Effect of nystatin drug on the level of immunoglobulin's in children with autism

***Alhaidary, A. F. **Mousa, R.F.**
*psychiatrist ,medical department,medical college, kerbala university**MSc biochemistry ,college of pharmacy,kerbala university

Keyword: autism ,immunoglobulin's,nystatin
Received (June) . Accepted(December)

ABSTRACT
Autism spectrum disorders (ASD) are a group of **heterogeneous, behaviorally defined disorders characterized by disturbances in both verbal and non-verbal communication, language, imagination, and social interaction, often with repetitive and stereotyped behavior . The aim of this study is to achieve serum level of immunoglobulin IgE near normal value in children with autism after nystatin administration . This study was performed on 30 child with autism spectrum disorders (ASD) that are go to psychiatric clinic at Al – Hussain teaching Hospital/kerbala –Iraq. The study revealed significant different on a level of immunoglobulins in autism spectrum disorders as compared with control group .This study indicate the reduction in level of IgG,IgA,IgM,IgE in autism child when compared with the same immunoglobulins in control group ,also this study showed an increase in the level of IgE level (p=0.01) in patient with autism and discus the relationship between it and Candida Albecan fungi for this reason the study treated this fungi by using anti fungi drug (nystatin) ,that lead to reduce the level of IgE in patient to be near the value in control group . The last result improve behavior of autism after treatment with nystatin** .

تأثير النستاتين على مستوى الممنعات في أمصال الأطفال المصابين بمرض التوحد

* **عامر فاضل الحيدري** ،كلية الطب ،جامعة كربلاء،العراق****رنا فاضل موسى** ،كلية الصيدلة ،جامعة كربلاء،العراق

الكلمات المفتاحية : التوحد، مستوى الممنعات ،نستاتين

الخلاصة

مرض التوحد هو عبارة عن طيف واسع من الأعراض المتغيرة سلوكيا والمتضمنة خلل بالتواصل أللفضي أو غير أللفضي ،اللغة ،الخيال، والتواصل الاجتماعي مع عادات سلوكية متكررة كالتصفيق وهز الرأس وغيرها الهدف من الدراسة هو قياس مستوى الممنعات ومن ثم دراسة تأثير إعطاء علاج مضاد للفطريات عليها ،أجريت هذه الدراسة على ثلاثون طفل مصاب بالتوحد ممن يراجعون أخصائي الأمراض النفسية في مستشفى الحسين (ع) التعليمي في محافظة كربلاء . و تم تقسيم المرض حسب الجنس والعمر لمعرفة تأثيرهما على المرض ومن ثم تم قياس مستوى الممنعات (IgG,IgA,IgM,IgE) حيث لوحظ انخفاض بمستويات الممنعات (IgG,IgA,IgM) كما لوحظ ارتفاع مؤثر بمستوى الممنع (IgE) وتمت السيطرة على هذا الارتفاع بإعطاء جرع محسوبة من مضاد للفطريات والمعروف باسم نستاتين حيث تبين من خلال استخدامه السيطرة على فطر الكانديدا البيكان والذي سبب الارتفاع الملحوظ بمستوى الممنع IgE مما انعكس ايجابيا على سلوك المرضى حسبما أكد آباء الأطفال المصابين.

1. INTRODUCTION

Autism is a spectrum of neuropsychiatric disorders characterized by deficits in social interaction and communication, and unusual repetitive behavior.[1] Autism spectrum disorders(ASD) is diagnosed between 2 and 4 years of age, when children begin to take part in structured social interactions. [2] While no specific autism genes have been identified to date, autism is believed to have a complex heritability involving several susceptibility genes that may impact both neurodevelopment and immune function. [3]

How genes associated with autism interact among themselves and how their phenotypic penetrance is influenced by epigenetic and environmental factors are poorly understood. [4-6]. The potential role of the immune system in the etiology of autism arises from emerging evidence of a dysregulated or abnormal immune response in children with ASD. Hypotheses that implicate the immune system in the etiology of a behavioral disorder are somewhat controversial. [7].

