

RESEARCH

Open Access

Neuroinflammation in autism spectrum disorders

Afaf El-Ansary^{1,2,3,5*} and Laila Al-Ayadhi^{2,3,4}

Abstract

Objectives: The neurobiological basis for autism remains poorly understood. However, research suggests that environmental factors and neuroinflammation, as well as genetic factors, are contributors. This study aims to test the role that might be played by heat shock protein (HSP)70, transforming growth factor (TGF)- β_2 , Caspase 7 and interferon- γ (IFN- γ) in the pathophysiology of autism.

Materials and methods: HSP70, TGF- β_2 , Caspase 7 and INF- γ as biochemical parameters related to inflammation were determined in plasma of 20 Saudi autistic male patients and compared to 19 age- and gender-matched control samples.

Results: The obtained data recorded that Saudi autistic patients have remarkably higher plasma HSP70, TGF- β_2 , Caspase 7 and INF- γ compared to age and gender-matched controls. INF- γ recorded the highest (67.8%) while TGF- β recorded the lowest increase (49.04%). Receiver Operating Characteristics (ROC) analysis together with predictiveness diagrams proved that the measured parameters recorded satisfactory levels of specificity and sensitivity and all could be used as predictive biomarkers.

Conclusion: Alteration of the selected parameters confirm the role of neuroinflammation and apoptosis mechanisms in the etiology of autism together with the possibility of the use of HSP70, TGF- β_2 , Caspase 7 and INF- γ as predictive biomarkers that could be used to predict safety, efficacy of a specific suggested therapy or natural supplements, thereby providing guidance in selecting it for patients or tailoring its dose.

Keywords: Autism, Neuroinflammation, Heat shock protein-70, Transforming growth factor- β , Interferon- γ , Caspase 7

Introduction

Autism is a complex neurodevelopmental disorder of early onset that is highly variable in its clinical presentation. Although the causes of autism in most patients remain unknown, several lines of research support the view that susceptibility to autism is clearly attributed to both genetic and environmental factors that influence the development of abnormal cortical circuitry that underlies autistic cognitive processes, social impairment and other behaviors [1]. Additionally, recent evidence points to inflammatory mechanisms contributing to autism. Vargas *et al.* [2] suggested neuroinflammatory processes are present in the autistic brain by showing that transforming growth factor (TGF- β_1), interleukin (IL) 6 and IL10 are increased in the brain of autistic patients. A number of studies have also shown that inflammatory

cytokines, including tumor necrosis factor (TNF) α , interferon (IFN) γ , IL1, IL6, IL8 and IL12, are elevated in blood mononuclear cells, serum, plasma and cerebrospinal fluid (CSF) of autistic subjects [2-8].

The role of extracellular 70 kDa heat shock protein 70 (HSP70) in central nervous system inflammation is vastly understudied, despite evidence supporting its ability to drive a pro-inflammatory state [9]. Heat shock proteins (HSPs) are induced in response to many injuries including stroke, neurodegenerative disease, epilepsy and trauma. The overexpression of HSP70 serves a protective role in several different models of nervous system injury, but has also been linked to a deleterious role in some diseases [10].

The transforming growth factor- β (TGF- β) superfamily is a multifunctional family of cytokines that has a critical role in the regulation of key events of development, disease and tissue repair in the nervous system. Accumulating evidence suggests that TGF- β has emerged as a crucial regulator of nervous system physiology, although it has been widely considered as an injury-

* Correspondence: elansary@ksu.edu.sa

¹Biochemistry Department, Science College, King Saud University, P.O. Box 22452, 11495 Riyadh, Saudi Arabia

²Autism Research and Treatment Center, Riyadh, Saudi Arabia

Full list of author information is available at the end of the article

related cytokine [11]. It is still unclear whether plasma TGF- β levels could reflect its brain concentration since it is shown that it can cross the disrupted but not the intact blood–brain barrier (BBB) [12].

It is well known that activation of cysteinyl aspartate-specific proteases (caspases) may underlie apoptotic cell death in the brain. Caspase 3, 6 and 7 likely contribute to such cell death in a stimulus- and cell type-specific manner [13]. Recent studies proved the activation of caspases 3, 7 and 12 in peripheral blood mononuclear cells (PBMCs) from 15 autistic children compared to age-matched normal healthy developing controls [14] In addition, El-Ansary *et al.* [8] recorded an elevation of Caspase 3 in plasma of Saudi autistic children compared to control subjects.

In a study done by Li *et al.* [15], the immune activities in the brain of autistic patients were significantly higher compared to matched normal subjects. Proinflammatory cytokines (TNF- α , IL-6), Th1 cytokine (IFN- γ) were the most significantly increased.

Considering the protective and/or the deleterious effect of HSP70, the key role of TGF- β in brain development [11], the possible role of caspase pathway, and the suggested role of localized brain inflammation and autoimmunity in the pathology of autism, it is of great interest to measure these parameters in plasma of the Saudi population compared to controls in an attempt to understand and clarify their roles in the etiology of this disorder.

Subjects and methods

The study protocol followed the ethical guidelines of the most recent Declaration of Helsinki (Edinburgh, 2000). All 20 autistic subjects enrolled in the study had written informed consent provided by their parents and assented to participate if developmentally able. Subjects for this study were enrolled through the ART Center (Autism Research and Treatment Center) clinic. The ART Center clinic sample population consisted of children diagnosed with Autism Spectrum Disorder (ASD). The diagnosis of ASD was confirmed in all subjects using the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) and 3DI (Developmental, dimensional diagnostic interview). The ages of all autistic children ranged between 3 and 16 years old. All were non-verbal males. Intelligence quotient (IQ) for all autistic children was below 80. All were simple cases. All are negative for fragile \times gene study. The 19 healthy control subjects were recruited from the well-baby clinic at King Khaled University Hospital and they were 3 to 16 years old. All participating subjects were excluded from the investigation if they had dysmorphic features, tuberous sclerosis, Angelman syndrome, or other serious neurological (for example,

seizures), psychiatric (for example, bipolar disorder) or known medical conditions. All participants were screened via parental interview for current and past physical illness. Children with known endocrine, cardiovascular, pulmonary, liver, kidney or other medical disease were excluded from the study.

