the gut, as reported by Wakefield et al. (1998) They performed colonoscopy with histologic examinations in 12 children and reported that all had intestinal abnormalities, ranging from lymphoid nodular hyperplasia to aphthoid ulceration. The most frequently detected abnormalities in children with autistic disorder included a high prevalence of reflux esophagitis, hyperplasia of duodenal Paneth's cells, intestinal carbohydrate digestive enzyme deficiencies, (Horvath 1999)**.** Many parents of children with autism had reported gastrointestinal symptoms in their children, which might suggest malabsorption (Horvath 1999) as a possible cause of iron deficiency in autism.

2.1.7 Maternal and neonatal factors

Several studies involved the creation of scores summarizing various combinations of maternal and neonatal factors such as maternal age, parity, intrauterine bleeding, infection, caesarian delivery, breech presentation, Rh incompatibility, neonatal birth weight, gestational age, Apgar score, and meconium staining. Most of the studies using composite sub optimality scores reported less optimal pre, peri-, and neonatal experiences among children with autism compared with both population and sibling controls (Gillberg, 1983; Lord et al, 1991; Stein et al, 2006; Sugie, 2005).

2.2 Study objectives

- To explore the association between Autism and Iron deficiency in autistic children in the northern West Bank.
- To identify food selectivity and compare indices of food selectivity among children with autism, mental disordered and typically developing children (normal children).

2.3 Problem statement

- The iron has important role in cognition, behavior, mood, concentration and communication.
- It is not clear whether the person with autism has an iron deficiency due to disease itself or anything related to behavior, environment, parenting and growth issue.
- Because of autism may not be the only mental illness which has iron deficiency, so it's important to do research on other psychiatric disorders group (control group 1).
- Autism is not the first priority in the community mental health in Palestine.
- The author wishes to emphasize the neglected disorder (Autism), where we have many problems in diagnosis and treatment.
- Considering the absence of sufficient research around the world on the relationship between autism and the lack of iron, this study aims to relate autism and iron deficiency, as the first study in Palestine.

- The author wishes this study to be the first step for other researcher in the future.

Chapter 3

Methodology

- The study design: Case control study.

- Setting: The study was conducted in the North West Bank, and three cities were included (Jenin, Nablus, Tulkarm).

3.1.1 Participant:

Three groups were considered in the research: a study group, and two control groups (**Table 1).**

The study group: Thirty autistic children were participating from three geographical areas Jinen, Nablus, Tulkarm. Contact with children was implemented through several rehabilitation centers.

The second group: Control Group (1), 30 children were chosen from another mental disorder, but not autism from the same centers, or any other center in the same geographical areas.

The third group: Control group (2) 30 typically developing children (normal children) were chosen form public in the same geographical and cultural characteristic of the study group.

Table 1: Centers for study and control group\cities

City	Center for study group	Centers for control (1) group
Jinen	Al Ghad center	Governmental Community Mental Health center
Nablus	Farah center, Care for children with special needs society	Farah center, Care for children with special need society
Tulkarm	Autistic & learning disability rehabilitation society	Autistic & learning disability rehabilitation society

3.1.2 Sampling procedure:

Sample: Purposive sampling of the study, the researcher took 50% of autistic children in the northern West Bank (Jinen, Nablus, Tulkarm), the total number of diagnosed children for autism is about 60 and 30 of them were recruited in the study group by performing a simple random. The three groups were matched for gender, and age.

3.1.3 Inclusion criteria

For study group;

- Children who are diagnosed with Autism by (DSM-IV) and ICD (10) by a qualified psychiatrist.
- Children who do not take supplements of iron or vitamins.

For control group (1);

- Children who are diagnosed with mental disorders, but not autism
- Children who do not take supplements of iron or vitamins.

For control group (2);

- Children who are free from any disease and they do not takes any type of medications or supplements of iron or vitamins.

3.1.4 Exclusion criteria

For study group;

- Children with chronic neurological disorders or physical illness than autism.
- Since ferritin is a marker of inflammation, children with infection or other inflammatory conditions were excluded from the study
- Children who received iron supplements during the last 3 months and / or who were on any dietary restrictions.
- Children who are inpatients in rehabilitation centers.

For control group (1);

- Diagnosed with Autism, or having Autistic behavior.
- Children who are inpatients in rehabilitation centers.
- Children who have any type of infections, or acute illness.
- Children who received iron supplements during the last 3 months and / or who were on any dietary restrictions.

For control group (2);

- Children who have any dietary restrictions.
- Children who take any type of medications, or have any illness.

3.1.5 Assessment of questionnaire

None of the available assessment forms were sufficient for the purposes of this study, so the author developed a questionnaire includes specific food habits survey for the study in Arabic language and then the questionnaire translated to the English language for the purpose of research which should be written in English language.

After developing a thorough understanding of the research, the next step is to generate statements/questions for the questionnaire. In this step, content (from literature/theoretical framework) is transformed into statements/questions. In addition, a link among the objectives of the study and their translation into content is established. In Step 3, the focus is on writing statements/questions, selection of appropriate scales of measurement, questionnaire layout, format, question ordering, font size, and proposed data analysis. Scales are devices used to quantify a subject's response on a particular variable. As a result of Steps 1-3, a draft questionnaire is ready for establishing validity. Validity is the amount of systematic or built-in error in measurement (Norland, 1990). Validity is established using a panel of experts and a field test. The Arabic version was handed to the participants (Appendix 1). The questionnaire was given to 9 people, 2 doctors, 3 nurses, 3 researchers, and 1 statistician, who were

asked to judge whether or not the questions were appropriate and reasonable. After some changes the questionnaire was considered valid.

The following questions are addressed in Step 4:

1. Is the questionnaire valid? In other words, is the questionnaire measuring what it intended to measure?
2. Does it represent the content?
3. Is it appropriate for the sample/population?
4. Is the questionnaire comprehensive enough to collect all the information needed to address the purpose and goals of the study?
5. Does the instrument look like a questionnaire?

The next step is to conduct a field test using subjects not included in the sample. Make changes, as appropriate, based on both a field test and expert opinion. Now the questionnaire is ready to pilot test. In this final step, reliability of the questionnaire using a pilot test is carried out. Reliability refers to random error in measurement. Reliability indicates the accuracy or precision of the measuring instrument (Norland, 1990). The pilot test seeks to answer the question; does the questionnaire consistently measure whatever it measures?

The use of reliability types (test-retest) depends on the nature of data (nominal, ordinal, interval /ratio). To assess reliability of knowledge questions, test-retest is appropriate.

Reliability is established using a pilot test by collecting data from 20 subjects not included in the sample. Data collected from pilot test is analyzed using SPSS (Statistical Package for Social Sciences). The reliability coefficient (alpha) can range from 0 to 1, with 0 representing an instrument with full of error and 1 representing total absence of error. A reliability coefficient (alpha) of .70 or higher is considered acceptable reliability. Reliability of the survey was investigated with a test—retest. The test—retest correlation coefficient was 0.82. The questionnaire was described as appropriate and gave a correct picture of their experience by 100% of the participants.

3.1.6 Description of questionnaire

- The questionnaire (Appendix 1) was divided into three sets, first set for background information, the second set for parent information and the third set of 9 questions for patient information (food habits survey).
- The questions were open-ended questions. There is one close ended question about gastric dysfunction. The closed-ended question had options and the participant has to choose among these options the most appropriate answer.
- Thereafter the patients were asked to report other things that were not mentioned before.
- Parents were interviewed about their child's dietary habits. Parents also completed a demographic questionnaire and were instructed by the

author to complete a food habit survey. The completed questionnaire record was returned to the researcher.

3.1.7 Laboratory measurements

Ferritin, hemoglobin, hematocrit, Mean corpuscular volume (MCV), and Red Cell Distribution Width (RDW) values were measured.

Serum ferritin level is taken as an indicator of ID, because ID is the only cause of low ferritin concentration. Serum ferritin level reliably shows iron levels in body tissues including brain and is also an early precursor of ID (Worwood , 1997).

There is no consensus in the literature about the cutoff value for low serum ferritin in children (Cortese, 2009). The auther used ferritin cutoff of <10 ng/mL for preschoolers and <12 ng/mL for school-aged children to estimate iron deficiency since this was a widely used criterion in previous studies (Dosman, 2006; Latif, 2002).

The following cutoffs were used based on our hospital laboratory values: HGB Hemoglobin<110g/l (De Maeyer et al., 1989), hematocrit, <35%; mean corpuscular volume (MCV), <80 fL; and red cell distribution width (RDW), >14.5%. Ferritin, hemoglobin, hematocrit, MCV, and RDW values were measured in fasting blood in the morning at the university Laboratory using standard measurement assays. All subjects with iron deficiency were informed.

3.1.8 Statistical analysis:

Data were analyzed using SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). Demographic data and Clinical measurement were shown as means, frequency, and SD or percentages. Descriptive analyses of hematological values were conducted.

- **Chi-square test**

A chi-square test is used when you want to investigate if there is a relationship between two categorical variables. In SPSS, thechi-square option is used on the statistics subcommand of the crosstabs command to obtain the test statistic and its associated p-value.

- **One-way ANOVA**

A one-way analysis of variance (ANOVA) is used when you have a categorical independent variable (with two or more categories) and a normally distributed interval dependent variable and you wish to test for differences in the means of the dependent variable broken down by the levels of the independent variable

- **Two independent samples t-test**

An independent samples t-test is used when you want to compare the means of a normally distributed interval dependent variable for two independent groups. A probability level of $p<.05$ was used to indicate statistical significance.

Descriptive analyses of hematological values were conducted.

