Neurotoxicology 71 (2019) 1–5

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Full Length Article

Increased serum levels of macrophage migration inhibitory factor in autism spectrum disorders

Jun Ning[1], Li Xu[1], Chang-Qing Shen*, Yu-Yan Zhang, Qing Zhao

Department of pediatrics, Affiliated Hospital of Jining Medical University, Jining, China

ARTICLE INFO

Keywords:
Macrophage migration inhibitory factor
Autism spectrum disorder
Association

ABSTRACT

Objective: Macrophage migration inhibitory factor (MIF) has been suggested as a pivotal regulator of innate immunity and inflammatory. The aim of this study was to measure serum circulating levels of MIF in relation to the degree of the severity of autism spectrum disorders (ASD).

Methods: One hundred and two Chinese children with ASD and same their age-sex matched typical development children were included. Concentrations of MIF were tested by Quantikine Human MIF Immunoassay. Serum levels of homocysteine (HCY), C-reactive protein (CRP) and serum Interleukin 6 (IL-6) were also tested. The influence of serum levels of MIF on ASD risk and ASD severity were performed by binary logistic regression analysis.

Results: The serum levels of MIF in the children with ASD (24.7 ± 08.9 ng/ml) were significantly higher than those of control subjects (18.3 ± 5.5 ng/ml) ($t = 6.134$, $P < 0.001$). Levels of MIF increased with increasing severity of ASD as defined by the CARS score ($P < 0.001$). In multivariate model, MIF was associated with an increased risk of ASD (OR 1.11, 95% CI: 1.05–1.17; $P < 0.001$). MIF improved the combined model (HCY/CRP/IL-6) to predict ASD ($P < 0.001$). At admission, 68 children (66.7%) had a severe autism. In these children, the mean serum level of MIF was higher than in those children with mild to moderate autism (28.1 ± 8.5 ng/ml VS. 17.9 ± 4.7 ng/ml; $t = 6.482$, $P < 0.001$). In multivariate model, MIF was still associated with an increased risk of severe ASD (OR: 1.15, 95% CI: 1.04–1.19; $P < 0.001$). MIF improved the combined model (HCY/CRP/IL-6) to predict severe ASD ($P < 0.001$).

Conclusions: These results identify high serum MIF levels are associated with severity of ASD. Further study is warranted on the precise involvement of MIF in ASD, and the mechanism by which MIF contributes to ASD pathogenesis.

1. Introduction

Macrophage migration inhibitory factor (MIF) has been suggested as the physiologic counter-regulator of glucocorticoid action within the immune system (Baugh and Bucala, 2002). In recent studies, MIF has proposed a role as a pivotal regulator of innate immunity (Calandra and Roger, 2003). It is an important mediator of the innate immune response with potential roles in the pathophysiology of inflammatory (Flaster et al., 2007), autoimmune (Assis et al., 2014), cancer (Nobre et al., 2017) and cardiovascular disease (Zernecke et al., 2008).

Autism-spectrum disorders (ASD) are neurodevelopmental disorders whose etiologies were unknown even though the gene–gene and gene–environment interactions role had been suggested (Tordjman et al., 2014). Recently, an increasing prevalence of ASD has been reported worldwide, wherein 1 in 68 children is diagnosed with ASD (Anon., 2014). A recently study reported that the prevalence of ASD in 3-year-old Chinese children was 1.11% (Wu et al., 2018).

Previous studies had identified a role for MIF in the physiology of the central nervous system (CNS) due to its role in the immune and inflammatory responses (Leyton-Jaimes et al., 2018). The role of MIF in ASD appears to be adversary effects (Leyton-Jaimes et al., 2018). MIF roles may either be beneficial such as in the case of neurodevelopment, or adversary such as in the case of spinal cord injury (SCI), tumirogenesis, Alzheimer's disease (AD) and ASD (Leyton-Jaimes et al., 2018). The neuroimmunopathogenic responses play a fundamental role in ASD had been suggested (Zimmerman et al., 2005), while some studies reported that innate rather than adaptive neuroimmune responses are associated with ASD (Pardo et al., 2005). Thus, we

* Corresponding author at: No. 79 Guhuai Road, Jining, 272029, Shandong Province, PR China.
E-mail address: Tujykkthu@126.com (C.-Q. Shen).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.neuro.2018.11.015
Received 16 April 2018; Received in revised form 30 August 2018; Accepted 28 November 2018
Available online 29 November 2018
0161-813X/

proposed that MIF, a mediator of the innate immune response might play a fundamental role in ASD. Interestingly, (Grigorenko et al. (2008)) identified MIF as a susceptibility gene for ASD. Comparably little, however, is known about MIF blood levels in Chinese children with ASD. This study aims to investigate whether serum circulating levels of MIF in children with ASD (i) were higher than in control or (ii) were associated with severity of autistic symptomatology.