Ethics approval and consent

This work was ethically approved by the ethical committee of King Khalid Hospital, King Saud University (Approval number is 11/2890/IRB). A written consent was obtained from the parents of each individual case, according to the guidelines of the ethical committee.

Samples collection

After an overnight fast, 10 ml blood samples were collected from both groups in test tubes containing heparin as an anticoagulant. Centrifugation was done; plasma was obtained and deep frozen (at -80°C) until analysis time.

Chemicals and kits

All chemicals and kits used in this study were of analytical grade, a product of Sigma-Aldrich Corp, St Louis, USA. Uscn LifeScience Inc, Wuhan, China; Quantikine, R & D Systems, Inc, Minneapolis, USA and Thermo-scientific (Rockford, IL, USA).

Biochemical assays

Assay of heat shock protein 70 (HSP70)

HSP70 was measured using an ELISA kit, product of Uscn Life Science Inc., Wuhan, China, according to the manufacturer's instructions. The microtiter plate provided in this kit has been pre-coated with an antibody specific for HSP70. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for HSP70. Next, avidin conjugated to horseradish peroxidase (HRP) is added to each microplate well and incubated for two hours at 37°C . Then, a 3,3', 5,5' tetramethylbenzidine (TMB) substrate solution is added to each well. Only those wells that contain HSP70, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450\text{ nm} \pm 10\text{ nm}$. The concentration of HSP70 in the samples is then determined by comparing the optical density of the samples to the standard curve. The minimum detectable level of HSP70 detected is less than 0.045 ng/ml.

Table 1 HSP70 (ng/ml), TGF-β (pg/ml), Caspase-7 (ng/ml) and INFγ (ng/ml) of control and autistic groups

Parameters	Groups	N	Mean ± S.D.	P-value
HSP70 (ng/ml)	Control	19	10.17 ± 2.05	0.001
	Autistic	20	15.82 ± 2.21	
TGF-β (pg/ml)	Control	19	68.30 ± 10.35	0.001
	Autistic	20	101.80 ± 8.86	
Caspas-7 (ng/ml)	Control	19	5.63 ± 1.07	0.001
	Autistic	20	8.74 ± 1.43	
INF-γ (ng/ml)	Control	19	50.85 ± 5.71	0.001
	Autistic	20	85.33 ± 9.06	

Table 1 describes the independent T-Test between the control and autistic groups in HSP70 (ng/ml), TGF-β (pg/ml), Caspase-7 (ng/ml) and INF-γ (ng/ml).

Assay of TGF-β₂

The Quantikine Human TGF-β₂ ELISA kit used in the present study is designed to measure activated TGF-β₂ in serum and plasma. A monoclonal antibody specific for TGF-β₂ has been pre-coated onto a microplate. A total of 100 μl standards and samples were pipetted to each well and incubated for two hours at room temperature. After washing away any unbound substances, 200 μl of HRP-linked polyclonal antibody specific for TGF-β₂ is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a 200 μl substrate solution is added to the wells and color develops in proportion to the amount of TGF-β₂ bound in the initial step. The color development is stopped using 50 μl stop solution and the intensity of the color was measured within 30 minutes at 540nm. The minimum detectable level of TGF-β₂ is less than 7.0 pg/ml.

Assay of Caspase7 (CASP7)

CASP7 was measured using an ELISA kit, a product of Uscn Life Science Inc., Wuhan, China, according to the manufacturer's instructions. The microtiter plate provided in this kit has been pre-coated with an antibody specific to CASP7. Standards or samples are then added to the appropriate microtiter plate wells with a

biotin-conjugated polyclonal antibody preparation specific for CASP7 and incubated for two hours at room temperature. Next, avidin conjugated to HRP is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain CASP7, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm² nm. The concentration of CASP7 in the samples is then determined by comparing the O.D. of the samples to the standard curve. The minimum detectable level of CASP7 detected is less than 0.065 ng/ml.

Assay of INFγ

INFγ was measured using an ELISA kit, a product of Thermo Scientific (Rockford, IL, USA) according to the manufacturer's instructions. This assay employs a quantitative sandwich enzyme immunoassay technique that measures INFγ in less than five hours. A polyclonal antibody specific for human INFγ has been pre-coated onto a 96-well microplate. INFγ in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for INFγ, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured at 550 nm and subtracted from absorbance at 450 nm. The minimum level of INFγ detected by this product is less than 2 pg/ml.

Statistical analysis

A SPSS (Statistical Package for the Social Sciences) computer program was used. Results were expressed as mean ± S.D. and all statistical comparisons were made by means of independent t-test with $P \leq 0.05$ considered as significant. ROC analysis was performed. Area under the curve, cutoff values selected by the program together