3.1.9 Procedure:

Ethical approval was obtained from the Institutional Review Board (IRB) of An-Najah National University, and the permission was obtained to conduct the study in rehabilitation centers. The study purposes were explained to all parents. The parents who agreed to participate in the study have read the participant`s letter, and give written informed consent. The demographic data was taken and the blood samples were withdrawn. "The amount of blood taken from each of the child is 2 ml. Volume of 2ml is enough, 1 ml in Edita tube and 1 ml in the plane tube. Blood tubes were sent immediately to An Najah National University Laboratory after putting them in ice to keep them valid. "

The control group (1) mental disorder but not autism was chosen from the same rehabilitation center or other centers with a matching to the same criteria for age and number of male and female as much as possible. The process of participation was the same for the study group.

For the control group (2) typically developing children, was selected from public as the closest geographic area, and matching was performed as much as possible to the study group. The process of participation was same for the study and control group (1) group. Lab results were sent back to rehabilitation centers and families. A food habit survey was used to record the diet of children. The sample collection started at the beginning of

September in 2012 and finished at the end of December in the same year. Statistical analysis was conducted for all groups and the results were compared with each other, and the conclusion was made.

3.2 Ethical considerations

- Ethical approval was obtained from the Institutional Review Board (IRB) of An-Najah National University.
- A letter from the university to obtain permission to conduct the study in the rehabilitation centers was obtained.
- The process and purpose of the study was described in detail to the child and their care givers and care givers who agreed to participate in the study were asked to sign an informed consent.
- Participation was voluntary and they were able to withdraw from the study at any time without any negative consequences.
- The information kept confidential.
- Safety and security during the procedure (drawing of blood samples) was taken.
- Care was taken into account in order to ease the pain and any complication of needle sticks.
- The procedure of blood taking was done by professionals.

Chapter 4

Results

4.1 Demographic characteristics of the study sample

The number of study sample meeting the inclusion criteria were 90. 33.3% (30/90) are autistic children, 33.3% (30/90) are mental disorders children, and 33.3% (30/90) are typically developing children (normal children).

For all groups the gender was 67.8% (61/90) for males, and 29/90 (32.3%) for females. School age ≥ 6years old (from 6 to 13 years old) was 62.2% (56/90) and pre- school age < 6 years old (from 3 to less than 6 years old) was 37.8% (34/90).Method of delivery: 74.4% (67/90) for normal delivery and 25.6% (23/90) for cesarean. Maternal age at delivery (<19 years): 2.2% (2/90), (20-29 years): 55.6% (50/90), (30-39 years): 37.8% (34/90), (40-49 years): 4.4% (4/90). Length of pregnancy: 91.1% (82/90) for full term, and 8.9% (8/90) for premature. The demographic details are shown in (**Table 2).**

Table 2: Demographic characteristics of the whole group (n=90). Figures are as frequency (%).

Category	Variable	Frequency (%)
Group n (%)	Autism group	30 (33.3)
	Mental disorder	30 (33.3)
	Typically developing children	30 (33.3)
Gender n (%)	Male	61(67.8)
	Female	29 (32.3)
Age n (%)	School age ≥6	56 (62.2)
	Pre- school age < 6	34 (37.8)
Method of Delivery n (%)	Normal	67 (74.4)
	Cesarean	23 (25.6)
Maternal age at delivery n (%)	<19 years	2 (2.2)
	20-29 years	50 (55.6)
	30-39 years	34 (37.8)
	40- 49 years	4 (4.4)
Length of pregnancy n (%)	Full term	82 (91.1)
	Premature	8 (8.9)

All patients completed the study protocol. The response rate was 100%. The three groups were similar with respect to demographic characteristics. The results showed by using Crosstab/Chi-Square test that there was no significant difference between the groups in term of age, sex, method of delivery, maternal age at delivery, length of pregnancy (**Table 3).**

Table 3: Age, sex, method of delivery, maternal age at delivery, length of pregnancy are distributed by the groups n (%)

Category	Variable	Group			Sig*
		Autism group n=30	Mental disorder n=30	Typically developing children n=30	
Gender n (%)	Female	8 (26.7%)	13 (43.3%)	8 (26.7%)	0.280
	Male	22 (73.3%)	17 (56.7%)	22 (73.3%)	
Age n (%)	School age ≥6	18 (60%)	19 (63.3%)	19 (63.3%)	0.954
	Pre- school age < 6	12 (40%)	11 (36.7%)	11 (36.7%)	
Maternal age at delivery n (%)	Normal	22 (73.3%)	21 (70%)	24 (80%)	0.664
	Cesarean	8 (26.7%)	9 (30%)	6 (20%)	
Mothers Age on delivery n (%)	< 19 years	1 (3.3%)	1 (3.3%)	0 (0%)	0.557
	20-29 years	16 (53.3%)	16 (53.3%)	18 (60%)	
	30-39 years	10 (33.3%)	12 (40%)	12 (40%)	
	40- 49 years	3 (10%)	1 (3.3%)	0 (0%)	
Length of pregnancy n (%)	Full term	27 (90%)	27 (90%)	28 (93.3%)	0.872
	Premature	3 (10%)	3 (10%)	2 (6.7%)	

The results showed also that no significant (p=0.441) differences among the autism, mental disorder and typically developing children groups for the frequencies of meals per day (**Table 4)**.

Table 4: Frequency of meals per day and group type

Frequency of meals per day	Group type		
	Autism group	Mental disorder	Typically developing children
2	2	4	6
3	25	25	23
4	1	1	1
5	2	0	0
Total	30	30	30

The results demonstrated also a significant difference in the frequency of snacks per day (≥ 4) in autistic children (n = 12/30) compared with both mental disorder (n = 5/30) (p = 0.006) and typically developing children (n = 2/30) groups (p = 0.001) (**Figure 1).**

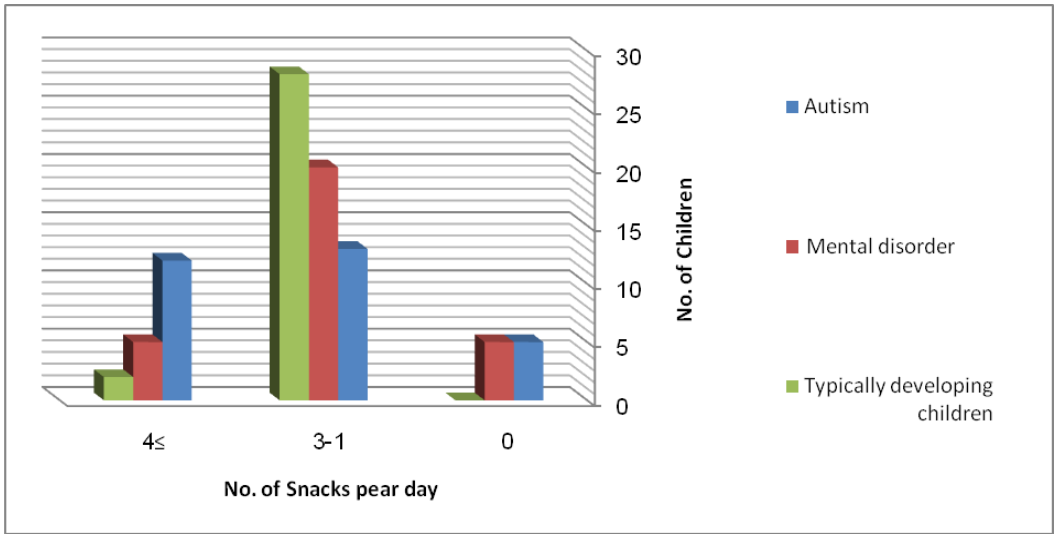

Figure 1: Frequency of snacks pear day**.**

Consistent relationship was found between dietary iron intake and ferritin. Low ferritin was more prevalent among the autistic children who ate food preferences according to red color compared to the prevalence among the entire sample of children). Related to the issue of the favorite color of food,

there was a significant difference between children in the autistic group who chose foods with red color as favorite 23% (7/30) compared to the other two control groups 0%, respectively (p= 0.0001); **(Figure 2).**

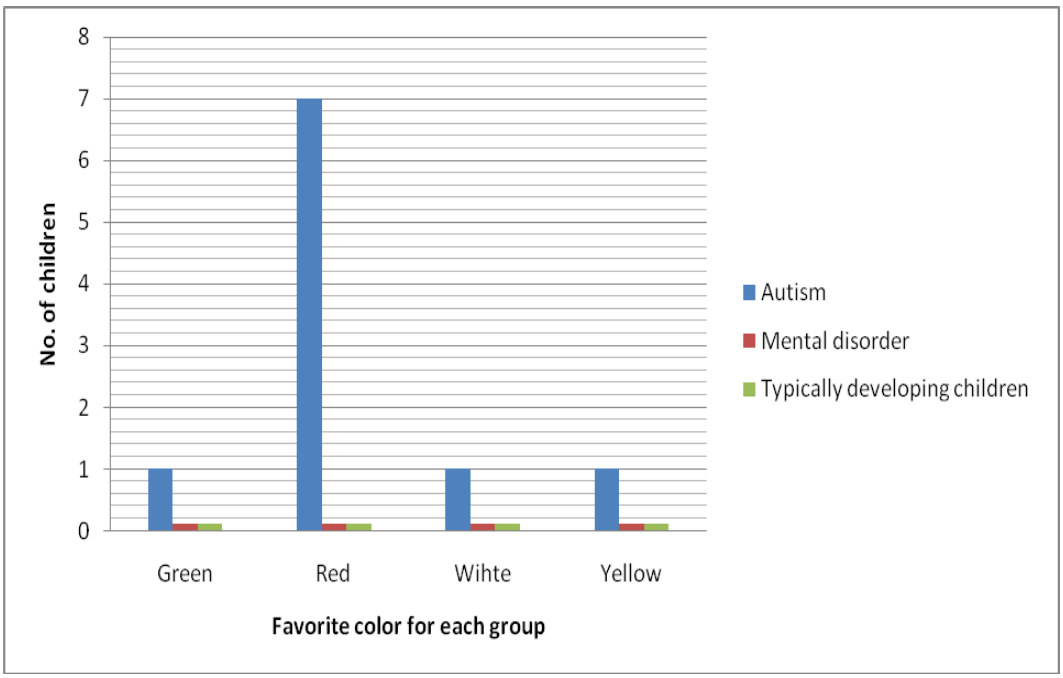

Figure 2: Favorite color of food

Regarding to gastric dysfunction "Unusual annoying symptoms" there are no significant (p=0.386) differences between groups (**Table 5)**.

