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Increase of cytosolic phospholipase A2 as hydrolytic enzyme of phospholipids and autism cognitive, social and sensory dysfunction severity

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Abstract

Background: Autism is neurodevelopmental disorder that is characterized by developmental, behavioral, social and sensory abnormalities. Researchers have focused in last years in immunological alteration and inflammation as a hot subject in autism field. This work aims to study the alteration in phospholipids (PE, PS, and PC) together with the change in cPLA2 concentration as the main phospholipid hydrolytic enzyme in autistic patients compared to control. It was also extended to find a correlation between these biomarkers and severity of autism measured as childhood autism rating scale (CARS), Social responsiveness scale (SRS), and Short sensory profile (SSP).

Methods: Phospholipids (PE, PS, PC) and cPLA2 as biochemical parameters were determined in the plasma of 48 Saudi autistic male patients, categorized as mild-moderate and severe as indicated by their Childhood Autism Rating Scale (CARS), social responsiveness scale (SRS) and short sensory profile (SSP) and compared to 40 age- and gender-matched control samples.

Results: The reported data demonstrate significantly lower levels of PE, PS, and PC together with a significant increase in cPLA2. While association between severity of autism and impaired phospholipid concentration was completely lacked, an association between cPLA2 and impaired sensory processing was observed.

Conclusions: The impaired phospholipid level and remarkable increased in cPLA2 concentration asserted their roles in the etiology of autism. Receiver operating characteristic analysis together with predictiveness diagrams proved that the measured parameters could be used as predictive biomarkers of clinical symptoms and provide significant guidance for future therapeutic strategy to re-establish physiological homeostasis.

Keywords: Autism, Phospholipids, Cytosolic phospholipase A2, Neuroinflammation, Oxidative stress, Short sensory profile, Childhood autism rating scale, Social responsiveness scale

Background

Autism is a complex neurodevelopmental disorder that manifests before three years and considered as one of the most widespread disorder of childhood with high rate of morbidity, impact on the family and cost to society. According to recent epidemiological data, around one in fifty children is affected with autism [1]. The disorder is

characterized by developmental, behavioral, social and sensory abnormalities [2, 3]. There is a significant gender bias in autism, with approximately 4:1 male/female ratio [4]. Its cause remains unknown, but it considered as a multi-factorial disorder that is influenced by genetic, immunological, and environmental factors with oxidative stress as a mechanism linking these risk factors [5].

Individual with autism exhibited cognitive disability, memory reduction and they have self-focused attention [6]. In general, cognitive level was associated with autism severity [7]. It's not surprising that all autistic children have unusual response to auditory, visual and tactile

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stimuli [8, 9]. There are a few studies that examined sensory profile in autistic children. Children with autism had more sensory dysfunction (i.e., tactile sensitivity, auditory, taste and smell) compared to children with other developmental delays when they measured by SSP [10, 11].

Neuroinflammation are characterized by brain cell activation (i.e., microglia and astrocyte) and increase in cytokine production as major causes of cell damage in autistic children [12, 13]. Evidence suggest that mast cells activation participate to a modulation of blood brain barrier (BBB) [14, 15]. Biochemical studies have shown elevated inflammatory cytokines such as IL-1, IL-6, IL8, IL12, and tumor necrosis factor (TNF- α) in serum, plasma, and cerebral spinal fluid in autistics [13, 16, 17]. It has been proposed that abnormal activation of neuroinflammation considered as potential mechanism in autism pathogenesis.

Several studies have been concluded the impairment in glutathione (GSH) associated pathway in autism [18–20]. These finding have shown that brain cells induce and release diverse inflammatory mediators in response to oxidative stress [21–23]. Phospholipase A2 and cyclooxygenase-2 are target proteins for inflammation and usually induced by proinflammatory factors such as cytokines, infections and peroxidants [24–26].

Neuronal membranes are rich in lipids, and can contain up to 80% lipid by weight [27]. These membranes are composed of glycerophospholipids [phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI)], sphingolipid and cholesterol [28]. The cellular membrane plays a protective, anti-inflammatory role and indirectly an antioxidant role, favoring physiological defense processes against free radicals. In addition to their role as structural components of the cell membrane, phospholipids serve as precursors for various second messengers such as arachidonic acid (AA), docosahexaenoic acid (DHA), ceramide, 1,2-diacylglycerol, phosphatidic acid, and lysophosphatidic acid [29]. Neuronal membrane phospholipids are more susceptible to reactive oxygen species ROS because they are rich in polyunsaturated fatty acids (PUFAs) that are labile to peroxidation and oxidative damage [30]. In addition, it is not particularly enriched in antioxidant defenses [20, 31].

Reduced level of polyunsaturated fatty acids have been associated with autism [32–35]. Autism also implies to be associated with changed in lipid metabolism which can be participating in the pathogenesis of this disease. These changes aggravate alterations in the cell membrane phospholipid function and structure. A significant reduction in phospholipid levels in the plasma of autistic patient have been previously identified [33, 36].

In addition, children with autism have been shown higher phospholipase A2 (PLA2) activity compared to

their matched control [32]. Evidence proposes that the instability observed in fatty acid levels may be caused by an increase in PLA2 activity, perhaps in association with the high oxidative stress found in autistic patients [37]. Recent studies have been proved that deregulation of lipid metabolism due to PLA2 over activation is associated with nervous system dysfunction and cognitive impairment [34, 35, 38].

Childhood Autism Rating Scale (CARS), Social Responsiveness Scale (SRS) and Short Sensory Profile (SSP) have been used as scales to define children with autism and determined the severity and abnormalities of autistic behaviors beside build strong background about their social and psychological problem [39–41]. In this context, the aim of this study was to evaluate the relationship between impaired plasma phospholipid levels, cPLA2 activity and the age, cognitive, social and sensory profiles of children with autism compared with healthy control subjects.