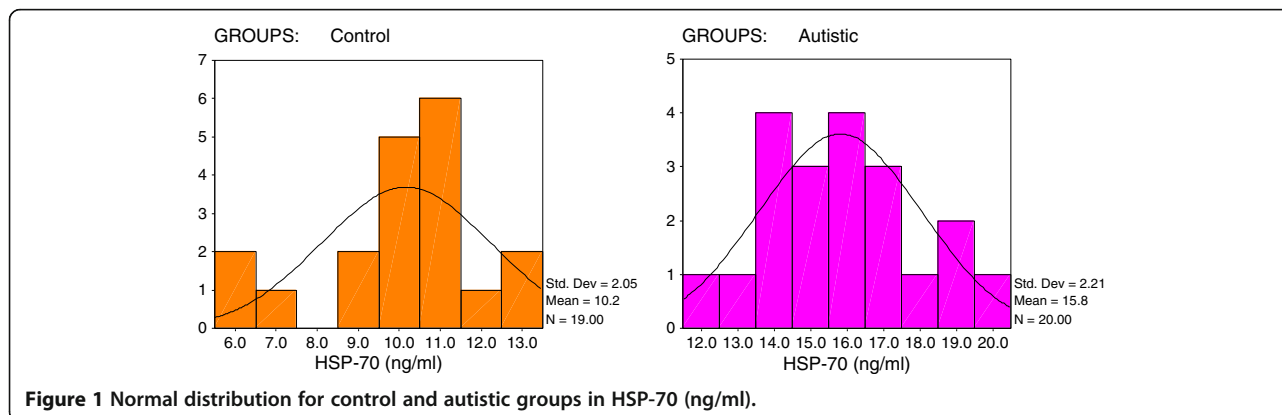
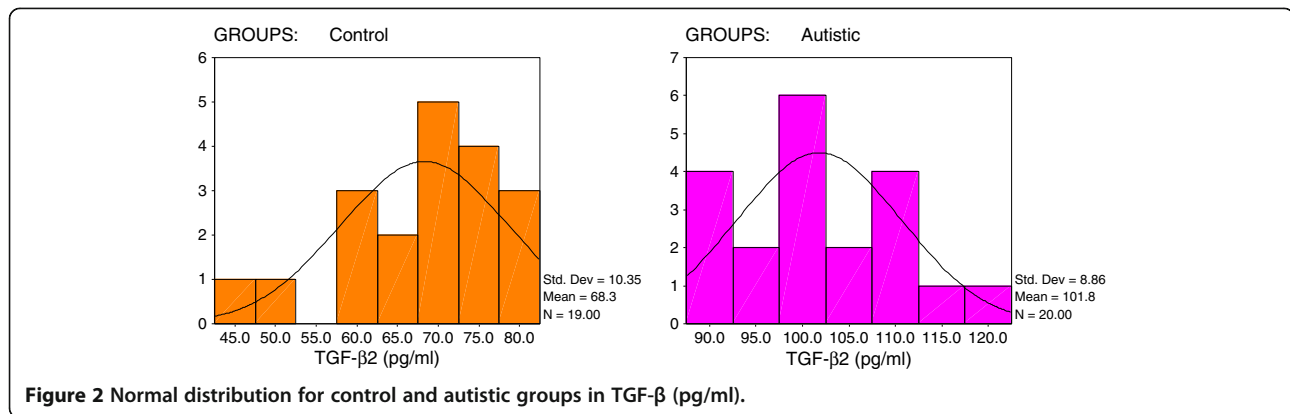


Figure 1 Normal distribution for control and autistic groups in HSP-70 (ng/ml).



with degree of specificity and sensitivity were calculated. Moreover, the predictiveness diagrams of the four measured parameters were drawn in which the x axis represents percentile rank of the biomarker, y axis represents the probability of identifying the disease and the horizontal line is the prevalence of the disease using a Biostat 16 computer program.

Results

Table 1 and Figures 1, 2, 3, 4 demonstrate the significant increase of the four measured parameters in autistic patients compared to healthy age- and gender-matched control subjects. Figure 1 shows that 19/20 of the autistic samples recorded a HSP-70 concentration greater than 12 ng/ml while 16/19 of the controls show values remarkably lower than this value. All autistic patients recorded values greater than 80 pg/ml or 7.5 ng/ml as the maximum concentration seen in control subjects for TGF- β and Caspase-7, respectively (Figures 2 and 3). Additionally, 10/20 of autistics recorded concentrations of INF- γ greater than 85ng/ml while 15/19 of the controls show values lower than 57.5 ng/ml (Figure 4).

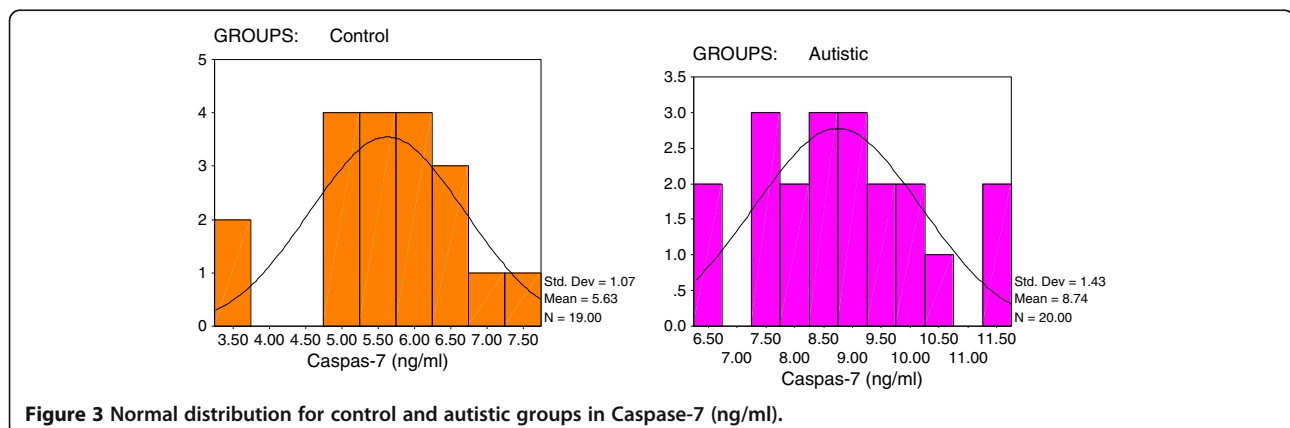
Figure 5 demonstrate the percentage increase in the measured parameters. It could be easily noticed that INF- γ recorded the highest increase (67.8%) while TGF-

β 2 recorded the lowest increase (49.04%). Table 2 and Figures 6 and 7 demonstrate the ROC analysis of the measured parameters (area under the curve, specificity and sensitivity).

Figure 8 shows the predictiveness diagrams of the four measured parameters in relation to the prevalence of autism in Saudi Arabia, which was most recently recorded as 18 per 10,000 persons [16].