Table 5: Differences between groups and unusual annoying symptoms.

Unusual annoying symptoms	Group type		
	Autism	**Mental disorder**	**Typically developing children**
Diarrhea	2	2	0
Constipation	2	4	2
Vomiting	0	1	0
Gazes	1	0	2
Total	**5**	**7**	4

4.2 Clinical measurement

The study group was divided into two groups as group 1, cases <6 years old (n=12), and group 2, cases ≥6 years old (n=18).The proportion of children amongst autism group with serum ferritin less than the cutoff value of "10" for pre-school age and "12" for school-age children are significantly lower by 20% (6/30) (p = 0.002), by using Crosstab/chi-square test, compared with a mental disturbance 0% (0/30) and typically developing children 0% (0/30) groups (**Figure 3).**

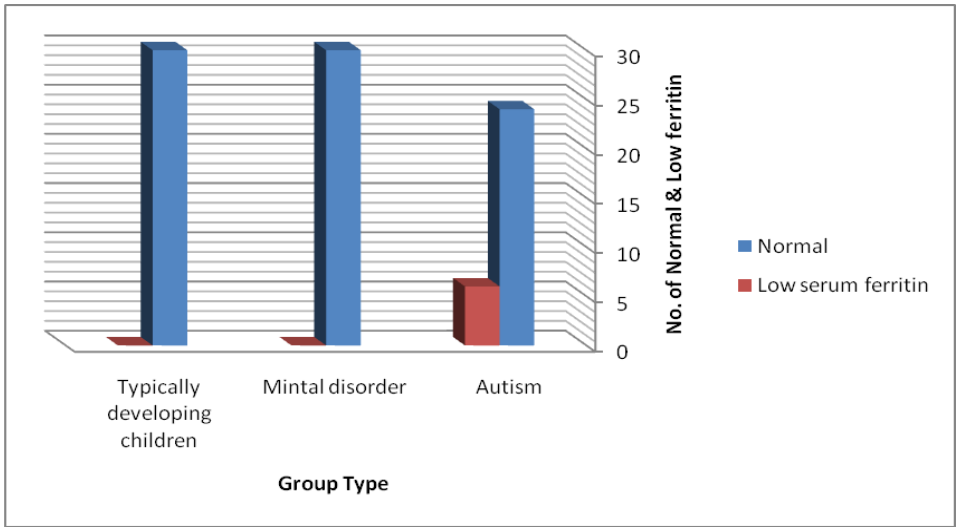

Figure 3: "Normal & Low serum Ferritin" for each group

Anemia was defined as hemoglobin<110g/l for children under the age of 6 years and hemoglobin<120g/l for children 6 to 13 years of age (De Maeyer et al 1989). Serum ferritin levels below 12µg were taken as evidence of iron deficiency. It was found that 20% (6/30) of patients in the autistic group have low ferritin. When we analyzed the other laboratory measurements for them we found that 66.6% (4/6) of children (two males are pre-school children (HGB is less than 110g/l), and the other two, one male and one female are school children (HGB is less than 120g/l)) have iron deficiency anemia. The result was indicated for who have anemia that these differences are for the males 75% (3/4) **(Table 6)**.

Table 6: Laboratory tests for children who have low serum ferritin and their classification to iron deficiency and iron deficiency anemia based on measurements of serum ferritin and hemoglobin (HGB).

No	Age	sex	HGB g/l	HCT	MCV	RDW	Ferritin	Classification
1	School	male	110	39.0	75.3	19.0	7.2	anemia
2	Pre-school	male	100	29.3	63.6	17.8	2.1	anemia
3	Pre-school	male	109	33.3	74.3	15.2	8.6	anemia
4	Pre-school	female	123	36.2	71.9	15.1	9.5	Iron deficiency
5	School	female	114	34.7	76.8	15.0	7.4	anemia
6	Pre-school	male	118	34.9	73.0	16.9	5.6	Iron deficiency

The results showed that the number of children who have less than the cut-off point of HGB (<110g / l) 9/30 is significant difference compared to typically developing children group (1/30) p = 0.018; **Table 7.**

Table 7: Number of affected children who have less than the cut off points of laboratory measurements in the three groups.

Lab tests	Cut off points of laboratory measurements	Number of affected children in Autistic group (n=30)	Number of affected children in mental disorder group (n=30)	Number of affected children in typically developing children. (n=30)	p-value
Hemoglobin (HGB)	<110g/l *	9	6	1*	0.018
Mean corpuscular volume (MCV),	<80 fL	21	17	23	0.631
Hematocrit (HCT)	<35%	19	13	9	0.058
Red cell distribution width (RDW)	>14.5%	10	12	7	0.467

Analysis of variance (ANOVA) was used to analyze differences between the three groups with respect to the mean of HGB, HCT, MCV and RDW. If a difference was found, a post hoc analysis using LSD test was applied.

The result demonstrated that HGB level differed between the three groups [F (4.371), p=0.016]. Further analysis indicated that the HGB in autism group is lower compared to the typically developing children group ($P < 0.05$). The result indicated also that HCT level differed between the three groups [F (4.330), p=0.016]. Further analysis showed that the HCT in autism group is lower compared to typically developing children group ($P < 0.05$). The result indicated that MCV level differed between the three groups [F (3.051), p=0.052]. Further analysis indicated that the MCV in

autism group is lower compared to typically developing children group (P = 0.052) (**Table 8).**

No difference was demonstrated between the groups regarding RDW (p=0.106). The result showed the mean of Ferritin in Autistic (29.630) and Mental disorder groups (29.513) are near to each other but its lower than typically developing children (35.880).The differences are not significant (p= 0.316) (**Table 8).**

Table 8: One Way ANOVA test for the association between the clinical measures of (HGB, HCT, MCV, RDW and Ferritin) between the groups.

Measure/ Group	**Means**			**(F)**	**Sig.***
	Autism group N:30	Mental disorder N:30	Typically developing children N:30		
HGB	11.543	11.960	12.250	4.371	0.016*
HCT	34.320	36.453	35.707	4.330	0.016*
MCV	76.597	80.243	78.213	3.051	0.052
RDW	14.373	14.630	13.850	2.302	0.106
Ferritin	29.630	29.513	35.880	1.167	0.316

*** The mean difference is significant at the 0.05 level.**

When analyzing the ferritin level related to the gender; it was shown that males have lower rate of ferritin level than females rate in Autistic group but it wasn't significant (p=0.697), but the males in typically developing children have higher rate of ferritin level than females rate and it was significant (p=0.017) (**figure 4).**

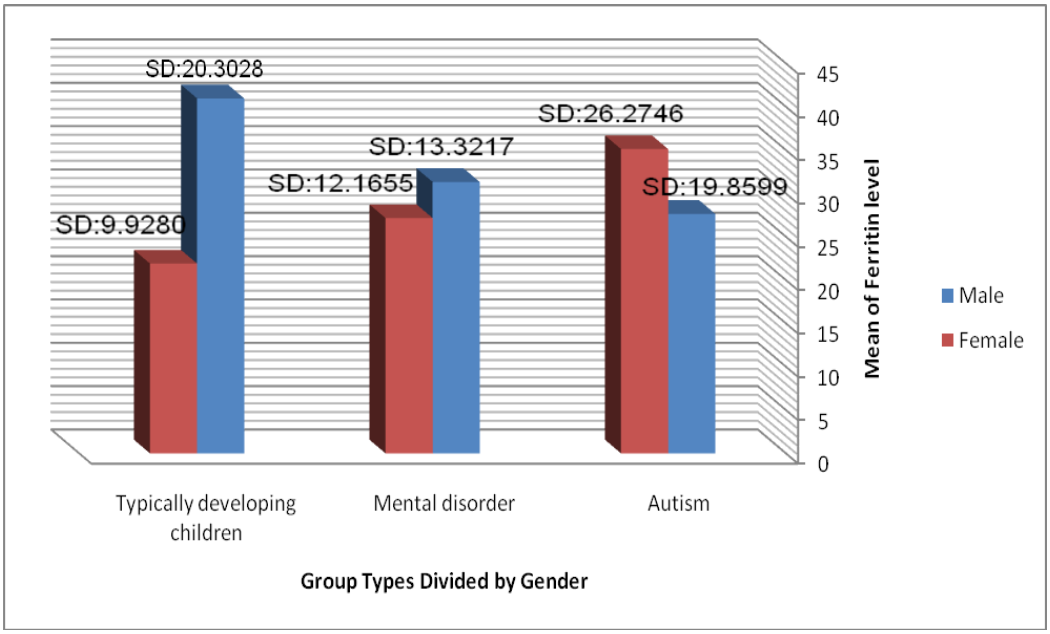

Figure 4: Ferritin levels in the three groups divided by gender

The T- test analysis of the age variable in relation to clinical measures **(HGB, HCT, MCV, RDW and Ferritin)** of the Autism group indicated that there were significant differences between school age ≥ 6 years & under school age<6 years groups in MCV measure (P=(0.004). Also, the test indicated that these differences are for school age ≥ 6 years (**Table 9).**

Table 9: T-test for the association between the clinical measures (HGB, HCT, MCV, RDW and Ferritin) and the age group for the Autism group.