2. Methods

In this study, one hundred and two Chinese children with ASD and same their age-sex matched typical development children were included. From March 2017 to March 2018, consecutive children with ASD admitted to our hospital were identified. Our hospital was an autism treatment center serving the city. The ASD diagnosis were made by two developmental pediatricians based on the Diagnostic and Statistical Manual of Mental Disorders (First, 2013), 5th Edition. All the included ASD should be newly diagnosed, drug-naïve, and exhibited symptoms within the typical triad of autistic traits. The included children with ASD stick to DSM-5 criteria for ASD (which includes Asperger's).

The typical development children from a kindergarten near the hospital were assigned to as the controls. In order to exclude the possibility that the controls could have any sub-clinical autistic features, all control subjects were also clinically examined by the above two pediatricians. The exclusion criteria of the included children were (1) other congenital diseases(N = 2), or an acute or chronic infectious disease during the previous three months(N = 4); (2) any food or drug allergy(N = 3); (3) use of any medication to address behavior/focus/attention during the previous six months(N = 1); and (4) known genetic disease(N = 1). The protocol and informed consent for this study were reviewed and approved by the Institutional Review Board of the Affiliated Hospital of Jining Medical University. The details of the study were explained to the parents of the participating children, and written informed consents were obtained from the parents.

At admission, demographic data (age and sex), body mass index (BMI, kg/m2), family history of ASD and time from ASD onset to admission were obtained. The severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS) score using the Chinese version (Zhang et al., 2014) (The CARS comprise 15 items, and a scale from 1 to 4 in each of 15 areas). The severity of ASD was divided into two groups according to CARS scores. Mild-to-moderate autism was defined as a total score of 30–37, whereas more than 37 denotes severe autism (Esnafoglu and Ayyıldız, 2017).

Fasting serum samples were collected at first morning at 8:00 for measurement of MIF. Concentrations of MIF were tested by Quantikine Human MIF Immunoassay using a commercially available ELISA kit (Catalog Number DMF00B; R&D Systems, Inc. Minneapolis, USA). The minimum detectable dose (MDD) of human MIF ranged was 0.068 ng/ml. The measuring range of the MIF is between 0.2 ng/ml and 10 ng/ml (defined by the lower detection limit and the maximum of the master curve). Serum and platelet-poor plasma samples require a 10-fold dilution. Thus, the test range of the MIF is between 2 ng/ml and 100 g/ml. The coefficients of variation (CV) for the intra-and inter-assay reproducibility were 4.5%–6.0% and 8.4–9.8%, respectively. In addition, serum levels of homocysteine (HCY), C-reactive protein (CRP) were also tested using an enzyme cycling method by OLYMPUS AU2700 (OLYMPUS, Tokyo, Japan), and serum Interleukin 6 (IL-6) was also tested by ELISA method.

3. Statistical analysis

All statistical analysis was performed with SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA), and R version 2.8.1. Results are expressed as percentages for categorical variables and as mean (standard deviation, SD) for the continuous variables. Student's paired *t*-test was used to compare the values in control and autistic children. Correlations among continuous variables were assessed by the Pearson rank-correlation coefficient.

The influence of serum levels of MIF on ASD risk and ASD severity were performed by univariate and multivariate logistic regression analysis. In the multivariate logistic regression analysis, confounding factors including age, sex, BMI, the time from symptom onset to admission and serum levels of HCY, IL-6, and CRP were adjusted. The results are expressed as adjusted odds ratios (ORs) with the corresponding 95% confidence intervals (CIs).

Receiver operating characteristic (ROC) curves were utilized to evaluate the accuracy of serum MIF and other biomarkers to diagnose ASD risk and severity. Area under the curve (AUC) was calculated as measurements of the accuracy of the test. The combined model I (HCY/CRP/IL-6) and combined model II (HCY/CRP/IL-6/IMF) were assessed in the ROC curves. To test whether the MIF level improves score performance, we considered the two logistic regression models with combined model I and combined model II. Under the lower-dimensional sub-model, the difference in deviance between the two models has a X2 distribution with 1 ° of freedom. Furthermore, care was taken to adjust for the optimistic bias of in-sample prediction error estimates using a fivefold cross-validation scheme. Previous studies found that some inflammatory markers (CRP and IL-6) (Yang et al., 2015; Brown et al., 2014) and homocysteine (Tu et al., 2013) were associated with autism risk and severity. Thus, in this study, we included those biomarkers in the logistic regression analysis model and adjusted them. In addition, we also compared those factors with MIF in the ROC curves and the discriminatory ability was tested by AUC. Statistical significance was defined as $p < 0.05$.