Discussion

In a recent hypothesis proposed by Theoharides and Zhang [17], an association among neuroinflammation, mast cell activation and seizures, through secretion of pro-inflammatory mediators and regulation of the BBB permeability was suggested. Despite a large amount of research, the pathogenic mechanism of autism has not yet been clarified. Abnormal protein folding [17,18] oxidative stress [19], mitochondrial dysfunction [20], and apoptotic mechanisms [8] have all been reported as causes of neurodegeneration in association with neuroinflammatory mechanisms which, by generating deleterious molecules, could promote the cascade of events leading to autism. Heat shock proteins (HSPs) play a central role in preventing protein misfolding and



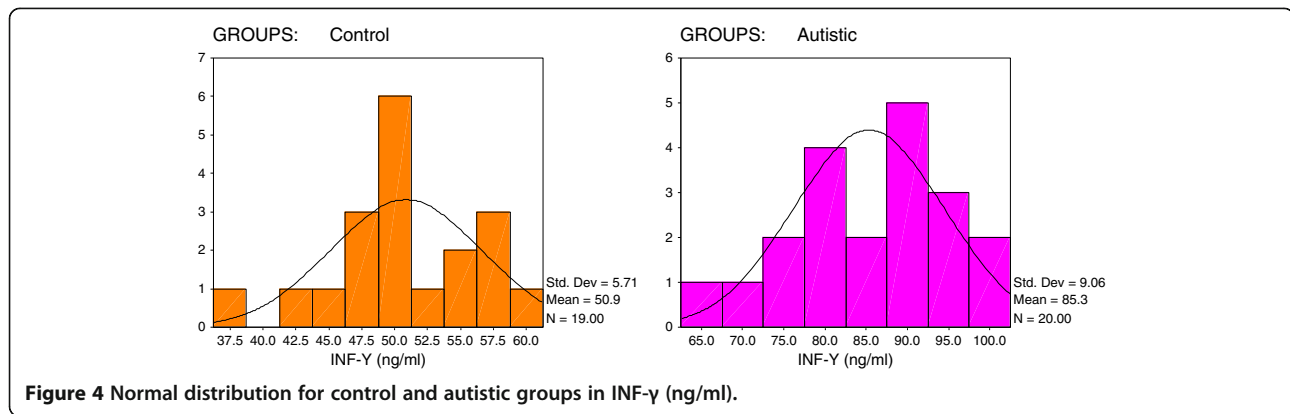


Figure 4 Normal distribution for control and autistic groups in INF-γ (ng/ml).

inhibiting apoptotic activity, and represent a class of proteins potentially involved in neurological disorders [10].

The significant increase in HSP70 reported in the present study could be easily related to oxidative stress as the most important mechanism involved in the etiology of autism. In spite of the protective role of HSPs, they are only effective when up-regulated in the right place (that is, be cell specific), at the right time and to a level and specificity that ensures that all the important binding partners, namely the co-chaperones, are also present at the appropriate levels [21]. So, even though heat shock proteins are known to inhibit various types of apoptosis, some studies show that heat or elevated HSP70 also potentiates cell death following specific stimuli. The unexpected elevation of HSP70 could be related to the etiology of autism by considering the fact that HSP70 initiates TNF-mediated apoptosis by binding IκB kinase (IKK) and impairing nuclear factor-kappaB (NF-κB) survival signaling due its inactivation after being phosphorylated [22]. This explanation could be supported through considering the recent work of El-Ansary *et al.* [8], in which they recorded a significantly lower TNF-α in the same plasma samples used for the current study. Moreover, a number of studies have also shown that heat shock or elevated HSP70 suppresses

NF-κB activity[23-27].Although these studies imply the possibility that HSP70 impairs NF-κB signaling, the exact molecular basis of the Hsp70 and NF-κB interaction is still not clarified. Elevation of HSP70 reported in the present study could be supported and related to the heavy metal toxicity (mercury) and the inability to adequately up-regulate metallothionein (MT) biosynthesis in response to the heavy metal challenge by autistic patients, which was recently recorded in the same investigated samples (unpublished work). Cultured lymphocytes from autistic children when challenged with 10 μM ethyl mercury responded in a different pattern than those of non-autistic siblings. Following the challenge, autistic cultured lymphocytes responded by up-regulating numerous heat shock protein transcripts, but not MTs [28].

The remarkable increase of TGF-β₂ in plasma of autistic patients reported in the present study could be related to brain injury in autism because it is well known that this cytokine is expressed in the lesioned brain [9] and is up-regulated in the central nervous system following ischemia-induced brain damage [29,30]. Although TGF-β₂ is often considered an anti-inflammatory molecule, we could propose that enhanced TGF-β₂ expression may play a certain role in promoting

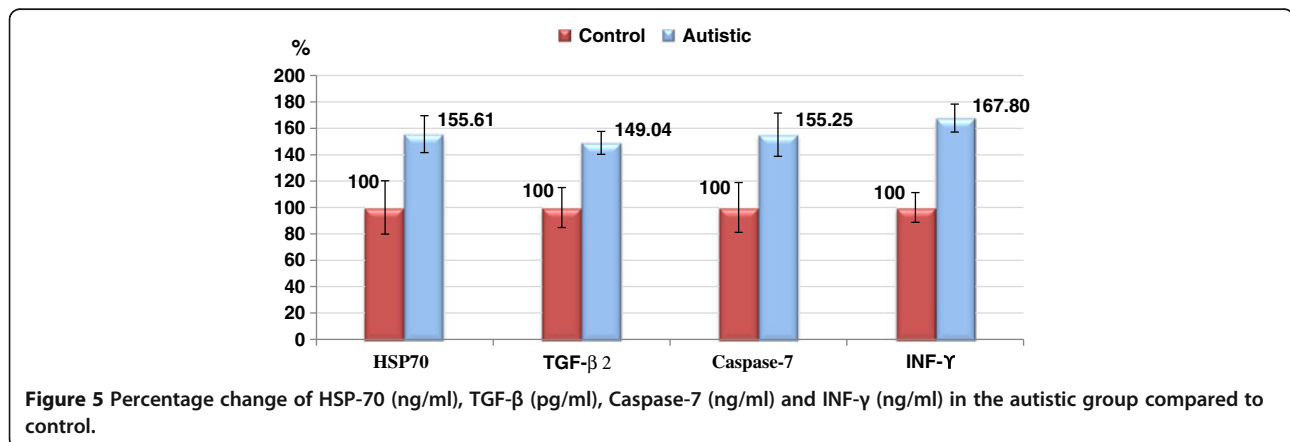


Figure 5 Percentage change of HSP-70 (ng/ml), TGF-β (pg/ml), Caspase-7 (ng/ml) and INF-γ (ng/ml) in the autistic group compared to control.