However, the molecular and cellular mechanisms that interconnect the immune and nervous systems are becoming more clearly understood. Elucidation of the mechanisms responsible for the observed abnormalities in immune function noted in autism may therefore provide valuable insights into the etiology of this disorder. [4,8]. One of the conditions that lead to recurrent otitis media or other recurrent infections is called immunodeficiency, meaning the presence of a weak or deficient immune system. [6].

Immunodeficiency can be caused by a deficiency of antibodies such as IgG, IgA, and IgM. Children with autism have a high frequency of abnormalities of these different kinds of antibodies [9].

Deficiencies of any of the total antibodies indicate aprobable immunodeficiency. In addition, the total amount of a particular antibody could be normal but the amount of a specific antibody might be deficient.this study and others found that the child with autism have high level of IgE . [9,10] IgE is the antibody most widely known for its involvement in all types of allergies and may also be involved in protecting the body from parasites. Elevated IgE in blood is associated with a history of excessive allergies and bacteria , this study found that the increase level of IgE related with type of bacteria know Candida ,so, Candida can be cilled by any antifungal drugs but we used nystatin because it is the most commonly used drug and it is one of the safest and most effective. The most common suspensions of nystatin are formulated to have 100,000 units per cc or ml. (1 cc equals 1 ml for water-based drugs.) Five cc or five ml is the amount in a teaspoon that tacked four time daily for three weak only [11] .

The main consideration in using nystatin is how to avoid the side-effects of the yeast die-off reaction. This can be accomplished by increasing the dosage of nystatin gradually so that the severity of the yeast die-off is minimized. [11] When this approach is used, the yeast are killed over a longer time period instead of during a very short time period. So we used dosage of nystatin step by step with carefully . the aim of this study is to reduce the level of IgE near the normal amount in the control group .

The aim of this study is to achieve serum level of immunoglobulin near normal value in children with autism after nystatin administration . Then improve behavior and breeding of child with autism after the treatment .

2. MATERIALS AND METHODS

The work is done in Al – Hussain teaching Hospital/kerbala Karbala and its laboratories. The total number of collected sample is 30 child with autism compared with 15 normal child (table 1). The cases are collected from the patient that are found in the hospital

and psychiatric clinic .The study involved with asking specialist doctor ,patient's family and reviewing the patients to get information about them .

then , sera was collected from children with autistic disorder (n=30),(21 male, 9 female)as shown in(table 3) and age-matched typically controls (n=15) as shown in (table 2). Samples were assayed for systemic levels of immunoglobulin (IgG, IgM, IgA,) by ELISA (enzyme-linked immunosorbent assay). [12] And IgE by tosoh instrument using (flursence dichromatic kinetic energy assay) .

[13] Subjects with autism were evaluated using the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview—Revised, and all subjects were scored on the Aberrant Behavior Checklist. Levels of total IgG, IgM, IgA, were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits purchased from ALerCHEK Inc. (Portland, ME). Kits were run according to the manufacturer's instructions. Briefly, samples were diluted 1:100,000 (IgG), 1:10,000 (IgM and IgA), and added to 96 well plates pre-coated with capture antibody. After 1-hr incubation and subsequent washing, horseradish peroxidase- conjugated detection antibodies were added and TMB (3,3′, 5,5′ tetramethyl benzidine)/peroxide substrate used for development. Data are reported as median mg/mL (IgG, IgM, IgA) Intra- and inter-assay variability was controlled for using control standards on each plate. The coefficient of variance was less than 10% on any given plate. [12].

IgE was measured by fluorescence kinetic immunoassay by using tosoh instrument. The procedure of this method involved very simple steps by putting conjugate fluorescent solution (antigen) in vial special for IgE then serum (antibody) was added to form complex , this complex added to substrate (dichromate) then the result was obtained . [13].