4. Results

Characteristics of the ASD and controls were presented in Table 1. The mean CARS score on admission was 42.3points (SD: 7.0). The mean time from symptom onset to admission was 139 days (SD: 75). In those children, 30.4% (n = 31) had onset age of autism symptoms in the first 3 months, while 26.5% (n = 27) were more than 6 months. There were no siblings in the ASD group. All autistic subjects were receiving special treatment.

Table 1
Characteristics of the ASD and control cases.

Variable	ASD(N = 102)	Control cases(N = 102)	p-value[‡]
Sex(female/male)	22/80	22/80	—
Age (years, Mean ± SD)	4.5(1.3)	4.5(1.3)	—
BMI (kg/m2, Mean ± SD)	16.6(1.7)	17.4(1.9)	0.12
The time from symptom onset to admission (days, Mean ± SD)	139(75)	—	—
Family history of ASD, n (%)	11(10.8)	1(0.98)	< 0.001
CARS (Mean ± SD)	42.3(7.0)	21.9(4.3)	< 0.001
Mild to moderate autism[#], N (%)	34(33.3)	—	—
Laboratory findings, Mean ± SD			
MIF (ng/ml,)	24.7(8.9)	18.3(5.5)	< 0.001
HCY(μmol/l)	13.2(4.5)	10.5(3.1)	< 0.001
CRP(mg/dl)	6.1(1.6)	5.0(1.3)	< 0.001
IL-6(pg/ml)	7.2(1.6)	5.9(1.4)	< 0.001

Data are presented as mean ± standard deviation (SD) or numbers (%).
ASD: Autism spectrum disorders; BMI, body mass index; CARS, Childhood Autism Rating Scale; HCY, homocysteine; CRP; C-reactive protein; IL-6; Interleukin 6; MIF, Macrophage migration inhibitory factor.
[‡] P value was tested by Student's paired *t*-test.
[#] A total score of between 30 and 36 indicates mild-to-moderate autism, whereas the interval between 37 and 60 denotes severe autism.

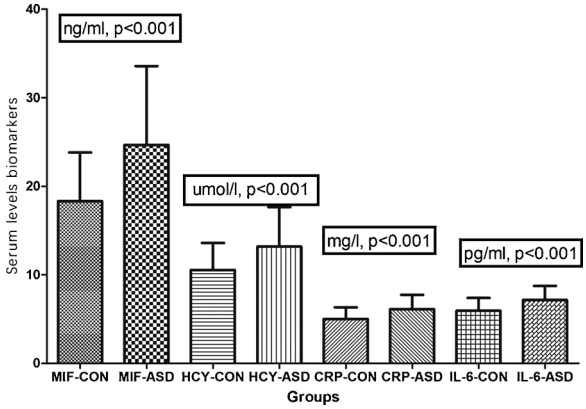

Fig. 1. Distribution of serum biomarkers in children with ASD and controls. All data are mean and standard deviation (SD). P values refer to Student's paired *t*-test for differences between groups. ASD: Autism spectrum disorders; HCY, homocysteine; CRP; C-reactive protein; IL-6; Interleukin 6; MIF, Macrophage migration inhibitory factor.

The serum levels of MIF in the children with ASD (24.7 ± 08.9 ng/ml) were significantly higher than those of control subjects (18.3 ± 5.5 ng/ml) (t = 6.134, P < 0.001; Table 1 and Fig. 1). In addition, serum levels of HCY, CRP and IL-6 were also higher in autistic children than in controls (P < 0.05; Table 1 and Fig. 1). Levels of MIF increased with increasing severity of ASD as defined by the CARS score. A statistically significant positive correlation was found between the serum levels of MIF and the CARS score indicating severity of autism (r = 0.496; P < 0.001). Furthermore, there was a modest positive correlation between levels of MIF and CRP (r = 0.264; P = 0.002), IL-6(r = 0.302; P < 0.001) and HCY (r = 0.241; P = 0.012). No statistically significant correlations between MIF values and age of diagnosis (P = 0.33), sex (P = 0.15) and BMI (P = 0.17) were found.