	Age	N	Mean	Std. Deviation	(t)	**Sig.***
HGB	school age ≥ 6 years	18	11.656	.7350	0.979	0.336
	under school age < 6 years	12	11.375	.8192		
HCT	school age ≥ 6 years	18	34.756	2.3012	1.272	0.214
	under school age < 6 years	12	33.667	2.2904		
MCV	school age ≥ 6 years	18	78.539	3.3729	3.114	0.004*
	under school age < 6 years	12	73.683	5.1945		
RDW	school age ≥ 6 years	18	14.050	1.5497	1.336-	0.192
	under school age < 6 years	12	14.858	1.7312		
Ferritin	school age≥6 years	18	30.911	18.7072	0.393	0.697
	under school age < 6 years	12	27.708	25.9719		

*** The mean difference is significant at the 0.05 level.**

The T- test analysis of the age variable in relation to clinical measures **(HGB, HCT, MCV, RDW and Ferritin)** of the Mental disorder indicated that there were significant differences between the groups of school age ≥ 6 years & under school age<6 years groups in HGB and HCT measures (p=0.047 and p=0.017) respectively. Also, the result demonstrates that these differences are for school age ≥ 6 years MCV; look **Table 10.**

Table 10: T-test for the association between the clinical measures (HGB, HCT, MCV, RDW and Ferritin) and the age group for the Mental disorder.

	Age	N	Mean	Std. Deviation	(t)	**Sig.***
HGB	school age ≥ 6 years	19	12.295	1.2394	2.082	0.047*
	under school age < 6 years	11	11.382	0.9928		
HCT	school age ≥ 6 years	19	37.726	4.0312	2.537	0.017*
	under school age < 6 years	11	34.255	2.6953		
MCV	school age ≥ 6 years	19	82.342	8.8138	1.892	0.069
	under school age < 6 years	11	76.618	6.2265		
RDW	school age ≥ 6 years	19	14.263	1.1056	1.573-	0.127
	under school age < 6 years	11	15.264	2.3855		
Ferritin	school age ≥6 years	19	30.947	14.5205	0.802	0.429
	under school age < 6 years	11	27.708	25.9719		

*** The mean difference is significant at the 0.05 level.**

The T- test analysis of the age variable in relation to clinical measures **(HGB, HCT, MCV, RDW and Ferritin)** of the Typically developing children indicated that there were no significant differences between the groups of school age ≥ 6 years & under school age<6 years groups (**Table11).**

Table 11: T-test for the association between the clinical measures (HGB, HCT, MCV, RDW and Ferritin) and the age group for the Typically developing children.

	Age	N	Mean	Std. Deviation	(t)	**Sig.***
HGB	school age ≥ 6 years	19	12.226	.7571	-.234-	0.817
	under school age < 6 years	11	12.291	.6745		
HCT	school age ≥ 6 years	19	35.642	2.0478	-.245-	0.808
	under school age < 6 years	11	35.818	1.5892		
MCV	school age ≥ 6 years	19	78.311	2.6658	.275	0.786
	under school age < 6 years	11	78.045	2.3227		
RDW	school age ≥ 6 years	19	13.779	.7307	-.710-	0.484
	under school age < 6 years	11	13.973	.7016		
Ferritin	school age ≥6 years	19	39.126	22.6661	1.183	0.247
	under school age < 6 years	11	30.273	12.9796		

*** The mean difference is significant at the 0.05 level.**

The T- Test analysis of the gender variable in relation to clinical measures **(HGB, HCT, MCV, RDW and Ferritin)** of the Autism group indicated that there were significant differences between groups in MCV measure (p=0.004).The test indicated that these differences are for females (**Table 12).**

Table 12: T-test for the association between the clinical measures (HGB, HCT, MCV, RDW and Ferritin) and the gender for the Autism group.

	Gender	N	Mean	Std. Deviation	(t)	**Sig.***
HGB	male	22	11.532	0.8191	0.979	0.336
	female	8	11.575	0.6585		
HCT	male	22	34.305	2.5638	1.272	0.214
	female	8	34.363	1.6133		
MCV	male	22	75.695	4.6450	3.114	0.004*
	female	8	79.075	4.4701		
RDW	male	22	14.532	1.7537	-1.336-	0.192
	female	8	13.938	1.3005		
Ferritin	male	22	27.636	19.8599	0.393	0.697
	female	8	35.113	26.2746		

*** The mean difference is significant at the 0.05 level.**

The T- test analysis of the gender variable in relation to clinical measures **(HGB, HCT, MCV, RDW and Ferritin)** of the Mental disorder indicated that there were no significant differences between groups in (HGB, HCT, MCV, RDW and Ferritin measures) (**Table 13).**

Table 13: T-test for the association between the clinical measures (HGB, HCT, MCV, RDW and Ferritin) and the gender for the Mental disorder.

	Gender	N	Mean	Std. Deviation	(t)	**Sig.***
HGB	male	17	12.318	1.3717	1.915	0.066
	female	13	11.492	0.8261		
HCT	male	17	37.512	3.9922	1.743	0.092
	female	13	35.069	3.5377		
MCV	male	17	80.382	5.7629	0.103	0.919
	female	13	80.062	11.1135		
RDW	male	17	14.629	1.9509	-0.002-	0.998
	female	13	14.631	1.4430		
Ferritin	male	17	31.318	13.3217	0.880	0.386
	female	13	27.154	12.1655		

*** The mean difference is significant at the 0.05 level.**

The T- test analysis of the gender variable in relation to clinical measures **(HGB, HCT, MCV, RDW and Ferritin)** of the Typically developing children indicated that there were significant differences between groups in Ferritin measure (p=0.017). The test indicated that these differences are for male (**Table 14).**

Table 14: T-test for the association between the clinical measures (HGB, HCT, MCV, RDW and Ferritin) and the gender for the Typically developing children.

	Gender	N	Mean	Std. Deviation	(t)	**Sig.***
HGB	male	22	12.300	.7185	0.627	0.536
	female	8	12.112	.7415		
HCT	male	22	35.818	1.6512	0.536	0.596
	female	8	35.400	2.4692		
MCV	male	22	78.464	2.3722	0.904	0.374
	female	8	77.525	2.9036		
RDW	male	22	13.895	.7700	0.571	0.572
	female	8	13.725	.5574		
Ferritin	male	22	40.964	20.3028	2.527	0.017*
	female	8	21.900	9.9280		

*** The mean difference is significant at the 0.05 level.**

One Way ANOVA test analysis of the favorite food groups variables (meat, milk, fruit, vegetables & grains) for the autism group in relation to clinical measures indicated that there were no significant differences between favorite food groups and (HGB, HCT, MCV, RDW and Ferritin measures) (**Table 15).**

Table 15: One Way ANOVA test for the association between the clinical measures (HGB, HCT, MCV, RDW and Ferritin) and the variable of the favorite food for the Autism group.

Measure Group	Means					(F)	Sig.*
	Meat n=4	Milk n=4	Fruit n=4	Vegetables n=4	Grains n=1		
HGB	11.275	12.325	11.250	11.750	11.900	1.317	0.319
HCT	33.025	36.100	34.750	34.725	35.700	0.716	0.597
MCV	78.650	76.700	76.575	74.200	77.400	0.338	0.847
RDW	12.925	14.175	15.150	15.150	14.200	1.032	0.430
Ferritin	39.400	22.975	27.275	31.000	23.500	0.288	0.880

*** The mean difference is significant at the 0.05 level.**

One Way ANOVA test analysis of the favorite groups food color variable for Autism group associated with clinical measures showed that there were significant differences between groups in (Ferritin measure) ($p = 0.031$) for green color with high ferritin levels than other colors. It must here taken into account that there are seven children in the autism group who eat foods with a red color compared to only one child who eats food with green color (**Table 16).**

Table 16: One Way ANOVA test for the association between the clinical measures (HGB, HCT, MCV, RDW and Ferritin) and the variable of the favorite food color for the Autism group.

Measure Group	Means				(F)	Sig.*
	Green n=1	Red n=7	White n= 1	Yellow n=1		
HGB	11.600	11.257	12.400	12.400	1.121	0.412
HCT	33.400	33.343	36.400	36.200	0.868	0.508
MCV	74.300	76.414	75.600	77.600	0.036	0.990
RDW	13.800	15.000	14.300	14.200	0.172	0.911
Ferritin	57.800	16.471	14.800	35.500	5.994	0.031*

*** The mean difference is significant at the 0.05 level.**

Chapter 5

Discussion

To our knowledge this is the first study in the Palestinian literature of iron deficiency in children with autism. In this study, it has demonstrated that 20% of children with autism had law serum ferritin, 13.3% had iron deficiency anemia. These findings confirmed that iron deficiency and anemia are common in autism, in parallel with previous reports (Bilgiç et al., 2010, Dosman et al., 2007, Latif et al., 2002).

Iron deficiency, with or without anemia, can impair cognition and affect, and is associated with developmental slowing in children and mood changes and poor concentration (Latif et al., 2002). Latif et al. (2002) showed high prevalence of iron deficiency in children with autism, which could potentially compromise further their communication and behavioral impairments. This finding makes a warning sign that iron deficiency has higher tendency to be iron deficiency anemia in future among autistic children; if not discovered and treated (Latif et al., 2002).