3. RESULTS

Children with ASD have a significantly reduced level of serum IgA (7,74± 0.22 mg/mL) as compared with control group (6.71±0.18 mg/mL $P<0.05$,), IgG (5.39±0.29 mg/mL) compared to the(TC=total control 7.72±0.28 mg/mL; $P<0.05$) .Children with autism also had a reduced level of plasma IgM (0.67± 0.1mg/mL)as compared to (total control 0.79±0.05 mg/mL; $P<0.05$). while,AU have high level of plasma IgE (8.72±0.44 mg/ML) as compared to the TC(6.14 ±0.02mg/mL; $P<0.001$) ,then after treatment with nystatin the level of IgE was reduced to be with normal value approximately (6.20 ± 0.04 mg/ml; $P<0.001$).(table 4).

4. DISSCUTION

The study showed that Autism affects with males more than females, but a role of genetics cannot be overlooked when ASD impact males significantly more than females. The ratio of males to females with ASD is about four to one. So, there are many plausible theories for this difference: Girls get two X chromosomes, boys get one X and one Y. Many autistic traits have X-linked faults and girls have two chances to get it right, boys have only one chance, Testosterone amplifies the neurotoxicity of mercury, aluminum and probably other toxins (all of which ASD individuals can have trouble detoxifying from the body), while estrogen has protective effects, males require more methylation, more homocysteine recycled to methionine, and more creatine than females because of specific muscle mass and other physiologic requirements. A defect in methylation capacity affects males more than females.Relevant to the oxidant-stress condition of autism, differences in innate immunity between human males and females may make males more prone to

inflammation and TNFα (tumor necrosis factor alpha) elevation than females. [2,6, 14]. Children with AU have significantly reduced levels of sera IgG ,IgA and IgM compared to controls, suggesting an underlying defect in immune function. [15]. This reduction in specific Ig levels correlates with behavioral severity. [16].

Our current data suggest that children with autism demonstrate decreased levels of IgG and IgM. Despite the strict diagnostic criteria we used to classify individuals with autism, there remains phenotypic heterogeneity of behavioral outcome in the study population . In previous studies, small sample sizes (range 30 autism subjects) and variations in the types of controls matched general population) could affect these results. Geographic location of the subjects and controls would also influence the levels of IgE in serum, as would the season during which the sample was obtained due to individual response to regional and seasonal allergens. In addition, as a highly heterogeneous disorder, the behavioral phenotype of the subjects studied may also affect the outcome. [16,17]. However, we believe that one of the most critical factors to consider with respect to Ig concentration is age. examined IgG, IgM, IgA, and IgE levels in the sera from subjects during a very narrow age window (2–9years of age) corresponding to the earliest time an official diagnosis could be obtained. This allowed us to better compare our index and control subjects. [1, 5,18].

The alterations of Ig levels observed in the current study are indicative of an underlying dysfunction in the immune system. While this may affect the way children manage a pathogenic insult, it is not our contention that host/pathogen or host/vaccine interactions are a causative factor in the etiology of autism.The immune system and the nervous system are highly interconnected. Beginning early in development, the relationship between the immune and nervous systems is exceedingly complex and continues throughout life Immune system factors, such as major histocompatibility complex I, cytokines, and chemokines are important during many stages of neuro-development and central nervous system plasticity, functioning, and maintenance[.[18]].. Likewise, several proteins associated with the nervous system, such as neuropeptides, have a broad range of effects on the development of the immune system and its function (suppression as well as activation), including the innervation of immune system-associated organs, such as the lymph nodes and spleen. [8 ,14,19].

Also increase level if IgE with antifungal was seen in Petridshes culture and decrease level of IgE and imprivment of mental behavior after treatment with nystatin and vita lactic acid due to the restoring the balance of amino acid (tartaric and malic acid) ,since there was alter metabolism .

CONCLUTION

The presence of yeast chemical in the urine or stool can be verified by measure a level of immunoglobulins like IgE ,symptoms such as skin problems, diarrhea , constipation . behavioral problems of autism patient are strong suggest an overgrowth of the intestine with the yeast Candida Albicans. So, using nystatin drug for two to four weeks will be beneficial to child with no adverse effect or risk and by using it the level of IgE can be reduced to be near the normal value ,this result improve behavior of autism child after treatment with nystatin .