In the univariate model matching for controls, MIF as a continuous variable was associated with an increased risk of ASD (OR 1.23, 95% CI:1.15-1.35; P < 0.001). In multivariate model, MIF was still associated with an increased risk of ASD (OR 1.11, 95% CI: 1.05–1.17; P < 0.001) even after adjusted for BMI, blood levels of HCY, Hs-CRP and IL-6, Table 2. Based on the ROC curve, the optimal cutoff value of serum MIF levels as an indicator for auxiliary diagnosis of ASD was

Table 2
Univariate and Multivariate logistic regression analysis for ASD risk and severity.

Parameter	Univariate Analysis		Multivariate Analysis [a]	
	OR (95%CI)	P	OR (95%CI)	P
ASD risk [#]				
MIF	1.23(1.15–1.35)	< 0.001	1.11(1.05–1.17)	< 0.001
HCY	1.26(1.07–1.48)	0.005	1.14(1.03–1.25)	0.012
CRP	1.69(1.10–2.18)	< 0.001	1.51(1.19–1.93)	0.001
IL-6	1.82(1.20–2.44)	< 0.001	1.63(1.28–2.06)	< 0.001
ASD severe severity [‡]				
MIF	1.24(1.13–1.37)	< 0.001	1.15(1.04–1.19)	< 0.001
HCY	1.18(1.06–1.32)	0.003	1.12(1.02–1.26)	0.025
CRP	1.39(1.05–1.84)	0.020	1.29(0.99–1.83)	0.053
IL-6	1.95(1.10–2.89)	0.004	1.72(1.14–2.60)	0.010

ASD: Autism spectrum disorders; BMI, body mass index; CARS, Childhood Autism Rating Scale; HCY, homocysteine; CRP; C-reactive protein; IL-6; Interleukin 6; MIF, Macrophage migration inhibitory factor.

[#] Adjusted for, BMI, Family history of ASD, serum levels of MIF, HCY, CRP and IL-6.

[‡] Adjusted for, age, sex, BMI, family history of ASD, the time from symptom onset to admission, serum levels of MIF, HCY, CRP and IL-6; A total score of between 30 and 36 indicates mild-to-moderate autism, whereas the interval between 37 and 60 denotes severe autism.

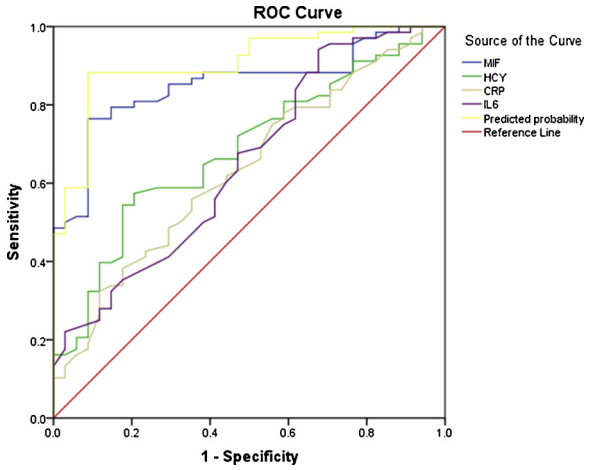

Fig. 2. Receiver operator characteristic curve demonstrating sensitivity as a function of 1-specificity for predicting the ASD based on the model incorporating all 4 biomarkers and the relative contribution of each biomarker alone (initial cohort). Combined model included HCY/IL-6/CRP/MIF. ASD: Autism spectrum disorders; HCY, homocysteine; CRP; C-reactive protein; IL-6; Interleukin 6; MIF, Macrophage migration inhibitory factor.

projected to be 27.3 ng/ml, which yielded a sensitivity of 40.3% and a specificity of 95.1% (Fig. 2), with the area under the curve at 0.71 (95%CI, 0.64-0.78; P < 0.001). With an AUC of 0.71, MIF showed a significantly greater discriminatory ability as compared with HCY (P = 0.03), while was in the range of CRP (P = 0.075) and IL-6(P = 0.69), Table 3 and Fig. 2. Interestingly, MIF improved the combined model I to predict ASD (AUC of the combined model I, 0.79; 95% CI, 0.74–0.83; AUC of the combined model II, 0.84; 95% CI, 0.78–0.89). This improvement was stable in an internal 5-fold cross validation that resulted in an average AUC (standard error) of 0.79 (0.032) for the combined model I and 0.84 (0.028) for the combined model II, corresponding to a difference of 0.05(0.004, P = 0.009), Table 3. The 5-fold cross-validated mean squared prediction error decreased from 0.185 (0.011) in the model I to 0.176 (0.012) in the model II, corresponding to an average decrease of 0.009 (0.009).