Table 2 ROC analysis of the measured parameters shows, area under the curves, specificity and sensitivity

Parameter	Area under the curve	Cutoff value	Sensitivity %	Specificity %
HSP-70 (ng/ml)	0.987	12.218	95.0%	84.2%
TGF- β (pg/ml)	1.000	78.649	100.0%	89.5%
Caspase-7 (ng/ml)	0.968	6.698	90.0%	89.5%
INF- γ (ng/ml)	1.000	56.558	100.0%	78.9%

inflammation in brain injury associated with autism. Because TGF- β_1 and TGF- β_2 exhibit a combination of specific and shared roles in the regulation of inflammation, this suggestion could find a support in the previous work which indicates that injection of an antiserum directed against TGF- β_1 reduces inflammation in the CNS after traumatic injury, and astroglial overproduction of TGF- β_1 enhances inflammatory CNS disease in transgenic mice [31-33]. The elevated TGF- β_2 reported in the present study is in good agreement with the previous work of Vargas *et al.* [2] in which they found TGF- β_1 among the neuroinflammatory cytokines elevated in the cerebral cortex, white matter and notably in the cerebellum of autistic patients. The recorded increase of plasma TGF- β_2 as a marker of elevated TGF- β_2 in the brain could be easily related to amyloid beta (A β) generation previously recorded in autistic patients [34,35]. This could be supported through considering the work of Lesne *et al.* [36], in which they show that TGF- β_1 added to human astrocyte cultures promotes perivascular inflammation, interactions with and increased production of amyloid beta precursor protein (A β PP) and subsequent A β generation.

Caspases as a family of cysteine proteases play central roles in coordinating the stereotypical events that occur during apoptosis. Because the major executioner caspases, Caspase-3 and Caspase-7, exhibit the most potent activity toward certain synthetic peptide substrates, this has led to the widespread view that both occupy critical

roles within the cell death machinery [37]. Additionally, Erener *et al.* [38] propose an apoptosis-independent regulatory role for Caspase 7-mediated cleavage of poly (ADP-ribose) polymerase family, member 1 (PARP1) as a DNA repair-associated enzyme that has multiple roles in cell death. The significant elevation of Caspase 7 reported in the present study could be easily related to the impaired NF- κ B signaling survival activity as previously attributed to HSP70 but through a different mechanism. Elevation of Caspase 7 again confirms the contribution of brain cell death and proinflammation in the etiology of autism as previously reported in our recent work in 2011 [8], in which Caspase 3 as a proapoptotic biomarker was significantly lower in plasma which might indicate its increase in the brain of Saudi autistic patients compared to age- and gender-matched controls. Moreover, both studies are consistent with the most recent work of Siniscalco *et al.* [14] in which they prove the increase of protein levels of caspase-3, -7 and -12 in autistic patients and suggest the possible role of the caspase pathway in autism clinical presentation and the use of caspases as potential diagnostic and/or therapeutic tools.

Immune factors, such as autoimmunity, have been implicated in the genesis of autism [3]. The increase of IFN- γ reported in the present study may indicate antigenic stimulation of Th-1 cells pathogenetically linked to autoimmunity in autism. The reported elevation of IFN- γ could support the previous work of Molloy *et al.* [5]