REFERENCES .

[1] Centers for Disease Control and Prevention (2007) Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, United States,. MMWR Surveill Summ ;61 (No.SS-3).
[2] Centers for Disease Control and Prevention. (2002)Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, Six Sites, United States, MMWR Surveill Summ; 56(No.SS-1).
[3] Centers for Disease Control and Prevention. (2008)Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States. MMWR Surveill Summ ; 56(No.SS-5).
[4] Centers for Disease Control and Prevention. (2010),Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, United States, MMWR Surveill Summ 58(No.SS- 10).
[5] Schieve LA, Boulet SL, Kogan MD, Yeargin-Allsopp M, Boyle CA, Visser SN, Blumberg SJ, Rice C. (2011)Parenting Aggravation and Autism Spectrum Disorders: 2007 National Survey of Children's Health. Disabil Health J. Jul;4(3):143-52.
[6] Kogan MD, Strickland BB, Blumberg SJ, Singh GK, Perrin JM, van Dyck PC.(2011) A national profile of the health care experiences and family impact of autism spectrum disorder among children in the United States, Pediatrics. Dec; 122(6):e1149–58.
[7] Amendah D, Grosse SD, Peacock G, and Mandell DS. (2011)The economic costs of autism: A review. In: Autism Spectrum Disorders. Oxford University Press Inc. June 23,
[8] Fombonne E. Epidemiologic surveys of autism and other pervasive developmental disorders(2012) an update. J Autism Dev Disord. 33(4):365–82.
[9] Rutter M.(2005) Incidence of autism spectrum disorders: changes over time and their meaning. Acta Paediatr. ; 94:2–15.
[10] American Psychiatric Association.(2000) Diagnostic and statistical manual of mental disorders, 4th ed, text revision. Washington (DC): APA.
[11] Kogan MD, Blumberg SJ, Schieve LA, Boyle CA, Perrin JM, Ghandour RM, Singh GK, Strickland BB, Trevathan E, van Dyck PC. (2009)Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US. Pediatrics. Nov;124(5):1395-403. Epub Oct 5.
[12] Boyle CA, Boulet S, Schieve L, Cohen RA, Blumberg SJ, Yeargin-Allsopp M, Visser S, Kogan MD.(2010) Trends in the prevalence of developmental disabilities in US children, . Pediatrics. Jun;127(6): 1034-1042.
[13] Individuals with Disabilities Education Act. Pub. L.(2004) No. 108-446.Dec. 3, , H.R. 1350. [cited 2011 Nov]. Available at: http://www.copyright.gov/legislation/ pl108-446.pdf
[14] American Academy of Pediatrics. Technical report(2001): the pediatrician's role in the diagnosis and management of autistic spectrum disorder in children [online article]. Pediatrics [Internet]; 107(5): E85. Available at: http://aappolicy.aappublications.org/ cgi/content/full/pediatrics;107/5/1221
[15] Committee on Educational Interventions for Children with Autism, (2001)National Research Council. Educating Children with Autism. Washington (DC): National Academies Press; . Available at: http://www.nap.edu/books/0309072697/html/.
[16] Schieve LA, Gonzalez V, Boulet SL, Visser SN, Rice CE, Van Naarden Braun K, Boyle CA.(2012) Concurrent medical conditions and health care use and needs among children with learning and behavioral developmental disabilities, National Health Interview Survey, 2006- 2010. Res Dev Disabil 2012 Mar-Apr; 33(2):467-476.

[17] Warren R, Odell J, Warren W, Burger R, Maciulis A, Daniels W, Torres A(2010). "Immunoglobulin A deficiency in a subset of autistic subjects. "J. Autism Develop Dis. 27:187-192.
[18] Warren R, Margaretten N, Pace N, Foster A: (1998)"Immune abnormalities in patients with autism." J. Autism Develop Dis. 16, 189-197.
[19] Warren R, Singh V, Cole P, Odell J, Pingree C, Warren L, DeWitt C, McCullough M,(1992) "Possible association of the extended MHC haplotype B44-SC30-DR4 with autism."Immunogenetics 36: 203-207, .