At admission, 68 children (66.7%) had a severe autism (CARS > 36). In these children, the mean serum level of MIF was higher than in those children with mild to moderate autism (28.1 ± 8.5 ng/ml VS. 17.9 ± 4.7 ng/ml; t = 6.482, P < 0.001; Fig. 3 and Table 4). Similarly, serum levels of HCY, CRP and IL-6 were also higher in severe autism than in mild to moderate autism

Table 3
Prediction of ASD risk and severity.

Parameter	AUC	
	OR (95%CI)	P
ASD risk		
MIF	0.71(0.64–0.78)	—
HCY	0.66(0.59–0.74)	0.03
CRP	0.70(0.63–0.77)	0.75
IL-6	0.72(0.65–0.79)	0.69
Combined model I (HCY/CRP/IL-6)	0.79(0.74–0.83)	< 0.001
Combined model II (MIF/HCY/CRP/IL-6)	0.84(0.78–0.89)	< 0.001
ASD severe severity		
MIF	0.85(0.78–0.93)	
HCY	0.68(0.58–0.79)	< 0.001
CRP	0.64(0.52–0.75)	< 0.001
IL-6	0.65(0.54–0.77)	< 0.001
Combined model I (HCY/CRP/IL-6)	0.73(0.66–0.79)	< 0.001
Combined model II (MIF/HCY/CRP/IL-6)	0.91(0.85–0.97)	0.002

ASD: Autism spectrum disorders; HCY, homocysteine; CRP; C-reactive protein; IL-6; Interleukin 6; MIF, Macrophage migration inhibitory factor.

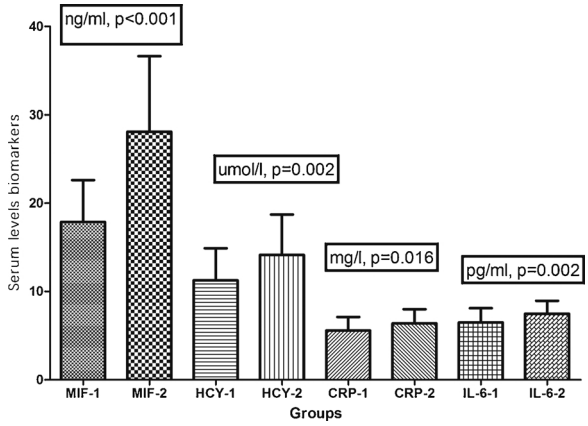

Fig. 3. Distribution of serum biomarkers in mild-to-moderate ASD and severe ASD. All data are mean and standard deviation (SD). P values refer to Student's paired *t*-test for differences between groups. A total score of between 30 and 36 indicates mild-to-moderate autism, whereas the interval between 37 and 60 denotes severe autism. 1= mild-to-moderate ASD(n = 34); 2= severe ASD (n = 68). ASD: Autism spectrum disorders; HCY, homocysteine; CRP; C-reactive protein; IL-6; Interleukin 6; MIF, Macrophage migration inhibitory factor.

Table 4
Characteristics of the ASD with mild-to-moderate severity and severe severity[#].

Variable	Moderate (N = 34)	Severe cases(N = 68)	p-value [‡]
Sex (Male/female)	5/29	17/51	0.24
Age (years, Mean ± SD)	4.6(1.3)	4.5(1.2)	0.85
BMI (kg/m2, Mean ± SD)	17.2(1.8)	16.3(1.4)	0.22
The time from symptom onset to admission (days, Mean ± SD)	150(100)	124(59)	0.11
CARS (Mean ± SD)	36.4(5.6)	45.3(5.6)	< 0.001
Laboratory findings, Mean ± SD			
MIF (ng/ml)	17.9(4.7)	28.1(8.5)	< 0.001
HCY(μmol/l)	11.3(3.6)	14.1(4.6)	0.002
CRP(mg/dl)	5.6(1.5)	6.4(1.6)	0.016
IL-6(pg/ml)	6.5(1.6)	7.5(1.5)	0.002

Data are presented as mean ± standard deviation (SD) or numbers (%).
ASD: Autism spectrum disorders; BMI, body mass index; CARS, Childhood Autism Rating Scale; HCY, homocysteine; CRP; C-reactive protein; IL-6; Interleukin 6; MIF, Macrophage migration inhibitory factor.