Methods

Participants

The study protocol followed the ethical guidelines of medicine Collage, King Saud University according to the most recent Declaration of Helsinki (Edinburgh, 2000). All subjects enrolled in the study (48 autistic children and 40 control males) had filled informed consent and signed by their parents. They were enrolled through the ART Center (Autism Research & Treatment Center) clinic in King Khalid University Hospital in Riyadh. The ART Center clinic population consisted of children diagnosed on the 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 Developmental, dimensional diagnostic interview (3DI). The mean of age of all autistic children participated in the study were between 7 ± 4 years old. All were simplex cases. All were negative for fragile x gene study. The control group recruited from pediatric clinic at King Saud medical city in Riyadh with mean age 7 ± 4 years old. Subjects were excluded from the investigation if they had dysmorphic features, or diagnosis of fragile X or other serious neurological (e.g., seizures), psychiatric (e.g., 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.

Behavioral assessment

The CARS score was fulfilled as a scale for autism severity. CARS assess the child on a scale from one to four in each of 15 dimensions or symptoms (relating to people;

emotional response; imitation; body use; object use; listening response; fear or nervousness; verbal communication; non-verbal communication; activity level; level and reliability of intellectual response; adaptation to change; visual response; taste, smell and touch response; and general impressions). Total Scores at or above 30 strongly suggest the presence of autism. Children who have scored 30–36 have mild to moderate autism ($n = 23$), while those with scores ranging between 37 and 60 points have severe autism ($n = 27$) [39]. SRS is the first widely used quantitative parent/teacher-report measure of autistic behaviors; it was completed in 15 to 20 min. A total score of 76 or higher is considered severe and strongly associated with a clinical diagnosis of autistic disorder. A score of 60–75 is interpreted as falling in the mild to moderate range of social impairment [40].

The Short Sensory Profile (SSP; Dunn 2001) is a 38-item questionnaire intended to rates a variety of sensory impairments. Each item on the SSP is measured on a 5-point Likert scale. Domain scores are measured in the areas of tactile sensitivity, taste/smell sensitivity, movement sensitivity, seeking sensation, auditory filtering, low energy levels, and visual/auditory sensitivity. Domain scores, as well as overall sensory response, are categorized as typical performance, probable difference from typical performance, and definite difference from typical performance. The score less than 142 consider as severe (definite difference), score from 142 to 152 consider as mild to moderate (probable difference) and score from 153 to 190 consider a typical performance. SSP can provide information about the sensory

processing skills of children with autism to assist occupational therapists in assessing and planning intervention for these children [41]. The studied control and autistic groups are illustrated in Fig. 1.

Laboratory assessment

Blood samples collection

After overnight fast, blood samples from autistics and matched controls were drawn by a qualified lab technician. Blood was taken into three ml blood collection tubes containing EDTA, and samples were immediately centrifuged at 4 °C at 3000 g for 20 min and stored at -80 °C until analysis.

Biochemical analysis

Chloroform and methanol used for phospholipid extraction, ammonia (NH₃) and water methanol for the mobile phase were 99% HPLC grade and obtained from Sigma-Aldrich (Taufkirchen, Germany). PC, PS and PE were obtained from Fluka, Sigma-Aldrich (Taufkirchen, Germany).

Phospholipids assay

Phospholipids measurement

Phospholipid separation was performed on a Kaneur Maxi Star HPLC system with four solvent lines, a degasser SEDEX 55 evaporating light detector (SEDEX 55 Lichtstreu detector, S.E.D.E.E., France) which was coupled with Apex M625 software (Autochrom, USA). As the nebulizing gaz, N₂ was used at a flow rate of 4 l/min, and a nebulizing temperature of 40 °C. The gain was set at 8 and 2.0 bar N₂.