Table (1) Ages of autism patient group

Age group	Number
2 year	**5**
3 year	**4**
4 year	**8**
5 year	**6**
6 year	**5**
9 year	**2**

Table (2) Age of control group that used to comparison

Age group	Number
4 year	**6**
7year	**4**
9 year	**5**

Table (3) The number of sex for patient with autism that used in the investigation

sex	Number
Male	21
Female	9

Table (4) Immunoglobulin's value to autism patient and control child

	*IgA mg/ml	IgG mg/ml	*IgM mg/ml	**IgE mg/ml before treatment	IgE mg/ml after tretmant
Patient mean ±SD	6.71±0.18	5.39±0.29	0.67±0.1	8.72±0.44	6.20±0.04
Control mean ±SD	7.74±0.22	7.72±0.28	0.79±0.05	6.14±0.20 Without treatment	no value

- **Statistical analysis by spss** (statistical package for social science program)for comparison between different experimental groups
- *** mean significant value (p < 0,05)**
- **** mean high significant value (p < 0.001)**

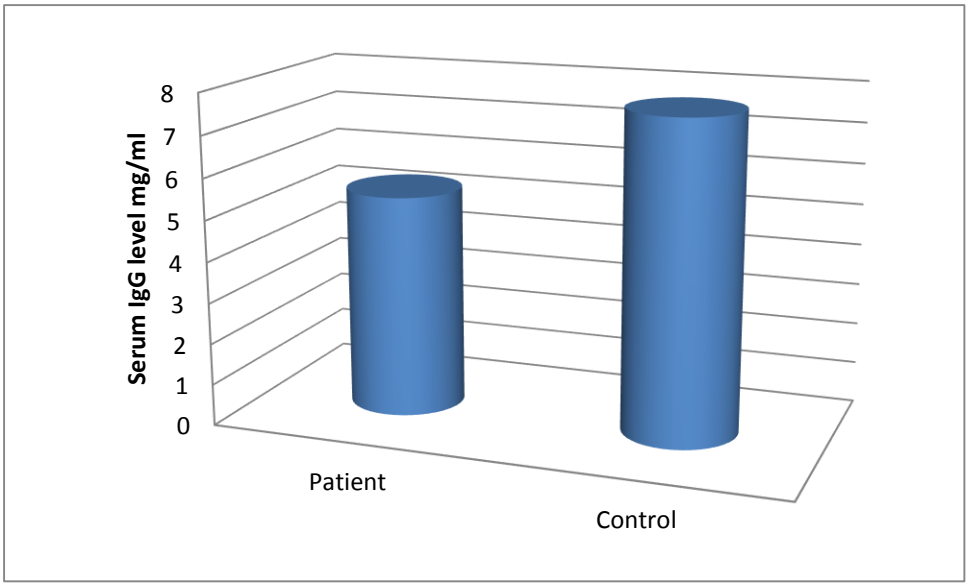

Fig. (1) COMPARESSION OF IgG LEVEL IN SERA OF CONTROL AND AUTISM PATIENT