This study was performed because of the important role of iron, lack of iron has related to negative cognitive, social, and emotional effects that may lead to irreversible behavioral sequels (Carter, 2010; Lozoff, 2007). Oski et al. (1983) stated that iron depletion means that erythropoiesis is normal but iron stores are reduced (serum ferritin <12µg/l), indicating a reduction of iron in the bone marrow, liver and other parts of the reticuloendothelial system. Iron plays an important part in brain function, especially during early development of the brain (Oski, 1993). The effect of

iron deficiency on psychomotor development is well documented (Lozoff., 1988; Moffatt et al., 1994; Walter et al., 1989).

In previous studies were investigating the iron status in children with autism there is a study in Turkey which is in agreement with this study; made by Herguner et al. (2012) who found that 24.1% of autistic children had low serum ferritin. While in other study the low serum ferritin results were “two times and half” higher than this study results; it was a study in UK, the first study performed in autism and iron deficiency, made by Latif et al. (2002) who was show a high prevalence of low serum ferritin among autistic children (52%). In Another study from Canada, Dosman et al. (2006) reported that ferritin level was low in 8.3% of 1–2-year-old children, in14.2% of 3–5-year-olds and in 20% of 6–10-years-old. In a study in Turkey, Bilgic et al. (2010) demonstrated that iron deficiency was detected in 32.3% of children with autism spectrum based on low serum ferritin level which is in agreement of this study. These results also about iron deficiency anemia 13.3% are in agreement with the results of Latif et al. (2002) who reported that 11.5% of the autistic children were shown to have iron deficiency anemia.

In this study the hemoglobin (HGB) and hematocrit (HCT) were significantly lower in autistic group than the other two groups. Low HGB and HCT levels are considering as the biggest risk that autistic children might face; that is the condition of anemia (Herguner et al., 2012).

In the current study the age was divided in two categories: pre-school age (from 3 to less than 6 years old), and school age (from 6 to 13 years old); the difference in incidence of iron deficiency between the categories was considerable: the pre-school aged children have low serum ferritin 33.3% than school aged children 11%. Similar results were obtained by Herguner et al. (2012) who reported that low serum ferritin was more prevalent in preschool-aged than school-aged children (32.4% vs. 20.3%).

In the autism group in this study, four children were anemic (13.3%) three were male and one was a female. Analyzing the results of gender differences within the affected children in the study group, there was an association between gender and iron deficiency anemia. This result indicates that these differences for males, this results are in contrast with the study of Latif et al. (2002) which showed that six children were anemic (11.5%) five were male and one was female.

Children with autism frequently have narrow food preferences, according to shape, texture, or color (Cornish, 1998) as it is the case in this study which was showed that selectivity of food confined in color of food (33.3%) from the autistic group; especially red color 23% (7/30), However Cermak (2010); Herndon (2009); Johnson (2008) and Xia (2010) reported that inadequate dietary iron intake was considered as a cause of iron deficiency, and low iron intake was thought to be associated with food selectivity which is commonly seen in children with autistic disorder.

In another research; feeding selectivity with food are a major concern in the majority of children of the autistic spectrum, there may be selectivity regarding the shape, color, smell and consistency of food and the way it is presented to the child (Baron-Cohen and Bolton; 1993). It was proposed that ID is associated with narrow food selection, and because this problem is more evident in preschool children. ID is more frequent in this age group (Cornish, 1998; Dosman et al., 2007).

This study report that autistic children had high frequency of snacks per day, one of the explanation may be related to behavioral therapy which has been noticed to be adopted in rehabilitation centers; the therapy would be based in these cases that given, as most positive reinforcement to the child, candies were given to the child when he/she performs an achievement to encourage him/her, and these several snacks based on candies per day would make the child replete when he takes his meal.

In this study regarding the gastric dysfunction that manifest by unusual annoying symptoms as diarrhea, constipation, vomiting, gazes or abdominal distention, there were no significant differences between autistic group and other two control groups, in contrast to Horvath (1999) who reported that “twenty-two” of “twenty-five” autistic children (88%) had symptoms such as nighttime awakening with irritability, signs of abdominal discomfort, or pushing on the. This study also is not congruent with a study of Wakefield et al. (1998) that described intestinal dysfunction in children with autistic spectrum disorder and D’Eufemia et al. (1996) that

reported increased intestinal permeability in 9 of 21 (43%) patients with autistic disorder. The report of Wakefield et al. (1998) represents the first effort to evaluate the gastrointestinal tract in children with autism.

5.1 Conclusion

Results of this study indicated that there is association between autism and the occurrence of iron deficiency and iron deficiency anemia; which may be explained by feeding difficulties and food selectivity.

5.2 Limitations & Strength

The first limitation is a restricted budget for the project that lead the researcher to limit the sample size of the study.

During the work the author felt a pressing need to ensure the diagnosis of the children and to evaluate the severity of autistic symptoms: unfortunately in our Palestinian health system there is an absence of scientific diagnostic tools for this specific porpoise.

The strength of this study is that it represents the first of its kind in Palestine. Internationally it's the only study design to take control groups. In the two Turkish studies conducted by Bilgic et al. (2010) & Herguner et al. (2012) correlation of results was made to another study measuring iron deficiency in typically developing children.

Another strength of this study is that it contained a food habit survey related to low serum ferritin. Which in previous studies were taken only

into consideration low serum ferritin alone or in relation with the severity of autistic symptoms was considered.

5.3 Recommendations

The author recommend that all children diagnosed with autism should have a full blood count and that their serum ferritin should be measured as part of their routine investigation, especially if they are selective with food and their diet has low iron content

Iron supplementation should continue to be recommended to all children with low serum ferritin; as well as to all children between 6 month and 2 years of age in order to avoid developing iron deficiency anemia in future.

Involvement of an experienced pediatric dietitian in children with autism is an important part of their management to enhance adequate dietary iron intake and other beneficial elements and proteins.

A large multicenter study is needed in order to determine the exact prevalence of iron deficiency in children with autism and its effect on their behavior and learning.

References

Abbott, R. (1998). **The effects of iron supplementation on cognitive function in infants and children.** Bibliotheca Nutritioet Dieta, 54, 67–75.

Ahearn, W. H., Castine, T., Nault, K., & Green, G. (2001). **An assessment of food acceptance in children with autism or pervasive developmental disorder–not otherwise specified.** Journal of Autism and Developmental Disorders, 31(5), 505–511.

AHMED, S., SINGH.V. & RAO. G.S. (1995) **'Releaseol lron from Ferrmn by 1,2,3- Banzenetriol'**, Chaniail and BioIoicaIlnierocioxas 96: 103—11.

VITERI, IE. (1998) **A New Concept in the Control of Iron Deficiency: Community. Based Preventive Supplementation of At-Risk Groups by the Vek1y Intake of Iron Supplements',** Biomdical and Environmernoi Sciences 11: 46—60.

Akman, M., Cebeci, D., Okur, V., Angin, H., Abali, O., &Akman, A. C. (2004). **The effects of iron deficiency on infants developmental test performance.** ActaPaediatrica, 93, 1391–1396.

American Psychiatric Association (2000). **Diagnostic and statistical manual of mental disorders: DSM-IV. 4 ed. Washington, DC**: American Psychiatric Association;. ISBN 0-89042-025-4. OCLC 768475353.Diagnostic criteria for 299.00 Autistic Disorder.

American Psychiatric Association (APA) (2013) **Diagnostic and statistical manual of mental disorders, 5th edn**. http://www.dsm5.org

American Psychiatric Association (APA) (1994) **Diagnostic and statistical manual of mental disorders, 4th edn**. American Psychiatric Association, Washington.

American Psychiatric Association. (1994**). Diagnostic Criteria for 299.00 Autistic Disorder;** Diagnostic and Statistical Manual of Mental Disorders: DSM IV

American Psychiatric Association. (2000). **Diagnostic and statistical manual of mental disorders: DSM-IV-TR (4th ed.).** Washington, DC: American Psychiatric Association.

Ashley-Koch, E., Wolpert, C. M., Menold, M. M., Zaeem, L., Basu, S., Donnelly, S. L., et al. (1999) **Genetic studies of autistic disorder and chromosome 7**. Genomics, 61(3), 227-236.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E. et al. (1995). **Autism as a strongly genetic disorder: Evidence from a British twin study.** Psychological Medicine, 25(1), 63-77.

BARON-COHEN, S. & BOLTON. P. (1993) **Autism: The Facts.** Oxford: Oxford University Press.

Beard JL (2001) **Iron biology in immune function, muscle metabolism and neuronal functioning.** J Nut 131((2S-2)):568S–579S.

Bilgiç A, Gürkan K, Türkoğlu S, Akça AF, Kılıç BG, Uslu R (2010) **Iron deficiency in preschool children with autistic spectrum disorders.** Res Autism SpectrDisord 4(4):639–644.

Bodfish, J. W., Symons, F. J., & Lewis, M. H. (1999). **Repetitive Behavior Scale—Revised.** Morganton, NC: Western Carolina Center Research Reports.

Buxbaum, J. D., Silverman, J. M., Smith, C. J., Kilifarski, M., Reichert, J., Hollander, E. et al. (2001**). Evidence for a susceptibility gene for autism on chromosome 2 and for genetic heterogeneity.** American Journal of Human Genetics, 68(6), 1514-1520.

Carcani-Rathwell, I., Rabe-Hasketh, S., &Santosh, P. J. (2006).**Repetitive and stereotyped behaviors in pervasive developmental disorders** [Electronic version]. Journal of Child Psychology and Psychiatry, 47(6), 573–581.