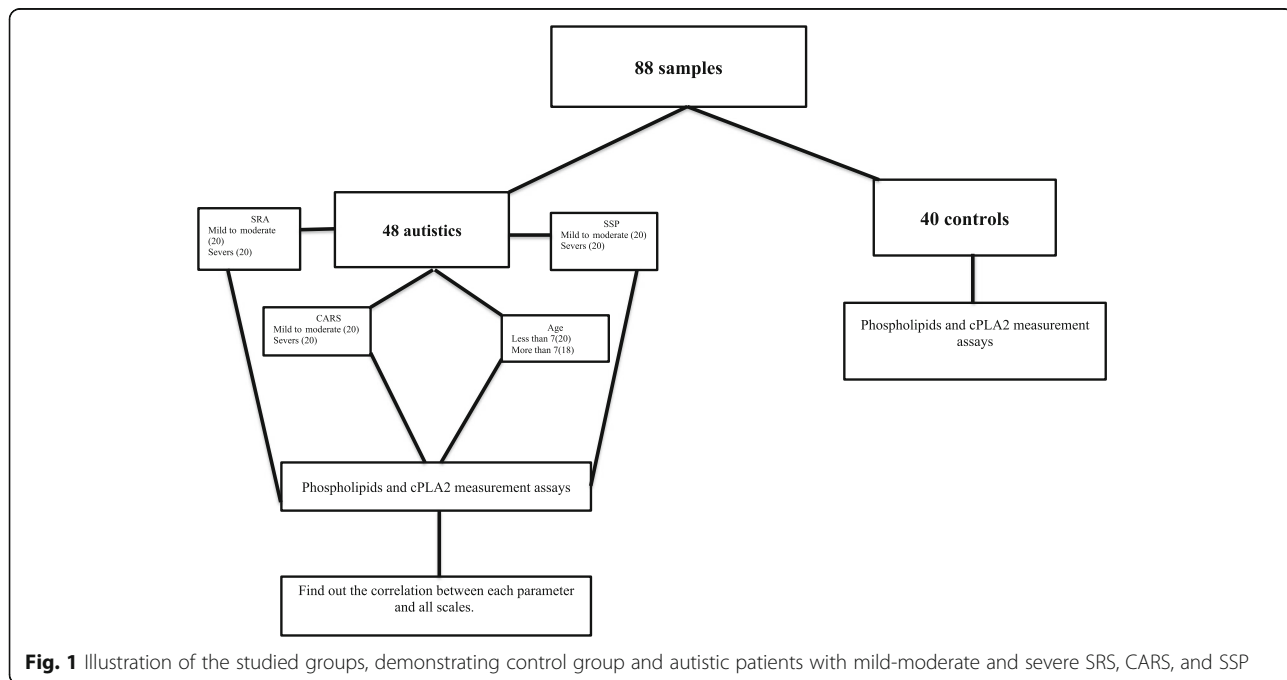


Fig. 1 Illustration of the studied groups, demonstrating control group and autistic patients with mild-moderate and severe SRS, CARS, and SSP

A 125 × 4.0 mm Si-60 column with 5 µm particle diameter (Lichrospher) was used. The elution program was a linear gradient with 80:19.5:0.5 (V/V) chloroform:methanol: water: ammonia (NH₃) at 22 min and the column was allowed to equilibrate until the next injection at 27 min. The injection volume was 50 µl. A liquid phase extraction procedure adapted from the method described by Bligh and Dyer [42] was used to extract the serum samples. Briefly, 50 µl of sample was diluted with 750 µl deionized water and mixed well. Then 2 ml of methanol and 1 ml of chloroform were added to the sample and mixed well. Then the mixture was homogenized (Rotary mixture 34,526, Snijders) for 15 min. The mixture was centrifuged for 5 min by 4000 rpm [42].

Assay of cPLA2

cPLA2 concentration was measured according to the manufacturer's instruction using a competitive enzyme immunoassay technique a product of Amsbio, Blue Gene Company. The detection range of the product was 1.56 ng/ml-100ng/ml.

Statistical analysis

SPSS computer program was used. Results were expressed as mean ± SD and all statistical comparisons were made by means of independent *t*-test with $P \leq 0.05$ considered as significant. To test the specificity and sensitivity of phospholipids and cPLA2 as markers of autism phenotype, receiver operating characteristics (ROC) analysis was performed. The correlation between the true positive rate (sensitivity) and the false-positive rate (1-specificity) was represented as a curve. The cutoff point was chosen to minimize the sum of false-positive and false-negative test results. The area under the curve (AUC) provides a useful metric to compare different biomarkers. Whereas an AUC value close to 1 indicates an excellent diagnostic and predictive marker, a curve that lies close to the diagonal (AUC = 0.5) has no diagnostic utility. AUC close to 1 is always accompanied by satisfactory values of specificity and sensitivity of the biomarker.

Predictivness curve offer the ability to provide the new risk for an individual based on biomarker test results. It is useful for assessing the fit of the risk model and the classification performance of the biomarker. A horizontal line of the disease prevalence is included as a reference for completely uninformative risk model. Better models will have larger area, below the horizontal disease prevalence line and above predictivness curve, and above the disease prevalence line and below the predictivness curve. In this regard, predictivness curves are the mirror images of ROC curve. Predictivness diagrams of the measured parameters were drawn in which the x axis represents percentile rank of the biomarker, the 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. Pearson's correlations analysis was used to determine the relationships between the parameters and scales.

Results

The significant difference noted in mean plasma concentration of cPLA2 between the autism and control individuals is presented in Table 1 and Fig. 2a. We can easily noticed that autistic patients showed an elevated mean of cPLA2 concentration of 3.242 ng/mL (SD ± 1.345), whereas the control individuals exhibited a much lower mean concentration of only 0.298 ng/mL (SD ± 0.174), $p < 0.001$.

In the same Table and Figure (b, c, and d), the mean value of phospholipid levels for individuals with autism and healthy control are also illustrated. The individuals with autism showed a decreased in mean concentrations of 0.029, 0.043 and 1.076 mmole/l for PE, PS and PC compared to control phospholipid of 0.057, 0.089, and 1.712 respectively (Table 1 and Fig. 2).

To assess the usefulness of these biomarkers in the diagnosis of autism, ROC analysis was performed. The optimal cut-off points for using PE, PS and PC as biomarkers for autism were 0.043, 0.061 and 1.447mmole/l respectively. These cut-off points were associated with a sensitivity of 97.5, 97.5 and 100% for PE, PS and PC respectively, and a specificity of 100% for all phospholipids have been measured in this study. The optimal cut-off point for using cPLA2as a biomarker for autism was 1.114 ng/ml. This cut-off point was associated with a sensitivity of 100% and a specificity of 100%. (AUC = 1.000) (Fig. 3a and Table 2).

For autistic individuals, the relationship between the levels of phospholipids and severity of autism measured by the CARS, SRS and SSP scores was also evaluated. There were negative correlation between cPLA2 and Phospholipids (i.e., PE, PS, and PC) ($R = -0.731, -0.757$ and -0.741 $p < 0.001$).

Figure 4 a–d demonstrates the predictiveness curves as an assessment of the performance of phospholipids (i.e., PE, PS and PC) and cPLA2 in autism risk prediction in the Saudi population. The four measured parameters showed adequate predictive power.