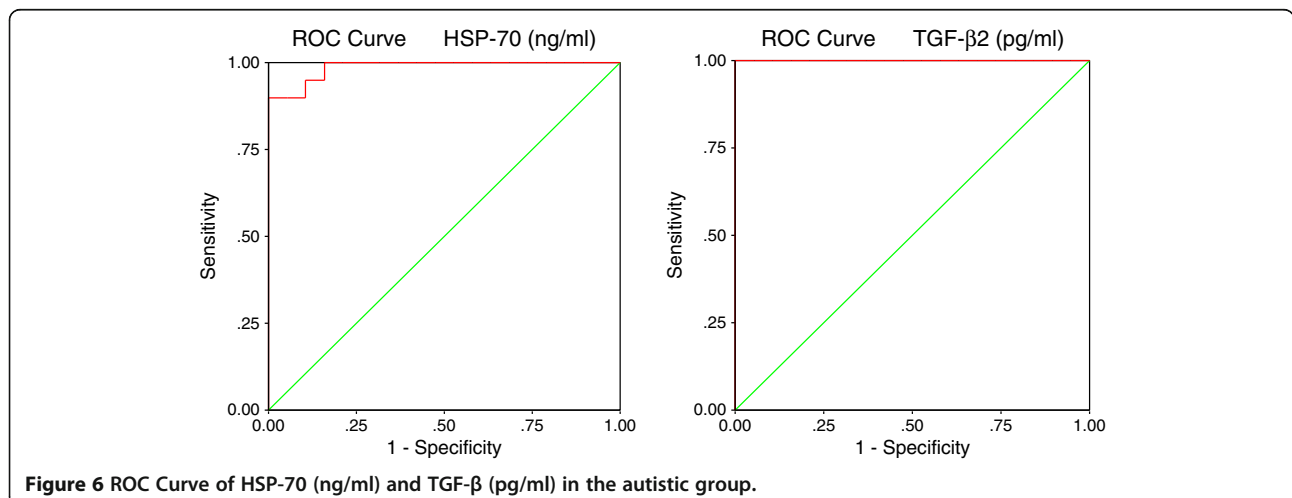
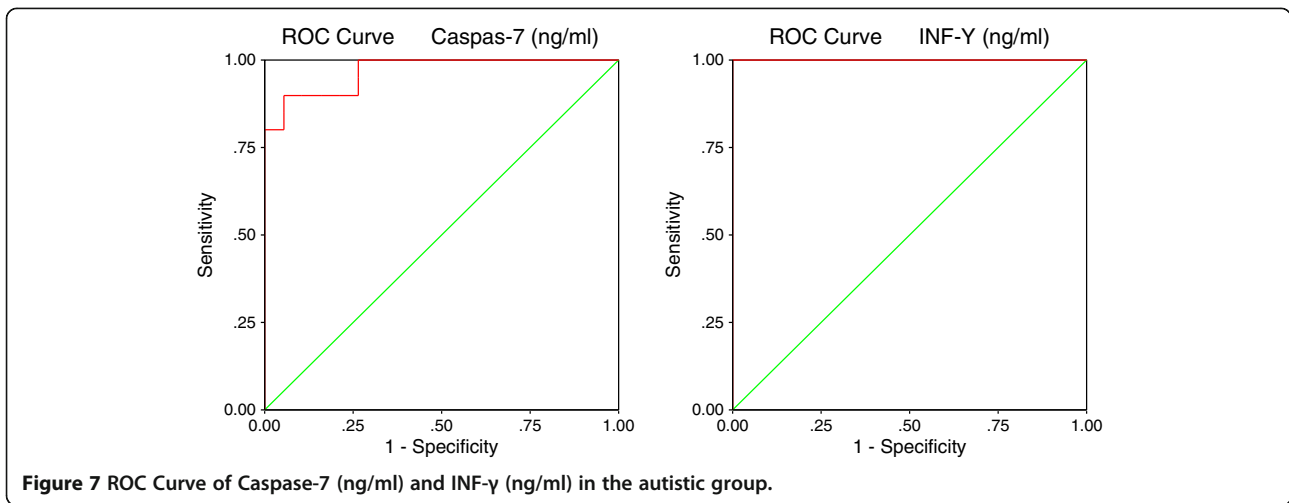
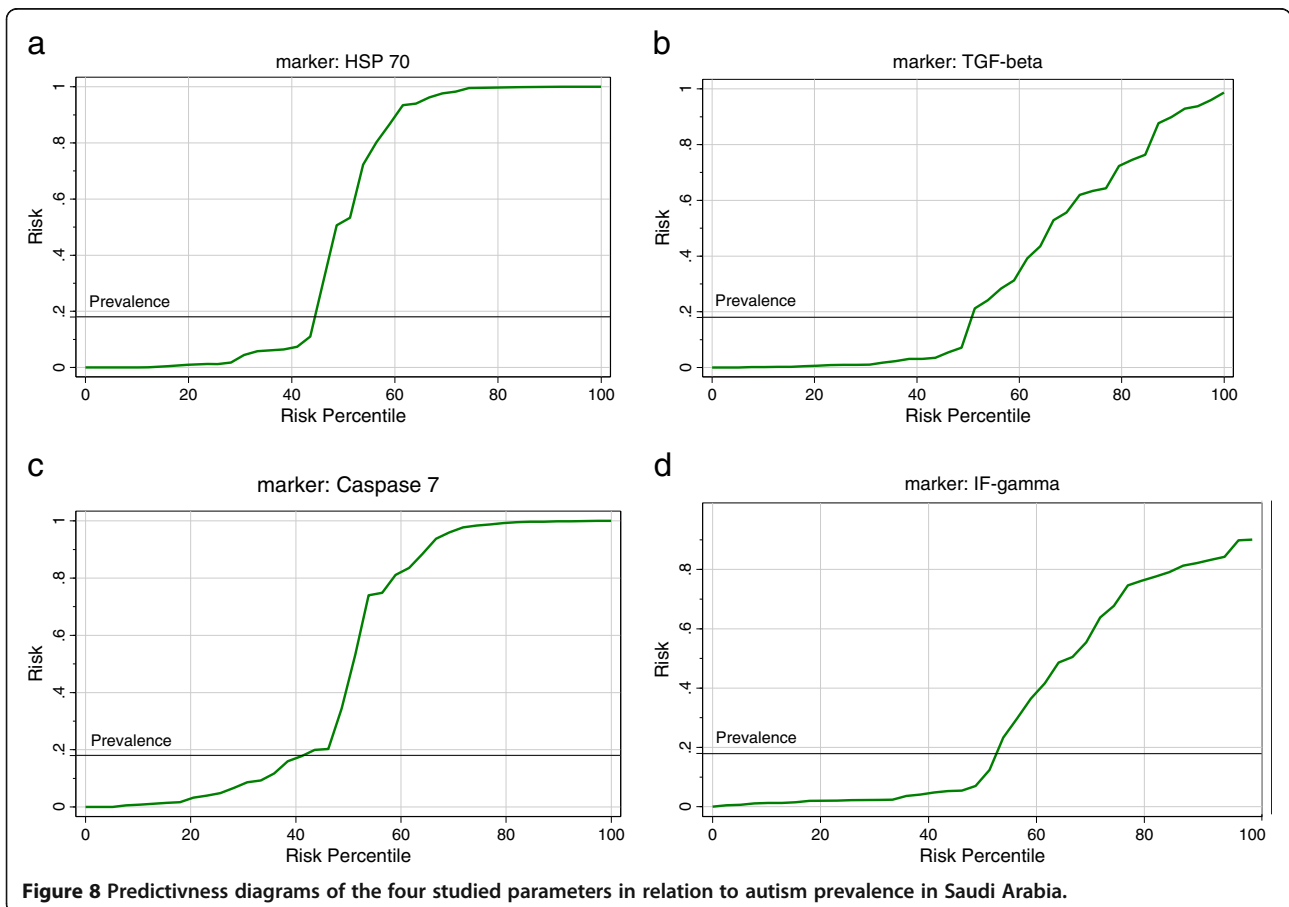


Figure 6 ROC Curve of HSP-70 (ng/ml) and TGF- β (pg/ml) in the autistic group.