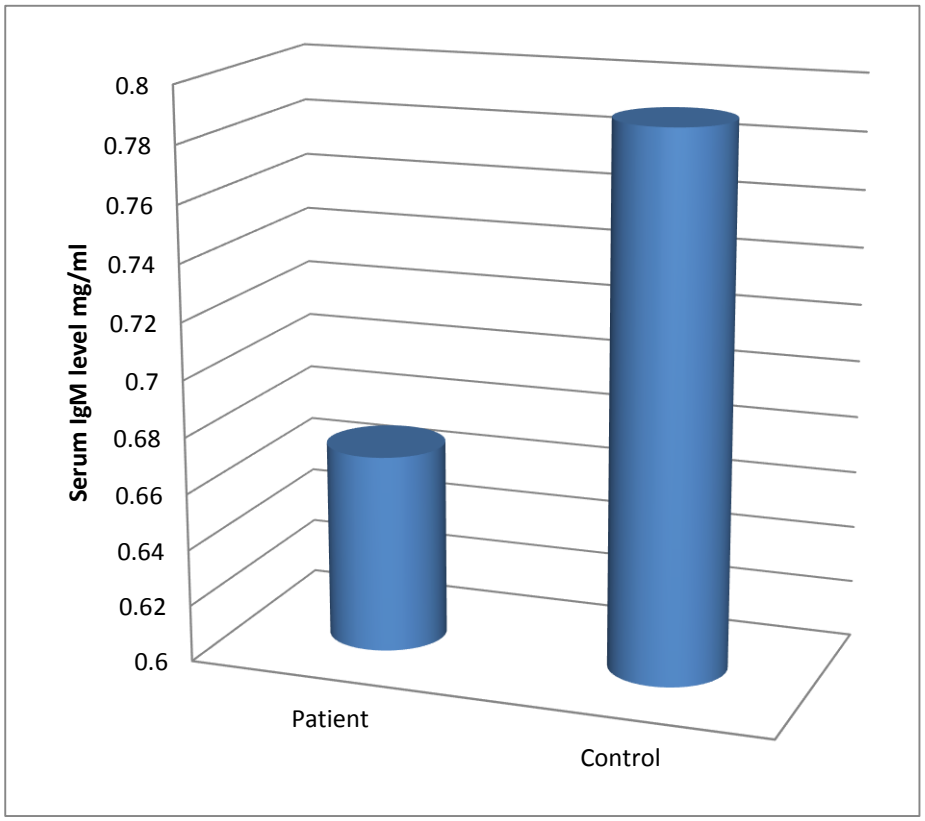

FIG.(2) COMPARESSION OF IgM LEVEL IN SEAR OF CONTROL AND AUTISM PATIENT

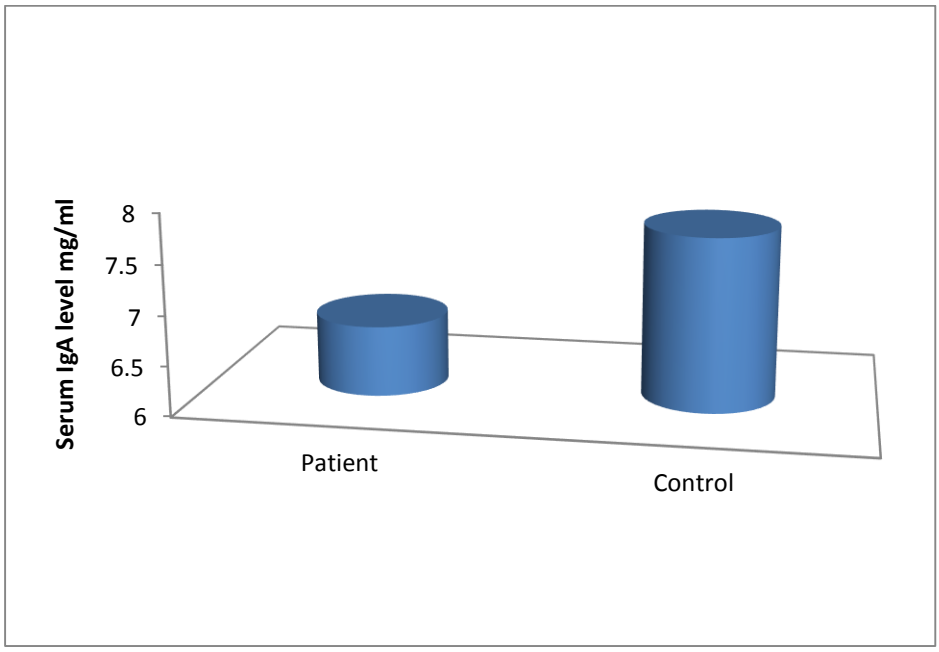

FIG. (3) COMPARESSION OF IgA LEVEL IN SERA OF CONTROL AND AUTISM PATIENT

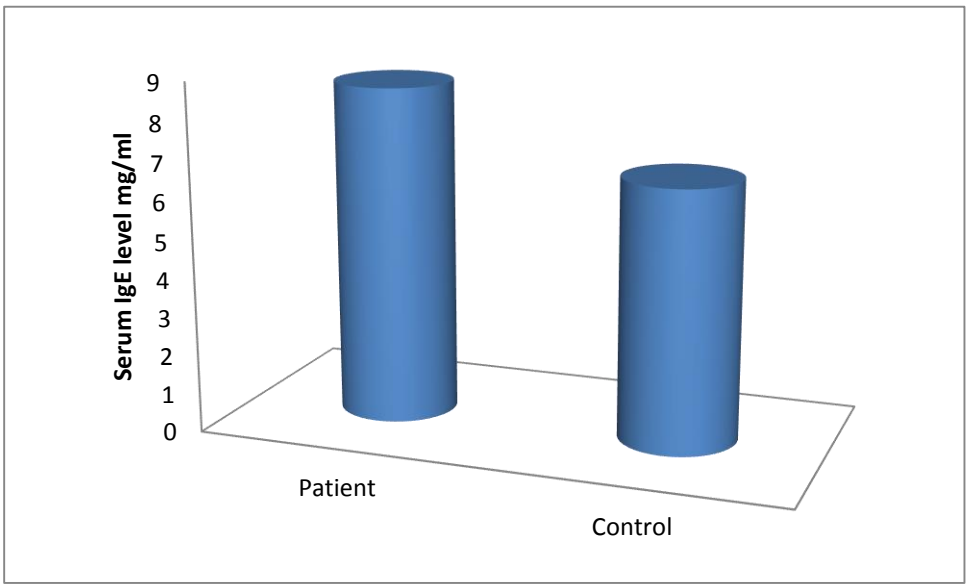

Fig. (4) COMPARESSION of IgE LEVEL IN SERA OF CONTROL AND AUTISM PATIENT before treatment

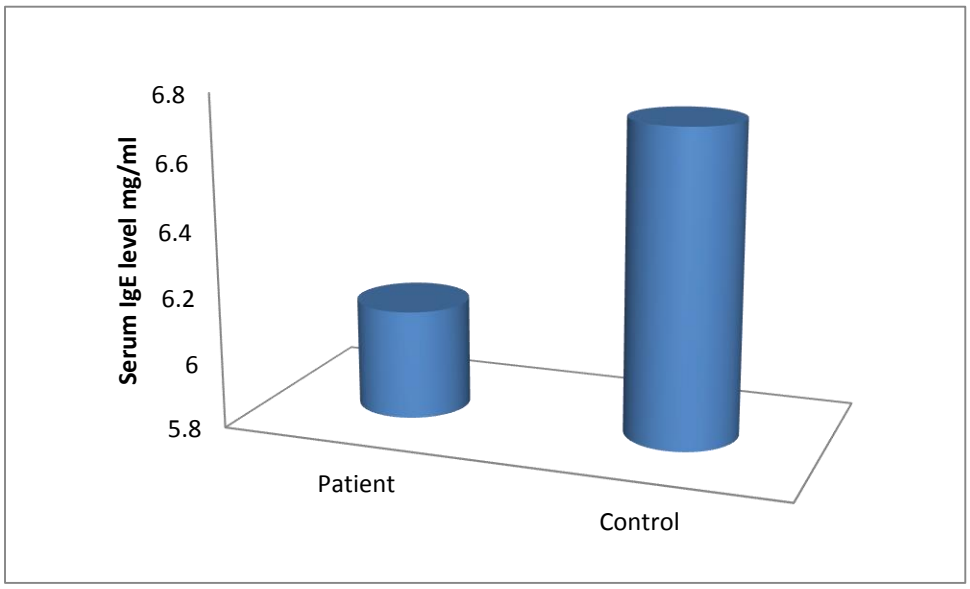

FIG. (5) COPRESSION OF IgE LEVEL IN SERA OF CONTROL AND AUTISM PATIENT AFTER NYSTATIN TRETMANT