Discussion

This study demonstrates that the levels of phospholipids in children with autism are significantly decreased when compared with typically developing children. Although, variation in phospholipid levels show positive correlation with autism diagnosis but their low level don't show any association with cognitive and behavioral measures such as stereotypy, hyperactivity, and communication evaluated as CARS and SRS scores. The significant decrease of PE, PS, and PC reported in the present study can find

Table 1 Levels of phospholipids and cPLA2 in autistic patients compared to control participants

Parameters	Group	N	Mean \pm S.D.	P value
cPLA2 (ng/ml)	Control	40	0.298 \pm 0.174	0.001
	Autistic Patients	48	3.242 \pm 1.345	
	Autism (severe in CARS)	24	2.943 \pm 1.408	0.083
	Autism (mild to moderate in CARS)	22	3.640 \pm 1.242	
	Autism (severe in SRS)	7	2.973 \pm 1.608	0.632
	Autism (mild to moderate in SRS)	15	3.304 \pm 1.430	
	Autism (mild to moderate in sensory)	25	2.932 \pm 1.178	0.050
	Autism (severe in sensory)	18	3.753 \pm 1.488	
	Age (less than 7 years)	24	3.234 \pm 1.472	0.830
	Age (more than 7)	22	3.322 \pm 1.265	
PE (mmole/l)	Control	39	0.057 \pm 0.008	0.001
	Autistic Patients	40	0.029 \pm 0.008	
	Autism (severe in CARS)	20	0.030 \pm 0.009	0.761
	Autism (mild to moderate in CARS)	20	0.029 \pm 0.006	
	Autism (severe in SRS)	6	0.031 \pm 0.009	0.762
	Autism (mild to moderate in SRS)	14	0.030 \pm 0.009	
	Autism (mild to moderate in sensory)	21	0.027 \pm 0.006	0.139
	Autism (severe in sensory)	14	0.030 \pm 0.008	
	Age (less than 7 years)	20	0.031 \pm 0.006	0.319
	Age (more than 7)	18	0.028 \pm 0.010	
PS (mmole/l)	Control	39	0.089 \pm 0.017	0.001
	Autistic Patients	40	0.043 \pm 0.011	
	Autism (mild to moderate in CARS)	20	0.043 \pm 0.009	0.605
	Autism (severe in CARS)	20	0.044 \pm 0.012	
	Autism (mild to moderate in SRS)	6	0.043 \pm 0.012	0.952
	Autism (severe in SRS)	14	0.043 \pm 0.014	
	Autism (mild to moderate in sensory)	21	0.044 \pm 0.011	0.815
	Autism (severe in sensory)	14	0.045 \pm 0.010	
	Age (less than 7 years)	20	0.044 \pm 0.012	0.831
	Age (more than 7)	18	99.10 \pm 11.86	
PC (mmole/l)	Control	39	1.712 \pm 0.184	0.001
	Autistic Patients	40	1.076 \pm 0.235	
	Autism (mild to moderate in CARS)	20	1.108 \pm 0.216	0.396
	Autism (severe in CARS)	20	1.044 \pm 0.253	
	Autism (mild to moderate in SRS)	6	1.045 \pm 0.323	0.455
	Autism (severe in SRS)	14	1.128 \pm 0.167	
	Autism (mild to moderate in sensory)	21	1.051 \pm 0.234	0.394
	Autism (severe in sensory)	14	1.125 \pm 0.268	
	Age (less than 7 years)	20	1.039 \pm 0.280	0.274
	Age (more than 7)	18	1.126 \pm 0.183	

support in many previous studies [36, 43, 44]. Choline plays an important role as a methyl-group donor in the synthesis of phosphatidyl choline that consider as one of essential building blocks for the membrane phospholipid

components as well as in the synthesis of the neurotransmitter acetylcholine. Hamlin et al. [45] have been studied the level of choline in plasma of autistic children and healthy controls, they found that autistics had low