Carter RC, Jacobson JL, Burden MJ, et al. **Iron deficiency anemia and cognitive function in infancy.** Pediatrics. 2010; 126(2):e427–e434. [PMC free article] [PubMed].

Centers for Disease Control and Prevention, **US Department of Health and Human Services.** Recommendations to prevent and control iron deficiency in the United States. MMWR 1998; 47(RR-3):1-23.

Cermak SA, Curtin C, Bandini LG (2010**) Food selectivity and sensory sensitivity in children with autism spectrum disorders.** J Am Diet Assoc 110(2):238–246.

Chess, S. (1997). **Follow-up report on autism in congenital rubella.** Journal of Autism & Developmental Disorders, 7(1), 69-81.

Cornish, E. (1998). **A balanced approach towards healthy eating in autism.** Journal of Human Nutrition Dietetics, 11, 501–509.

Cortese S, Konofal E, Bernardina BD, Mouren MC, Lecendreux M (2009) **Sleep disturbances and serum ferritin levels in children with attention-deficit/hyperactivity disorder**. Eur Child Adolesc Psychiatry 18(7):393–399

D'Eufemia P, Celli M, FinocchiaroR,Pacifico L, Viozzi L, Zaccagnini M, et al. **Abnormal intestinal permeability in children with autism.** ActaPaediatr 1996;85:1076-9.

Dales, L., Hammer, S. J., & Smith, N. J. (2001). **Time trends in autism and in MMR immunization coverage in California**. JAMA, 285(9), 1183-1185

DALLMAN, P.R. (1986) **'B1ochemcal Basis for the Manifestations of Iron Deficiency'.** Annual Reviews of Nutrition 6: 13—40

DALLMAN, P.R., LOOKER. AC., JOHNSON, C.L. & CARROLL. M. (1996**) 'Influence of Age on Laboratory Criteria for the Diagnosis of**

Iron Deficiency in Infants and Children', in L. HALLBERG & N.G. ASP (eds) Iron Nuritivn in Health and Disease, pp. 65—74. London: John Ubbe

DE MAEYER, E.M,, DALLMAN, P., GURNY. J.M., HALLBERG, I., SOUD, 5.K, & SRIKANTIA, S.G. (1989) **Prewnhing and Controlling Iron DeflciencyAneniio through Primary Health Care.** Geneva: WHO.

Dosman, C. F., Brian, J. A., Drmic, I. E., Senthilselvan, A., Harford, M. M., Smith, R. W., et al. (2007). **Children with autism: Effect of iron supplementation on sleep and ferritin.** Pediatric Neurology, 36, 152–158.

Dosman, C. F., Drmic, I. E., Brian, J. A., Senthilselvan, A., Harford, M. M., Smith, R. W., et al. (2006**). Ferritin as an indicator of suspected iron deficiency in children with autism spectrum disorder: Prevalence of low serum ferritin concentration.** Developmental Medicine & Child Neurology, 48, 1008–1009.

Erikson KM, Jones BC, Hess EJ, Zhang Q, Beard JL (2001**) Iron deficiency decreases dopamine D1 and D2 receptors in rat brain.** PharmacolBiochemBehav 69(3–4):409–418.

Ernsperger, L., &Stegen-Hanson, T. (2004). **Just take a bite: Easy, effective answers to food aversions and eating challenges**. Arlington, TX: Future Horizons.

Field, D., Garland, M., & Williams, K. (2003).**Correlates of specific childhood feeding problems.** Journal of Pediatric Child Health, 39, 299–304.

Freitag CM. **The genetics of autistic disorders and its clinical relevance: a review of the literature.** Mol Psychiatry. 2007;12(1):2-22. FULL TEXT | WEB OF SCIENCE | PUBMED.

Gillberg C, Gillberg IC. 1983. **Infantile autism: a total population study of reduced optimality in the pre, peri-, and neonatal period**. J. Autism Dev. Disord. 13:153–66

Gupta AR, State MW. **Recent advances in the genetics of autism**. Biol Psychiatry. 2007; 61(4):429-437. FULL TEXT | WEB OF SCIENCE | PUBMED.

Hallberg L, Hulthen L (2002) **Perspectives on iron absorption.** Blood Cells Mol Dis 29(3):562–573

Hergüner.S &Keleşoğlu.F.M&Tanıdır.C&Çöpür.M(2012) **Ferritin and iron levels in children with autistic disorder.** Eur J Pediatr 171:143–146

Herndon AC, DiGuiseppi C, Johnson SL, Leiferman J, Reynolds A (2009) **Does nutritional intake differ between children with autism spectrum disorders and children with typical development?** J Autism DevDisord 39(2):212–222.

Horvath K, Stefanatos G, Sokolski K, Wachtel R, Nabors L, Tildon JT. **Improved social and language skills after secretin administration in patients with autistic spectrum disorders.** J AssocAcad Minority Physicians 1998;9:9-15.

Horvath, K., Papadimitriou, J. C., Rabsztyn, A., Drachenberg, C., &Tildon, J. T. (1999).**Gastrointestinal abnormalities in children with autistic disorder**. Journal of Pediatrics, 135, 559–563.

Idjradinata, P., & Pollitt, E. (1993). **Reversal of developmental delays in iron deficient anaemic infants treated with iron.** The Lancet, 341, 1–4.

J Walker-Smith, J. **AndrewsAlpha-1-antitrypsin**, autism, and coeliac diseaseLancet, 2 (1972), pp. 883–884

Johnson CF, Handen BL, Mayer-Cosa M, Sacco K (2008) **Eating habits and dietary status in young children with autism.** J DevPhysDisabil 20:437–448.

Lainhart, J. E. (1999). **Psychiatric problems in individuals with autism, their parents and siblings.** International Review of Psychiatry, 11, 278–298.

Latif A, Heinz P, Cook R (2002) **Iron deficiency in autism and Asperger syndrome.** Autism 6(1):103–114.

Legge, B. (2002). **Can't eat, won't eat dietary difficulties and autistic spectrum disorders.** London: Jessica Kingsley.

Lord C, Mulloy C, Wendelboe M, Schopler E. 1991. **Pre- and perinatal factors in highfunctioning females and males with autism.** J. Autism Dev. Disord. 21:197–20

Lozoff B, Corapci F, Burden MJ, et al. **Preschool-aged children with iron deficiency anemia show altered affect and behavior.** Journal of Nutrition. 2007;137(3):683–689. [PubMed].

LOZOFF, 8.(1988) **'Behavioural Alteration in Iron Deficiency'**.Adances in &diairics 35: 33 1—60.

Lozoff, B. (2007). **Iron deficiency and child development**. Food and Nutrition Bulletin, 28(Suppl.), 560–571.

LOZOFF, B., JIMENEZ, B. & WOLF, A.W. (1992) **'LongTerm Developmental Outcome of Infants with Iron Deficiency'**, New England Journal of Medicine 325: 687—94

McCann, J. C., & Ames, B. N. (2007).**An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function.** The American Journal of Clinical Nutrition, 85, 931–945.

MOFFATT, M.E.K.. LANGESTAFFII, S., BESANT, J. & DURESKI, C. (1994) '**Prevention of Iron Deficiency and Psychomotor Decline in High-Risk Infants through Use of Iron-Fortified Infant Formula: A Randomized Clinical Trial'**. Journal of Pediatrics 125 (4): 527—33.

MS Goodwin, MA Cowen, TC. **Goodwin Mal absorption and cerebral dysfunction: a multivariate and comparative study of autistic children J** Autism Child Schizophr, 1 (1971), pp. 48–62

NELSON.C. • ERIKSON.K. • PINERO.D.J. & BEARD. J.L (1997) **'In Vivo Dopaminc Metabolism Is Altered in Iron-Deficient Anemic Rats'**, Journnlof Nutrition 127: 2282—8.

Newschaffer CJ, Croen LA, Daniels J et al.**The epidemiology of autism spectrum disorders [PDF]**.Annu Rev Public Health. 2007;28:235–58.doi:10.1146/annurev.publhealth.28.021406.144007. PMID 17367287.

Newschaffer, C. J., Fallin, D., & Lee, N. L. (2002). **Heritable and nonheritablerisk factors for autism spectrum disorders**. Epidemiologic Reviews, 24(2), 137-153.

Norland-Tilburg, E. V. (1990). **Controlling error in evaluation instruments.** Journal of Extension, [On-line], 28(2). Available at http://www.joe.org/joe/1990summer/tt2.html

OSKI. F.A., HONIG, A.S., HELtJ. B. & H0WANITZ, P. (1983)**'EffectoflronTherapy on Behavior Performance in Non-Anemic Iron Deficient Infants'**.&diatrics 71: 677—880.

OSKI.F.A. (1993) '**Iron Deficiency in Infancy and Childhood'**, NeiEnglnnd Journal of Mcdictnc 329: 190—3.05K I. F.A. & HONIG. A.S.

(1978) ‘The Effect ofTherapy on the Developmental Scores of Iron Deficient Infants’, Journal of FWiatr,cs 92: 2 I—S.

Otero, G. A., Aguirre, D. M., Porcayo, R., & Fernandez, T. (1999**).Psychological and electroencephalographic study in school children with iron deficiency**. The International Journal of Neuroscience, 99, 113–121.

Raiten, D. J., &Massaro, T. (1986).**Perspectives on the nutritional ecology of autistic children.** Journal of Autism and Developmental Disabilities, 16(2), 133–143.

Rapin I. Autism. N Engl J Med 1997;337:97-104.

Risch, N., Spiker, D., Lotspeich, L., Nouri, N., Hinda, D.,Hallmayer, J., et al. (1999). **A genomic screen of autism: Evidence for a multilocus etiology.** American Journal of Human Genetics, 65(2), 493-507.