showing that PBMNC of autistic children produce remarkably high levels of IL-12 and IFN- γ , or express higher than normal levels of mRNA for IFN- γ [39] and the most recent work of Tostes *et al.* [40] that plasma levels of vasoactive intestinal peptide (VIP), IFN- γ and NO were significantly higher in children with autism, compared to the healthy subjects and that a positive

correlation between plasma levels of NO and IFN- γ exists. Moreover, they suggested additional evidence that higher levels of IFN- γ may be associated with increased oxidative stress, a phenomenon greatly involved in the etiology of autism [19]. Collectively, the present study together with the previously mentioned studies confirm the existence of Th-1 type of immune response in



autistic children and that would also be consistent with an autoimmune pathology, simply because IFN- γ is among the cytokines well known for inducing autoimmune diseases. Based on the fact that the predictiveness curve is better if it is farther away from the prevalence line and useless if it is close to the prevalence line, the predictiveness curves of the four measured parameters (Figure 8a-d), varies significantly from the baseline risk depending on whether HSP70, TGF- β_2 , Caspase 7 and IFN γ concentrations were low or very high. This shows their usefulness as predictive biomarkers. This could be supported by the high sensitivity and specificity recorded through ROC analysis (Figures 6 and 7).

Abbreviations

A β : Amyloid beta; ADI-R: Autism Diagnostic Interview-Revised; HSP-70: Heat shock protein-70; IFN- γ : Interferon- γ ; TGF- β : Transforming growth factor- β ; TNF- α : Tumor necrosis factor- α ; ROC: Receiver Operating Characteristics.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AE designed the study and drafted the manuscript. LA provided samples and participated in the diagnosis of the autistic samples. Both authors have read and approved the final manuscript.

Acknowledgements

The authors would like to thank Shaik AL-Amodi Autism Research Chair, NPST - Medical Centers and the parents of autistic children, without whom this work was not possible. This work was supported by King Abdul Aziz City for Science and Technology (KACST).

Author details

¹Biochemistry Department, Science College, King Saud University, P.O. Box 22452, 11495 Riyadh, Saudi Arabia. ²Autism Research and Treatment Center, Riyadh, Saudi Arabia. ³Shaik AL-Amodi Autism Research Chair, King Saud University, Riyadh, Saudi Arabia. ⁴Department of Physiology, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia. ⁵Medicinal Chemistry Department, National Research Centre, Dokki, Cairo, Egypt.

Received: 13 October 2012 Accepted: 28 November 2012

Published: 11 December 2012

References

1. Abrahams BS, Geschwind DH: **Advances in autism genetics: on the threshold of a new neurobiology.** *Nat Rev Genet* 2008, **9**:341–355.
2. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA: **Neuroglial activation and neuroinflammation in the brain of patients with autism.** *Ann Neurol* 2005, **57**:67–81.
3. Singh VK: **Plasma increase of interleukin-12 and interferon-gamma: pathological significance in autism.** *J Neuroimmunol* 1996, **66**:143–145.
4. Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M: **Activation of the inflammatory response system in autism.** *Neuropsychobiology* 2002, **45**(suppl 1):1–6.
5. Molloy CA, Morrow AL, Meinen-Derr J, Schleifer K, Dienger K, ManningCourtney P, Altaye M, Wills-Karp M: **Elevated cytokine levels in children with autism spectrum disorder.** *J Neuroimmunol* 2006, **172**:198–205.
6. Ashwood P, Wakefield AJ: **Immune activation of peripheral blood and mucosal CD3a lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms.** *J Neuroimmunol* 2006, **173**:126–134.
7. Chez MG, Burton Q, Dowling T, Chang M, Khanna P, Kramer C: **Memantine as adjunctive therapy in children diagnosed with autistic spectrum disorders: an observation of initial clinical response and maintenance tolerability.** *J Child Neurol* 2007, **22**:574–579.
8. El-Ansary A, Ben Bacha AG, Al-Ayadhi LY: **Proinflammatory and proapoptotic markers in relation to mono and di-cations in plasma of autistic patients from Saudi Arabia.** *J Neuroinflammation* 2011, **8**:142.
9. Moore SA, Kim MY, Maiolini A, Tipold A, Oglesbee MJ: **Extracellular hsp70 release in canine steroid responsive meningitis-arteritis.** *Vet Immunol Immunopathol* 2012, **145**:129–133.
10. Turturici G, Sconzo G, Geraci F: **Hsp70 and its molecular role in nervous system diseases.** *Biochem Res Int* 2011, **2011**:618127.
11. Gomes FCA, Sousa Vde O, Romao L: **Emerging roles for TGF- β 1 in nervous system development.** *Int J Dev Neurosci* 2005, **23**:413–424.
12. Kastin AJ, Akerstrom V, Pan W: **Circulating TGF-beta1 does not cross the intact blood-brain barrier.** *J Mol Neurosci* 2003, **21**:43–48.
13. Meller R, Skradski SL, Simon RP, Henshall DC: **Expression, proteolysis and activation of caspases 6 and 7 during rat C6 glioma cell apoptosis.** *Neurosci Lett* 2002, **324**:33–36.
14. Siniscalco D, Sapone A, Giordano C, Cirillo A, de Novellis V, de Magistris L, Rossi F, Fasano A, Maione S, Antonucci N: **The expression of caspases is enhanced in peripheral blood mononuclear cells of autism spectrum disorder patients.** *J Autism Dev Disord* 2012, **42**:1403–1410.
15. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, Ji L, Brown T, Malik M: **Elevated immune response in the brain of autistic patients.** *J Neuroimmunol* 2009, **207**:111–116.
16. El-Tarras AE, Awad NS, Mitwaly N, Alsulaimani AA, Said MM: **Association between polymorphisms of SLC6A3 and DRD1 genes and autism among Saudi Arabia Taif population using PCR-restriction fragment length polymorphism (PCR-RFLP).** *Afr J Biotechnol* 2012, **11**:11665–11670.
17. Theoharides TC, Zhang B: **Neuro-inflammation, blood-brain barrier, seizures and autism.** *J Neuroinflammation* 2011, **8**:168.
18. De Jacobo A, Comoletti D, Kovarik Z, Gaietta G, Radic Z, Lockridge O, Ellisman MH, Taylor P: **A mutation linked with autism reveals a common mechanism of endoplasmic reticulum retention for the alpha, beta-hydroxylase fold protein family.** *J Biol Chem* 2006, **281**:9667–9676.
19. Al-Gadani Y, El-Ansary A, Attas O, Al-Ayadhi L: **Oxidative stress and antioxidant status in Saudi autistic children.** *Clin Biochem* 2009, **42**:1032–1040.
20. Al-Mosalem OA, El-Ansary A, Attas O, Al-Ayadhi L: **Metabolic biomarkers related to energy metabolism in Saudi autistic children.** *Clin Biochem* 2009, **42**:949–957.
21. Kalmar B, Greensmith L: **Induction of heatshockproteins for protection against oxidative stress.** *Adv Drug Deliv Rev* 2009, **61**:310–318.
22. Ran R, Lu A, Zhang L, Tang Y, Zhu H, Xu H, Feng Y, Han C, Zhou G, Rigby AC, Sharp FR: **Hsp70 promotes TNF-mediated apoptosis by binding IKK γ and impairing NF- κ B survival signaling.** *Genes Dev* 2004, **18**:1466–1481.
23. Feinstein DL, Galea E, Reis DJ: **Suppression of glial nitric oxide synthase induction by heat shock: effects on proteolytic degradation of I κ B- α .** *Nitric Oxide* 1997, **1**:167–176.
24. Guzova IV, Darieva ZA, Melo AR, Margulis BA: **Major stress protein Hsp70 interacts with NF- κ B regulatory complex in human T-lymphoma cells.** *Cell Stress Chaperones* 1997, **2**:132–139.
25. Curry HA, Clemens RA, Shah S, Bradbury CM, Botero A, Goswami P, Gius D: **Heat shock inhibits radiation-induced activation of NF- κ B via inhibition of I- κ B kinase.** *J Biol Chem* 1999, **274**:23061–23067.
26. Andres D, Diez-Fernandez C, Castrillo A, Cascales M: **Relationship between the activation of heat shock factor and the suppression of nuclear factor- κ B activity in rat hepatocyte cultures treated with cyclosporine A.** *Biochem Pharmacol* 2002, **64**:247–256.
27. Malhotra V, Wong HR: **Interactions between the heat shock response and the nuclear factor- κ B signaling pathway.** *Crit Care Med* 2002, **30**:S89–S95.
28. Walker SJ, Segal J, Aschner M: **Cultured lymphocytes from autistic children and non-autistic siblings up-regulate heat shock protein RNA in response to thimerosal challenge.** *Neurotoxicology* 2006, **27**:685–692.
29. Lindholm D, Castrén E, Kiefer R, Zafra F, Thoenen H: **Transforming growth factor-beta 1 in the rat brain: increase after injury and inhibition of astrocyte proliferation.** *J Cell Biol* 1992, **117**:395–400.
30. Buisson A, Lesne S, Docagne F, Ali C, Nicole O, MacKenzie ET, Vivien D: **Transforming growth factor-beta and ischemic brain injury.** *Cell Mol Neurobiol* 2003, **23**:539–550.
31. Logan A, Berry M, Gonzalez AM, Frautschy SA, Sporn MB, Baird A: **Effects of transforming growth factor beta 1 on scar production in the injured central nervous system of the rat.** *Eur J Neurosci* 1994, **6**:355–363.