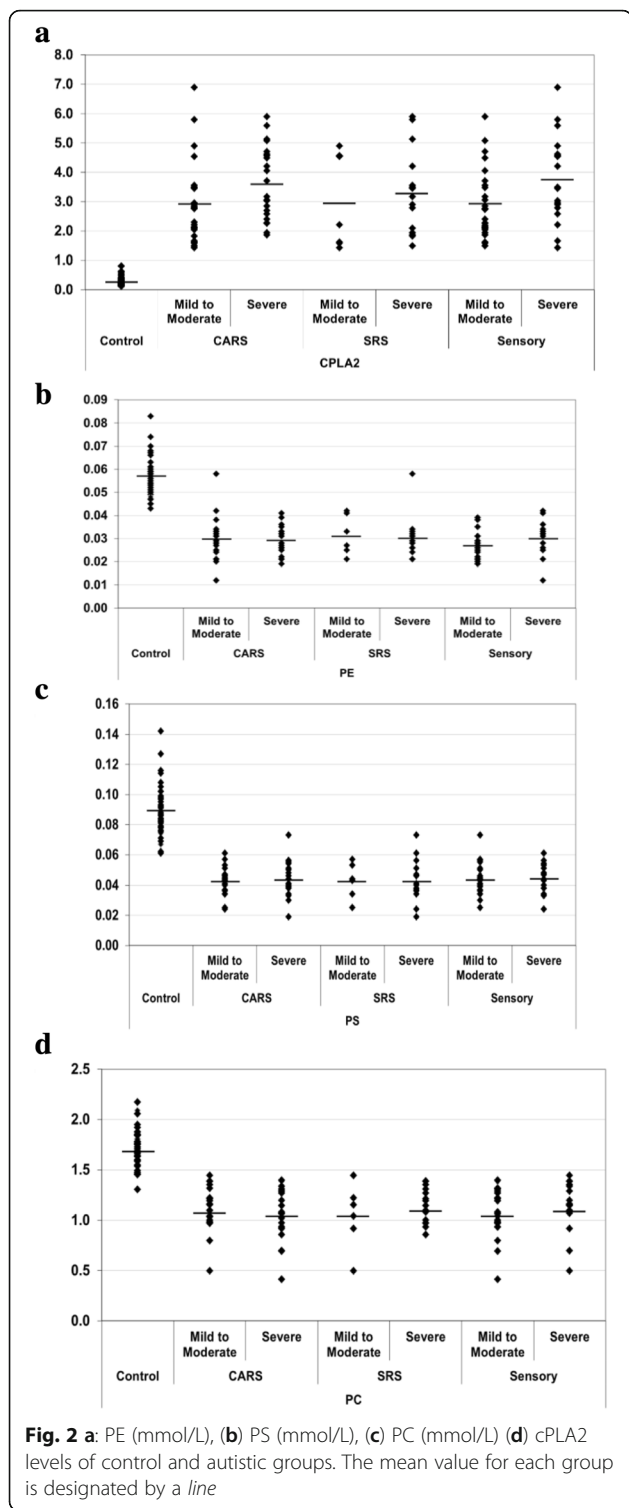


Fig. 2 a: PE (mmol/L), **(b)** PS (mmol/L), **(c)** PC (mmol/L) **(d)** cPLA2 levels of control and autistic groups. The mean value for each group is designated by a line

level of choline compared to controls. Consistent with low choline level, the present study found that PC was significantly lower in the autistic group compared to control.

This study adds further support for a possible role of phospholipid impairment in the neuroinflammation as a

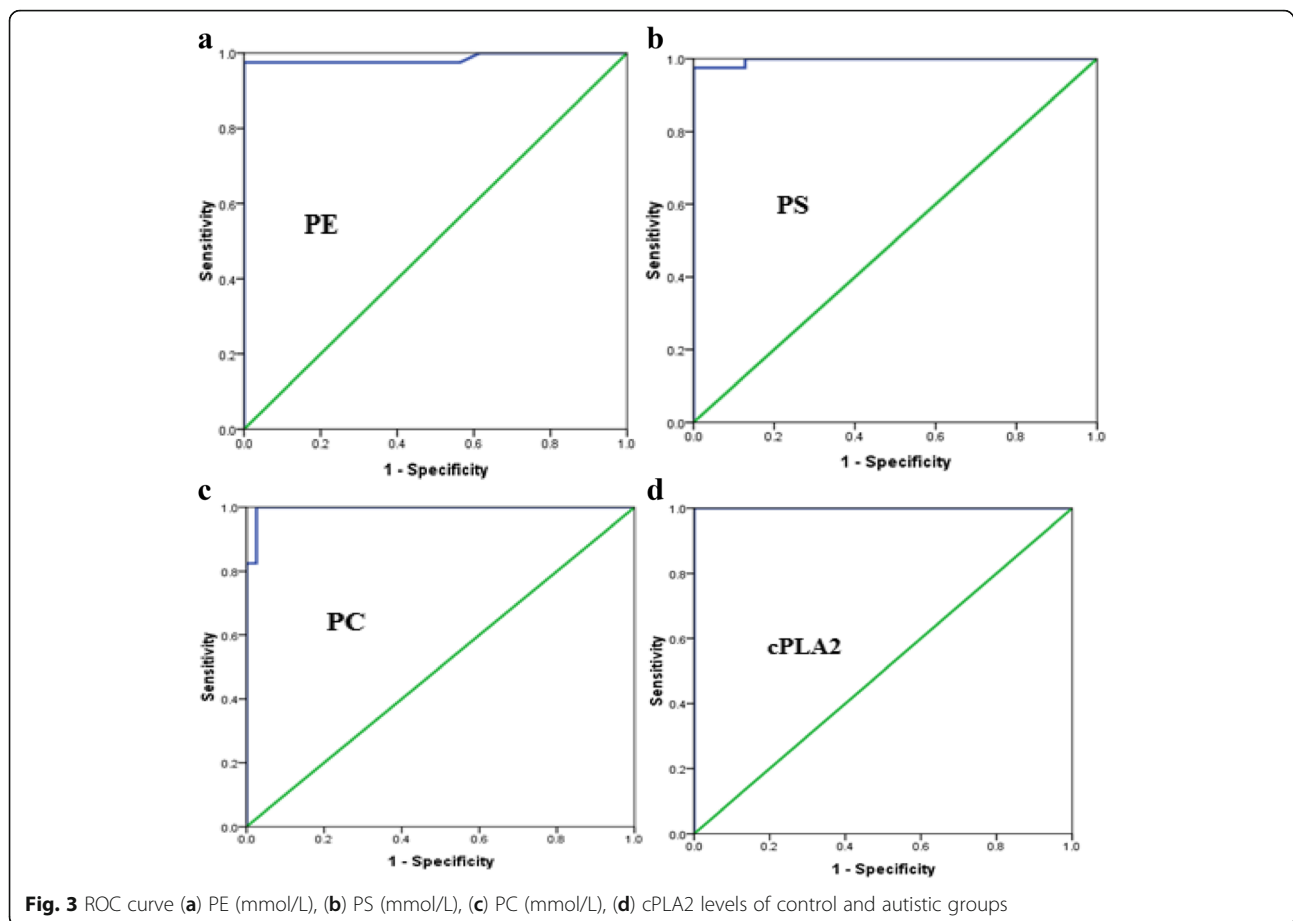
pathological mechanism related to autism [46]. This is consistent with reduced levels of PE and enhanced copper-mediated oxidation which was recorded in lymphoblasts from autistic subjects than from control [47, 48]. The significant decrease of PE reported in the present study can be easily related to H₂O₂ oxidative stress previously reported in the same Saudi autistic patients, and attributed to the over-expression of SOD together with diminished activity of catalase [49] This can find more support in the work of Glzman et al. [50] which prove that overexpression of Cu/Zn-SOD is correlated to phospholipid depletion.