Rodier, P. M., & Hyman, S. L. (1998).**Early environmental factors in autism.** Mental Retardation and Developmental Disabilities Research Reviews, 4(2), 121-128.

Schreck, K. A., & Williams, K. (2006).**Food preferences and factors influencing food selectivity for children with autism spectrum disorders** [Electronic version]. Research in Developmental Disabilities, 27(4), 353–363.

Schreck, K. A., Williams, K., & Smith, A. F. (2004).**A comparison of eating behaviors between children with and without autism.** Journal of Autism and Developmental Disorders, 34(4), 433–438.

Scott M. Myers, MD, Chris Plauche´ Johnson, MD, MEd. (2007). **Management of Children With Autism Spectrum Disorders**. American Academy of Pediatrics, 1162-1163.

Shao, Y., Raiford, K. L., Wolpert, M., Cope, H. A., Ravan, S. A., Ashley-Koch, A. A. et al. (2002). **Phenotypic heterogeneity provides increased support for linkage on chromosome 2 in autistic disorder.** American Journal of Human Genetics, 70(4), 1058-1061.

Smalley SL, Asarnow RF, Spence MA (1988) **Autism and genetics:a decade of research.** Arch Gen Psychiatry 45:953–961

Spiker, D., Lotspeich, L., Kraemer, H. C., Hallmayer, J., McMahon, W., Petersen, P. B., et al. (1994). **Genetics of autism: Characteristics of affected and unaffected children from 37 multiplex families**. American Journal of Medical Genetics, 54(1), 27-35.

Stein D, Weizman A, Ring A, Barak Y. 2006. **Obstetric complications in individuals diagnosed with autism and in healthy controls**. Compr. Psychiatry 47:69–75

Steyaert JG, De la Marche W (2008) **What's new in autism?** Eur J Pediatr 167(10):1091–1101

Stubbs, E. G., Ash, E., & Williams, C. P. S. (1984). **Autism and congenital cytomegalovirus.** Journal of Autism & Developmental Disorders, 14(2), 183-189.

Sugie Y, Sugie H, Fukuda T, Ito M. 2005. **Neonatal factors in infants with autistic disorder and typically developing infants**. Autism 9:487–94

Szatmari, P., Georgiades, S., Bryson, S., Zwaigenbaum, L., Roberts, W., Mahoney, W., et al. (2005). **Investigating the structure of the restricted, repetitive behaviors and interests domain in autism [Electronic version].** Journal of Child Psychology and Psychiatry, 47(6), 582–590.

Taylor, B., Miller, E., Lingam, R., Andrews, N., Simmons, A., & Stowe, J. (2002). **Measles, mumps, and rubella vaccination and bowel problem or developmental regression in children with autism**: population based study. BMJ, 324(7334), 393-396.

The American Society of Health-System Pharmacists (2011), "Risperidone - PubMed Health". **Ncbi.nlm.nih.gov**. Retrieved 2012-03-23.

Turner, M. (1999). **Annotation: Repetitive behavior in autism: A review of psychological research**. Journal of Child Psychology & Psychiatry, 40(6), 839–849.

Wakefield, A. J., Murch, S., Anthony, A., Linnell, J., Casson, D. M., Malik, M., et al. (1998).**Ileal-lymphoid-nodular hyperplasia, non-**

spesific colitis and pervasive developmental disorder in children. The Lancet, 351, 637–641.

WALTER, T., DE AMDRACA, I., CHADUD, P. & PERAI.FS, C.G. (1989) **'Iron Deficiency Anemia: Adverse PfIk'cts on Infant Psychomotor Development'.** Pediatrics 84: 7—17.113

Williams, K., Gibbons, B., &Schreck, K. (2005).**Comparing selective eaters with and without developmental disabilities.** Journal of Developmental and Physical Disabilities, 17(3), 299–309.

Williams, P. G., Dalrymple, N., & Neal, J. (2000).**Eating habits of children with autism.** Pediatric Nursing, 26(3), 259–264.

WORlD HEALTH ORGANIZATION (1993) **lCD-JO International Statistical Classihcation of Diseases and Rooted Health Problems, 10th** rev. Geneva: WHO.

World Health Organization, WHO. **Assessing the iron status of populations**. 2nd edition, including literature reviews, 2007,http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107.pdf.

World Health Organization. (1992). **International classification of diseases: Diagnostic criteria for research (10th edition).** Geneva, Switzerland

Worwood, M. (1997). **Influence of disease on iron status.** The Proceedings of the Nutrition Society, 56, 409–419.

Xia W, Zhou Y, Sun C, Wang J, Wu L (2010) **A preliminary study on nutritional status and intake in Chinese children with autism.** Eur J Pediatr 169(10):1201–1206.

YOUDIM, M.O., BEN-SHACHAR, D., ASHKENAZI, R. & YEHUDA, s. (1983) **'Brain Iron and Dopamine Receptor Function'**, Adiinca in Biochemistry and Psychopharmacology (37: 309—2) 114

Zwaigenbau, L., Szatmari, P., Jones, M. B., Bryson, S. E., Maclean, J. E., Mahoney, W. J., et al. (2002). **Pregnancy and birth complications in autism and liability to the broader autism phenotype.** Journal of American Academy of Child & Adolescent Psychiatry, 41(5), 572-579

Appendix

Questionnaire

- **Background information:**
  - Name:…….
  - Age:…..years old.
  - Gender: □Male □ Female
  - Method of delivery: □Normal delivery □Cesarean section
  - Age of mother on delivery:…….
  - Duration of pregnancy: □ Full term □ Premature
- **Parent information:**

Father:- **Mother:-**

Age**:……** Age:…….

Job**:……..** Job:……..

If the parents divorced or one of them died:…….

……...

- **Food habit :-**
  - Frequency of meals per day?.....
  - Frequency of snacks per day?.....
  - Is there a favorite food?.....If yes what?.....

    ……..

- Is there a favorite form of food?.....If yes what?.....

  ……..

- Is there a favorite color of food?.....If yes what?.....

  .....

- Is there a food allergy?.....If yes what?.....

  ……..

- Is there a food that child refuse to eat?.....If yes what?.....

  ……..

- Do you have unusual annoying symptoms?
  - Abdominal pain
  - Diarrhea
  - Constipation
  - Vomiting
  - Others…….

- Do you take permanent medications?.....If yes what?.....

  .....

بسم الله الرحمن الرحيم

جامعة النجاح الوطنية

كلية الدراسات العليا

نموذج موافقة على المشاركة في بحث

- الباحث: "ساجد فيصل العلي" الطالب في كلية الدراسات العليا / ماجستير تمريض الصحة النفسية المجتمعية، جامعة النجاح الوطنية.
- المشرف: الدكتورة عائدة أبو سعود القيسي رئيسة دائرة التمريض والقباله في كلية الطب والعلوم الصحية في جامعة النجاح الوطنية، و الدكتورة سابرينا روسو محاضرة في برنامج ماجستير تمريض الصحة النفسية المجتمعية في جامعة النجاح الوطنية.
- الجهة المشرفة: جامعة النجاح الوطنية / كلية الدراسات العليا / قسم التمريض / الصحة النفسية المجتمعية.
- عنوان البحث: **ارتباط التوحد مع نقص الحديد في الأطفال المصابين بالتوحد في شمال الضفة الغربية**.

Association between Autism and Iron deficiency in Autistic children in the Northern West Bank

يحتوي هذا الملف على : معلومات وتفاصيل البحث، و شهادة الموافقة على المشاركة في البحث، والاستبانة.

معلومات حول البحث

مقدمة

أخي/ أختيولي أمر المشارك/ة:

أنا الباحث ساجد العلي طالب ماجستير تمريض صحة نفسية مجتمعية في جامعة النجاح الوطنية، يسرني أن أدعوك إلى المشاركة في بحثي بعنوان"ارتباط التوحد مع نقص الحديد في الأطفال المصابين بالتوحد في شمال الضفة الغربية".

لك كامل الحرية والإرادة في المشاركة في هذا البحث، ولك الحق في أخذ الوقت الكافي للتفكير في المشاركة من عدمها، وسؤال الباحث عما تراه مناسبا، والتحدث لأي شخص أو جهة عن هذا البحث.

كما يمكنك الاستفسار عن أي جزء يتعلق في البحث الآن أو فيما بعد، وإذا كانت هناك كلمات أو أجزاء غير مفهومة بإمكانك سؤال الباحث وستجد/ين الوقت والإجابة الكافيتين.

هذا ويضمن الباحث سرية المعلومات المتعلقة بالمشاركة.

الهدف من البحث

يهدف هذا البحث لتبيين العلاقة بين التوحد ونقص الحديد في الأطفال المصابين بالتوحد في شمال الضفة الغربية مما سيؤدي إلى محاولة لإيجاد علاج لآثار التوحد. كما أن مشاركتك ودعمك لهذا البحث سيساهمان في تطوير وتعزيز الواقع الصحي في فلسطين.

طبيعة المشاركة في البحث

بعد الموافقة على المشاركة في البحث سنطلب من المشارك مقابلة شفوية لأخذ معلومات تتعلق بالعوامل الاجتماعية والديموغرافية وبعض الأسئله الأخرى تليها أخذ عينة دم منه لقياس مستوى الحديد في الدم و وبروتين الفراتين.

اختيار المشاركين

سيتم اختيار المشاركين من مرضى التوحد، والأطفال الذين يعانون من أمراض نفسية وعصبيةأخرى من عدة مراكز، وكذلك اختيار أطفال لا يعانون من المرض بشكل ملائم لأغراض البحث.