32. King VR, Phillips JB, Brown RA, Priestley JV: **The effects of treatment with antibodies to transforming growth factor beta1 and beta2 following spinal cord damage in the adult rat.** *Neuroscience* 2004, **126**:173–183.
33. Wyss-Coray T, Feng L, Masliah E, Ruppe MD, Lee HS, Toggas SM, Rockenstein EM, Mucke L: **Increased central nervous system production of extracellular matrix components and development of hydrocephalus in transgenic mice overexpressing transforming growth factor-beta 1.** *Am J Pathol* 1995, **147**:53–67.
34. Frackowiak J, Mazur-Kolecka B, Kuchna I, Nowicki K, Brown WT, Wegiel J: **Accumulation of Amyloid-Beta Peptide Species in Four Brain Structures in Children with Autism.** In *Proceedings of the 10th International Meeting for Autism Research 2011 (IMFAR): May 12–14, 2011; San Diego, California.*
35. Al-Ayadhi LY, Ben Bacha AG, Kotb M, El-Ansary AK: **A novel study on amyloid β peptide 40, 42 and 40/42 ratio in Saudi autistics.** *Behav Brain Funct* 2012, **8**:4.
36. Lesne S, Docagne F, Gabriel C, Liot G, Lahiri DK, Buée L, Plawinski L, Delacourte A, MacKenzie ET, Buisson A, Vivien D: **Transforming growth factor-beta 1 potentiates amyloid-beta generation in astrocytes and in transgenic mice.** *J Biol Chem* 2003, **278**:18408–18418.
37. Walsh JG, Cullen SP, Sheridan C, Lüthi AU, Gerner C, Martin SJ: **Executioner caspase-3 and caspase-7 are functionally distinct proteases.** *Proc Natl Acad Sci U S A* 2008, **105**:12815–12819.
38. Erener S, Pétrilli V, Kassner I, Minotti R, Castillo R, Santoro R, Hassa PO, Tschopp J, Hottiger MO: **Inflammasome-activated caspase 7 cleaves PARP1 to enhance the expression of a subset of NF- κ B target genes.** *Mol Cell* 2012, **46**:200–211.
39. Garbett K, Ebert PJ, Mitchell A, Lintas C, Manzi B, Mirnics K, Persico AM: **Immune transcriptome alterations in the temporal cortex of subjects with autism.** *Neurobiol Dis* 2008, **30**:303–311.
40. Tostes MH, Teixeira HC, Gattaz WF, Brandão MA, Raposo NR: **Altered neurotrophin, neuropeptide, cytokines and nitric oxide levels in autism.** *Pharmacopsychiatry* 2012, **45**:241–243.

doi:10.1186/1742-2094-9-265

Cite this article as: El-Ansary and Al-Ayadhi: **Neuroinflammation in autism spectrum disorders.** *Journal of Neuroinflammation* 2012 **9**:265.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