The anti-inflammatory effect of phospholipids was previously reported by Pandey et al. [51] who found that omega-6 phospholipids, e.g., PC demonstrates anti-inflammatory properties presented as inhibition of tumor necrosis factor (TNF- α) and H₂O₂ activated mitogen-activated protein kinase (MAPK) in neuronal SH-SY5Y cell line in addition to the prevention of nuclear factor-kappa B activation by phosphorylation.

Thomas et al. [52] have concluded that after infusion of rodents with propionic acid and butyric acid to be models for ASD, there was alteration in phospholipid profiles. In spite of the non-significant correlation between phospholipids and SSP reported in the present study, change in phospholipid metabolism in autism has been suggested to correlate with language deficits, and this can be observed in autistic children through sensory profile scale that was completed by their parents who always suffer from language difficulty and they need long time to understand what their children want [53].

The reported remarkable increase of cPLA2 concentration in autistic children compared to healthy control (Table 1) can be used to support the previously discussed reduction of phospholipids in autistic plasma. While phospholipids are not correlated with severity of autism, cPLA2 was significantly related to SSP but not CARS, SRS and age. There is evolving evidence for the involvement of cPLA2 in regulating neurite outgrowth and neuronal excitatory functions, both under physiological and pathological conditions. In cultured primary cortical neurons, the stimulation of ionotropic glutamate receptors by *N*-methyl- α -aspartic acid (NMDA) has been shown to activate cPLA2 and AA release [54]. Involvement of cPLA2 in neuronal excitotoxicity has been demonstrated by using neurons from cPLA2 knockout mice, which showed less NMDA-mediated injury as compared to the wild-type controls [55].

Although, there was no correlation between low level of phospholipids and the three measured scales (CARS, SRS, and SSP) as useful measure of cognitive, behavior impairment, and sensory dysfunction in autism [56], have recorded that increased anti-phospholipid antibodies in autism was associated with cognitive and



impaired behaviors. The lack of association between cPLA2, CARS and SRS is not in good agreement with certain studies which prove that deregulation of lipid metabolism due to PLA2 over activation is associated with nervous system dysfunction and cognitive impairment [30, 32, 34, 35] which might be attributed to the difference in ethnicity of the present study autistic participants.

A variety of cytokines such as IL-1, TNF- α and IFN γ have been shown to induce activation and increase synthesis of cPLA2 in diverse cell models [57]. In different studies on autistic patients, there were elevated level of those cytokines in autistic children compared to healthy controls which might explain the elevated level of cPLA2 in autistic children compared to control, reported in the present study [58]. In addition, increasing cytokine levels were associated with more impaired aberrant behaviors [59], and this could support the obtained correlation between elevated cPLA2 activity and SSP as measure of sensory processing dysfunction. It is well documented that cPLA2 is involved in injury of primary sensory neurons and pain behavior in the peripheral nervous system and that its inhibition decreases the levels of injurious lipid mediators, reduce pain [60, 61]. Again this can

support the obtained correlation between cPLA2 and SSP (Table 1).

Oxidant compounds such as H₂O₂ also led to activation of cPLA2, which in turn alters membrane molecular order and cytoskeletal arrangements. H₂O₂ causes astrocyte membranes to become more gel-like and induces actin polymerization and, subsequently, enhances formation of cytonemes and cell-to-cell connections, which can affect glutamate transporter and thus induce glutamate excitotoxicity [62]. Zhu et al. [63] suggested that oxidative stress may have an important impact on astrocyte membranes, signaling pathways and cytoskeletal arrangements. It is worth noting that under stimulated conditions, AA released by cPLA2 activity is a good target for lipid peroxidation, as lipid peroxides contribute to the increased oxidative pool in the cells [64]. These studies can be supported by Al-Gadani et al. [50] who suggested that autistic children are under H₂O₂ stress due to GSH depletion.

Collectively, the previous mentioned studies give strong evidence for the role of cPLA2 in oxidative and inflammatory signaling pathways and provide evidence for its link in the pathogenesis of ASD, and also activation of cytosolic PLA2 (cPLA2) has been shown to

Table 2 ROC curves of PE (mmole/l), PS (mmole/l), PC (mmole/l), cPLA2 (ng/ml) in autistic groups

Parameters	Patients with autism	CARS		SRS		SSP		
		Mild to moderate	Severe	Mild to moderate	Severe	Mild to moderate	Severe	
PE (mmole/l)	AUC	0.985	0.971	1.000	1.000	0.985	1.000	1.000
	Best cutoff value	0.043	0.043	0.042	0.043	0.039	0.041	0.043
	Sensitivity%	97.5	95	100	92.9	100	100	100
	Specificity%	100	100	100	100	100	100	100
PS (mmole/l)	AUC	0.997	1.000	0.994	1.000	0.991	0.994	1.000
	Best cutoff value	0.061	0.061	0.059	0.059	0.061	0.059	0.061
	Sensitivity%	97.5	100.0	95.0	100.0	92.9	95.2	100.0
	Specificity%	100.0	100.0	100.0	100.0	100.0	100.0	100.0
PC (mmole/l)	AUC	0.996	0.995	0.996	0.996	0.995	0.996	0.993
	Best cutoff value	1.447	1.447	1.422	1.447	1.419	1.422	1.447
	Sensitivity%	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Specificity%	97.4	97.4	97.4	97.4	97.4	97.4	97.4
cPLA2 (ng/ml)	AUC	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Best cutoff value	1.114	1.114	1.332	1.114	1.149	1.149	1.114
	Sensitivity%	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Specificity%	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Demonstrates the ROC analysis data as AUC, cutoff values, specificity, and sensitivity of the measured parameters. All parameters exhibited AUC values close to 1 and satisfactory values of accuracy presented as high specificity and sensitivity