المشاركة طوعية واختيارية وبإمكان المشارك الانسحاب من البحث في أي وقت ودون الحاجة لإبداء الأسباب وبدون أي تبعات.

المدة المتوقعة لإنهاء إجراءات البحث

عشرون دقيقة لكل مشارك.

شهادة الموافقة على المشاركة في البحث

إقرار من المشارك في البحث:

قمت بقراءة المعلومات الواردة في ورقة معلومات البحث، وأتيحت لي الفرصة أن أسال أي سؤال، وقد تمت الإجابة على كافة أسئلتي بشكل كاف، وبناء على ذلك أوقع طوعيا على المشاركة في هذا البحث.

إسم المسؤول في المركز:..... إسم ولي أمر المشارك:.....

توقيع المسؤول:..... توقيع ولي أمر المشارك:.....

التاريخ:...../...../..... التاريخ:...../...../.....

إقرار من الباحث:

لقد قمت بقراءة المعلومات الواردة في ورقة معلومات البحث بطريقة صحيحة وواضحة، وبذلت جهدي أن يعي المشارك أن البحث سيتضمن:

مقابلة المشارك في البحث والتحدث إليه شفوياً؛ لأخذ المعلومات المتعلقة بالعوامل الاجتماعية والديموغرافية، و أخذ عينة دم منه.

أؤكد على أن المشارك أخذ الفرصة الكافية للإجابة على اسفساراته بشكل واضح وصحيح، وبذلت ما بوسعي لتحقيق ذلك.

اؤكد أن المشارك لم يجبر على التوقيع على الورقة وأن مشاركته كانت بمحض إرادته وكامل اختياره.

الباحث ساجد فيصل العلي:

توقيع الباحث:.....

التاريخ:...../...../.....

(الاستبانة)

- **معلومات أساسية:-**
  - الاسم:.....
  - العمر:.....سنة
  - الجنس: □ ذكر □ انثى
  - طريقة الولادة: □ ولادة طبيعية □ ولادة قيصرية
  - عمر الأم عند الولادة:.....سنة
  - مدة الحمل: □ مدة حمل كامله (9 أشهر) □ ولادة مبكره

- **معلومات عن الوالدين:-**

الأم

- العمر:.....
- العمل:.....

الأب

- العمر:.....
- العمل:.....

- هل الوالدين منفصلين، أو أحد منهما متوفي؟

.....

.....

- **عادات الغذاء:-**
  - عدد الوجبات الرئيسة خلال اليوم؟.....
  - عدد الوجبات الخفيفة خلال اليوم؟.....

- هل هناك أكل مفضل؟.....إذا نعم ما

هو؟.....

.....

- هل هناك شكل أكل مفضل ؟.....إذا نعم ما

هو؟.....

.....•

- هل هناك لون أكل مفضل؟.....إذا نعم ما

هو؟.....

.....

- هل هناك حساسية من أطعمة معينة؟.....إذا نعم ما

هي؟.....

.....

هل هناك أطعمة يرفض الطفل تناولها؟.....إذا نعم ما

هي؟.....

- هل هناك أعراض مزعجة غير طبيعية:-
  - آلام في البطن
  - إسهال
  - إمساك
  - تقيؤ
  - غير

ذلك.....

- هل تأخذ أدوية بشكل دائم؟.....اذا نعم ما

هي؟.....

Budget

Table 17 Budget

Material	Costs
Lab tests (CBC, Serum Ferritin)	1000$
Transportation	200$
Printin	200$
SPSS Analyses	100$
Total cost	**1500$**

بسم الله الرحمن الرحيم

An-Najah
National University
Faculty of Medicine

جامعة
النجاح الوطنية
كلية الطب

IRB Approval letter

Study title:
Association between Autism and Iron deficiency in Autistic children in northern West Bank

Submitted by:
Sajed Faisal Al – Ali

Date Reviewed:
May 6, 2012

Date approved:
June 24, 2012

Your study titled " Association between Autism and Iron deficiency in Autistic children in northern West Bank " Was reviewed by An-Najah National University IRB committee & approved on June 24, 2012

IRB

Samar Musmar,MD, FAAFP
IRB Committee Chairman,
An-Najah National University

نابلس - ص.ب ٧٠٧,٧ هاتف: ٢/٤/٧/٨/١٤ ٢٣٤٢٩٠٢(٠٩)(٩٧٢)، فاكسميل ٢٣٤٩٧٣٩(٠٩)(٩٧٢)
Nablus - P.O.Box 7,707 - Tel. (972)(09)2342902/4/7/8/14 - Facximile (972)(09)2349739
Web Site:www.najah.edu

جامعة النجاح الوطنية

كلية الدراسات العليا

ارتباط التوحد مع نقص الحديد في الأطفال المصابين بالتوحد في شمال الضفة الغربية

إعداد

ساجد فيصل العلي

إشراف

د. عائدة ابو السعود القيسي

د. سابرينا روسو

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في تمريض الصحة النفسية المجتمعية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس – فلسطين.

2013

ارتباط التوحد مع نقص الحديد في الأطفال المصابين بالتوحد في شمال الضفة الغربية

أعداد

ساجد فيصل العلي

إشراف

د. عائدة ابو السعود القيسي

مشرفاً ثانياً

د. سابرينا روسو

الملخص

خلفية الدراسة: للحديد دور مهم في الإدراك والسلوك، والنمو الحركي. وقد تمت الإشارة إلى ارتفاع معدل انتشار نقص الحديد في التوحد. ولذا فإن أطفال التوحد معرضون لخطر التعرض لنقص الحديد، وهذا قد يزيد من حدة المشاكل الحركية والسلوكية عند أطفال التوحد.

الهدف: إن الهدف من هذه الدراسة، هو دراسة العلاقة بين مرض التوحد ونقص الحديد في الأطفال المصابين بالتوحد في شمال الضفة الغربية، وتحديد الانتقائية الغذائية، ومقارنة مؤشرات الانتقائية الغذائية بين الأطفال الذين يعانون من التوحد، والأطفال مضطربين نفسياً، والأطفال الطبيعيي النمو (الأطفال الطبيعيين).

المشاركين وطريقة البحث: لقد شارك تسعون طفلاً من عمر ثلاث إلى ثلاث عشرة سنة، في "دراسة الحالة الضابطة"، موزعة على مجموعة الدراسة، والمجموعتين الضابطتين. **مجموعة الدراسة:** ثلاثون طفلاً مصابون بالتوحد وفقاً لDSM-IV و ICD-10، **والمجموعة الضابطة الأولى:** ثلاثون طفلا يعانون من اضطراب نفسي "غير التوحد"، **والمجموعة الضابطة الثانية:** ثلاثون طفلاً طبيعياً مأخوذة من المجتمع ، وجرى الربط بين المجموعات الثلاث عن طريق العمر والجنس والمنطقة الجغرافية. وتم قياس الفيريتين، والهيموغلوبين، والهيماتوكريت، ومعدل حجم الكرية الحمراء، وعرض توزيع الخلايا الحمراء.

النتائج: نسبة نقص الحديد عند الأطفال المصابين بالتوحد 20٪ (ن = 30/6) على أساس مستوى الفيريتين (SF <10µ / لتر)، مقارنة مع 0٪ لغيرها من **المجموعتين الضابطتين:** "اضطراب نفسي غير التوحد، والأطفال الطبيعيي النمو" على التوالي (P = 0.0001). وقد تم تعريف فقر الدم بـــ الهيموغلوبين أقل من 110غرام / لتر بالنسبة للأطفال الذين تقل أعمارهم عن 6 سنوات، والهيموغلوبين أقل من 120غرام / لتر بالنسبة للأطفال في سن 6 إلى 13 سنة من العمر، وعندما قمنا بتحليل الهيموغلوبين للأطفال الستة الذين يعانون من نقص الحديد وجدنا أن 66.6٪ أي أربعة من الأطفال الستة (هما طفلان من عمر أقل من 6 سنوات ، والاثنان الآخران أكثر من 6 سنوات) لديهم فقر الدم، ونسبة فقر الحديد عند مجموعة التوحد13.3%(ن=30/4). وقد وجدنا أيضا أن وتيرة انخفاض تناول الحديد عند هؤلاء الأطفال تترافق مع صعوبات التغذية والانتقائية الغذائية، وكان هناك اختلاف كبير بين مجموعة الأطفال الذين يعانون من التوحد الذين اختاروا الأطعمة المفضلة ذات اللون الأحمر 23٪ (ن=30/7) بالمقارنة مع المجموعتين الضابطتين 0٪، على التوالي (P = 0.0001). وأظهرت النتائج أيضا اختلاف كبير في وتيرة وجبات خفيفة في اليوم الواحد (أكثر من 4) في الأطفال المصابين بالتوحد 40٪ (ن=30/12) بالمقارنة مع كل من المضطربين نفسياً 16.7٪ (ن = 30/5) (P = 0.006) والأطفال الطبيعيي النمو 6.7٪ (ن = 30/2) المجموعات (P = 0.001).

الخلاصة: أكدت نتائج هذه الدراسة أن هناك علاقة بين مرض التوحد ونقص الحديد وفقر الدم. وأن انخفاض مستوى الفيريتين عند التوحد قد يكون علامة على نقص الحديد، ومؤشر مبكر لفقر الدم. والنتائج تشير إلى أن الانتقائية الغذائية هي أكثر شيوعا في الأطفال الذين يعانون من التوحد بالمقارنة مع الأطفال الطبيعيين. وهذه النتائج تشير إلى أن مستوى الفيريتين ينبغي أن يقاس في الأطفال المصابين بالتوحد كجزء من فحص روتيني.