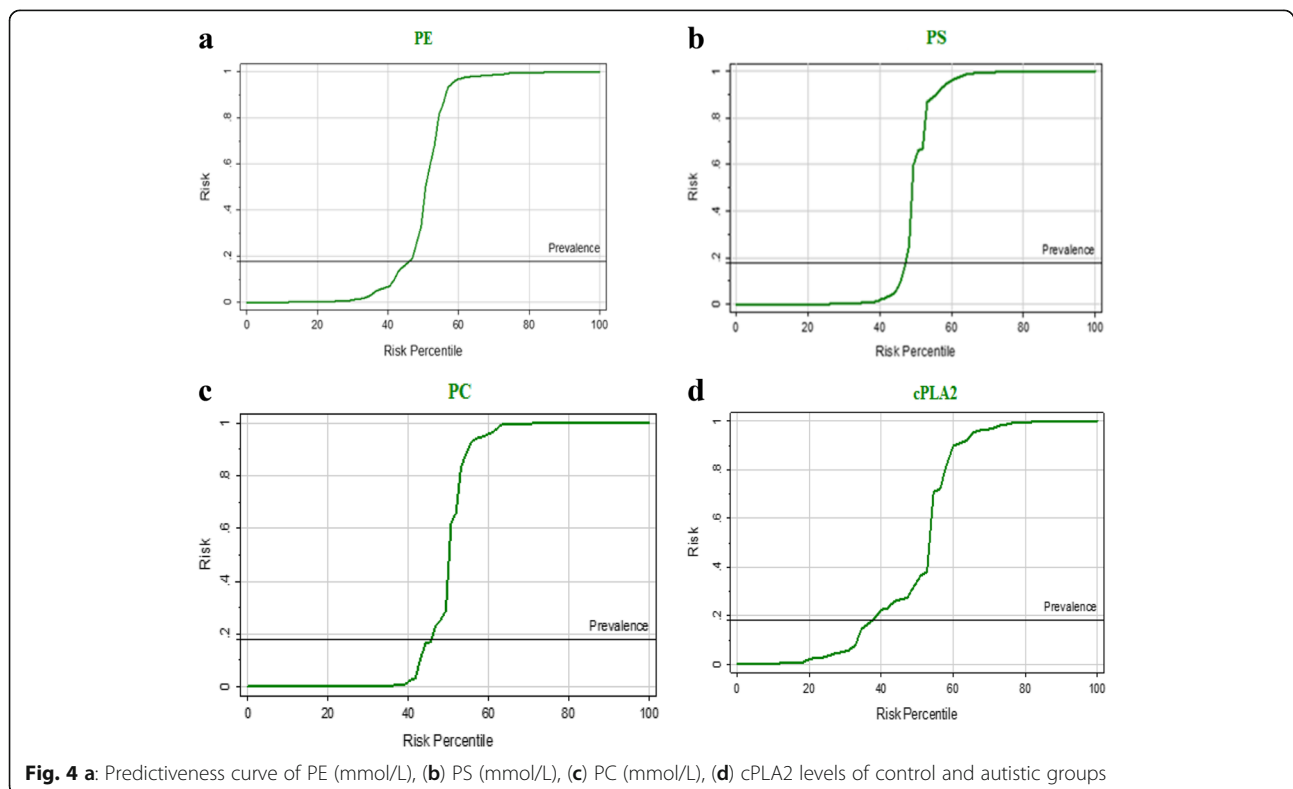


Table 3 Pearson’s correlations between age, CARS, SRS, SSP, and the four measured parameters

Parameters	R (Person Correlation)	Sig.	
SSP ~ Age	0.296*	0.043	P ^a
SSP ~ cPLA2	-0.319*	0.037	N ^b
cPLA2 ~ PE	-0.731**	0.001	N ^b
cPLA2 ~ PS	-0.757**	0.001	N ^b
cPLA2 ~ PC	-0.741**	0.001	N ^b
PE ~ PS	0.778**	0.001	P ^a
PE ~ PC	0.692**	0.001	P ^a
PS ~ PC	0.679**	0.001	P ^a

**Correlation is significant at $P < 0.01$ level, *Correlation is significant at $P < 0.05$ level

^aPositive Correlation, ^bNegative Correlation

produce lipoxidative toxicity, leading to inflammation and pain.

Administration of PS containing Omega3 long-chain polyunsaturated fatty acids to children with attention-deficit/hyperactivity disorder (ADHD) symptoms have been shown to reduce hyperactivity, attention deficits and behavior dysregulations as main characteristics of autism phenotype. Based on this, we can suggest that in spite of the absence of correlation between PS depletion and severity of autism, in the present study, PS supplementation can be used as treatment strategy [65].

The interesting positive correlation between SSP as useful scale of sensory abnormalities and age can be related to the remarkable improvement of autistic patients near adulthood (Table 3). This suggestion can find support in two previous studies that proved the improvement of majority of autistic patient’s phenotype during the transition to adulthood [66, 67].

The predictiveness curves of the four measured parameters (Fig. 4a–d), varies significantly from the baseline risk depending on whether PE, PS, PC, and cPLA2 concentrations were low or very high. So, these parameters can be used as predictive biomarkers in autism field. This is supported by the high sensitivity and specificity recorded through ROC analysis (Table 2).

Conclusion

In the present study, the decreased in phospholipid levels together with increased cPLA2 in children with autism and the correlation between cPLA2 levels and sensory abnormalities offer a potential new target for understanding the mechanisms involved in the pathogenicity of autism. The explained relationship between impaired phospholipid through the activation of cPLA2, oxidative stress, and neuroinflammation can be illustrated in Fig. 5.

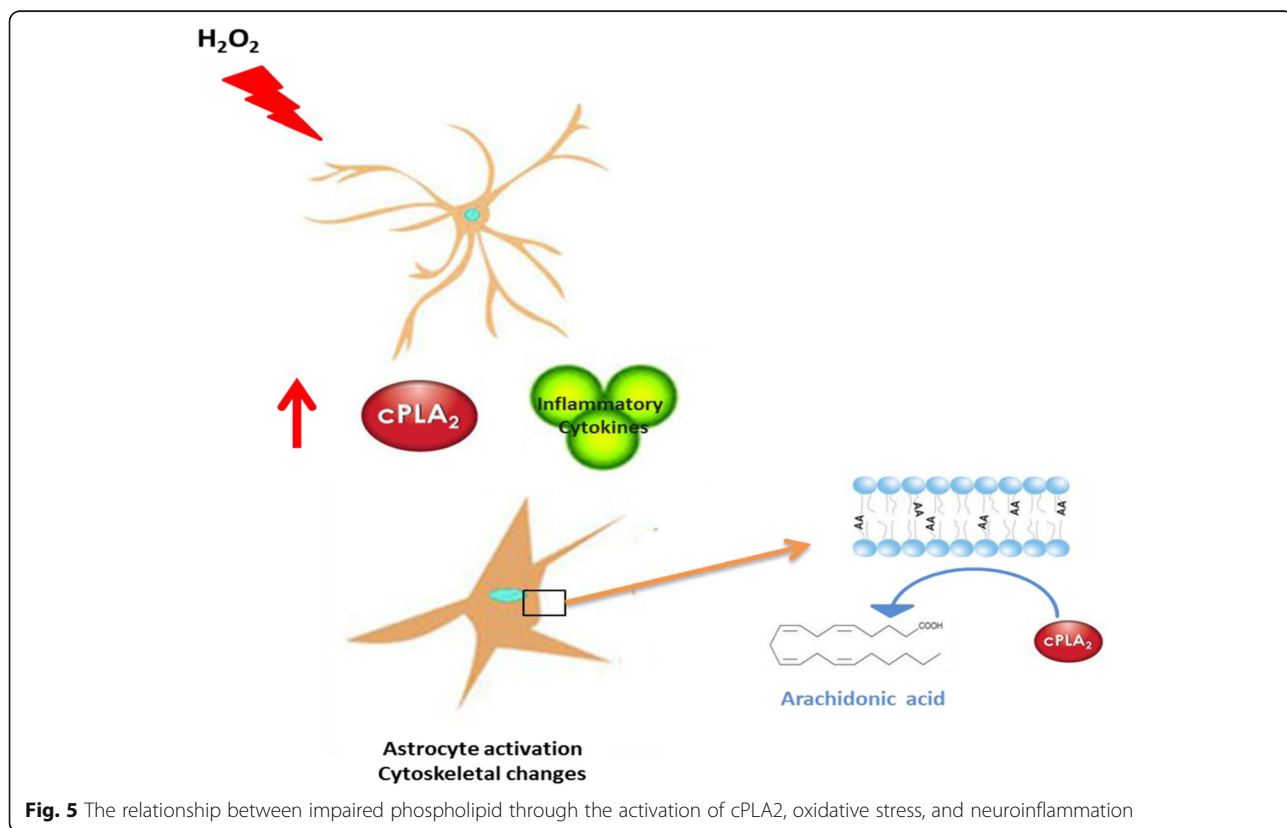


Fig. 5 The relationship between impaired phospholipid through the activation of cPLA2, oxidative stress, and neuroinflammation

Abbreviations

AA: Arachidonic acid; ADI-R: Autism diagnostic interview-revised; ADOS: Autism diagnostic observation schedule; CARS: Childhood autism rating scale; cPLA2: Cytosolic Phospholipase A2; GSH: Glutathione; IFN γ : Interferon gamma; IL-6: Interleukin-6; NMDA: N-methyl-D-aspartate; PC: Phosphatidyl choline; PE: Phosphatidyl ethanolamine; PS: Phosphatidyl serine; PUFAs: Polyunsaturated fatty acids; ROC-curve: Receiver operating characteristics curve; ROS: Reactive oxygen species; SOD: Superoxide dismutase; SRS: Social responsiveness scale; SSP: Short sensory profile; TNF- α : Tumor necrosis factor alpha

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Availability of data and materials

The data will not be shared because autistic patients from Autism Research and Treatment Centre, College of Medicine, King Saud University, did not give consent to the public release of their data but only to participate in the present study.

Authors' contributions

HQ: Performed the practical part and drafted the manuscript, LA: Provided the samples and participated in the diagnosis of the autistic participants. HA: Performed the statistical analysis and revised the manuscript. AE: suggested the work and co-drafted the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All subjects enrolled in the study had approved the participation in the study and filled informed consent which was signed by their parents.

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