[#] A total score of between 30 and 36 indicates mild-to-moderate autism, whereas the interval between 37 and 60 denotes severe autism.

[‡] P value was tested by Student's paired *t*-test or x2 test.

(P < 0.05; Table 4 and Fig. 3). In the univariate model, MIF as a continuous variable was associated with an increased risk of severe ASD (OR: 1.24, 95% CI: 1.13–1.37; P < 0.001). In multivariate model, MIF was still associated with an increased risk of severe ASD (OR: 1.15, 95% CI: 1.04–1.19; P < 0.001) even after adjusted for age, sex, BMI, family history of ASD, the time from symptom onset to admission, blood levels of HCY, Hs-CRP and IL-6.

Based on the ROC curve, the optimal cutoff value of serum level of MIF as an indicator for auxiliary diagnosis of severe ASD was projected to be 22.2 ng/ml, which yielded a sensitivity of 76.5% and a specificity of 91.2% (Fig. 4), with the area under the curve at 0.85 (95%CI, 0.78-0.93; P < 0.001). With an AUC of 0.85, MIF showed a significantly greater discriminatory ability as compared with HCY (P < 0.001), CRP (P < 0.001) and IL-6(P < 0.001), Table 3 and Fig. 4. Interestingly, MIF improved the combined model I to predict ASD severity (AUC of the combined model I, 0.73; 95% CI, 0.66–0.79; AUC of the combined model II, 0.91; 95% CI, 0.85–0.97). This improvement was stable in an internal 5-fold cross validation that resulted in an average AUC (standard error) of 0.73 (0.044) for the combined model I and 0.91 (0.030)

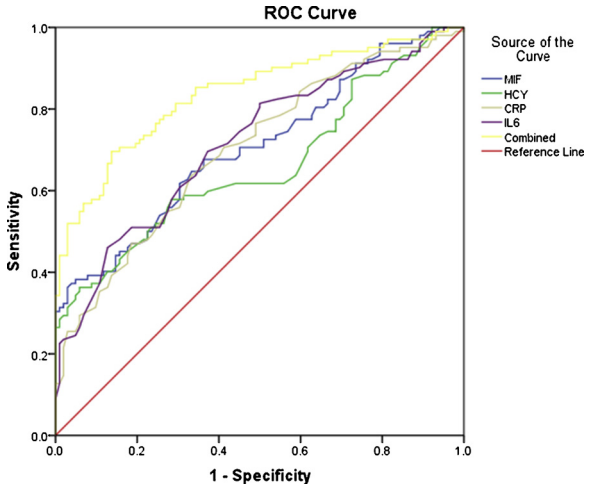

Fig. 4. Receiver operator characteristic curve demonstrating sensitivity as a function of 1-specificity for predicting the severe ASD based on the model incorporating all 4 biomarkers and the relative contribution of each biomarker alone (initial cohort). Combined model included HCY/IL-6/CRP/MIF. A total score of between 30 and 36 indicates mild-to-moderate autism, whereas the interval between 37 and 60 denotes severe autism. ASD: Autism spectrum disorders; HCY, homocysteine; CRP; C-reactive protein; IL-6; Interleukin 6; MIF, Macrophage migration inhibitory factor.

for the combined model II, corresponding to a difference of 0.14(0.014, P < 0.001), Table 3. The 5-fold cross-validated mean squared prediction error decreased from 0.198 (0.013) in the model I to 0.183 (0.012) in the model II, corresponding to an average decrease of 0.016 (0.003).

5. Discussion

MIF mediates both acute and chronic inflammatory responses (Twu et al., 2014), and it has potent paracrine and autocrine stimulatory effects on cell growth and survival and enhances the production of inflammatory cytokines (Funamizu et al., 2013). A previous study found that probands with autism spectrum disorder exhibited higher circulating MIF levels than did their unaffected siblings (Grigorenko et al., 2008). In this study, we found that elevated serum level of MIF was associated with higher risk of ASD and severe ASD in a Chinese cohort. These associations persisted after multivariate adjustment for traditional risk factors. To our knowledge, this was the first study to evaluate the relationship of MIF, a mediator of the innate immune response, with risk of ASD and severe ASD in Chinese children. These data identified MIF as a potential ASD susceptibility risk factors and support the previous suggestions of a role for innate immunity in the etiopathogenesis of ASD.

Human serum from depressed patients showed significantly elevated MIF levels (Musil et al., 2011). In addition, serum MIF level was suggested to be a potential pharmacodynamic and/or monitoring marker of schizophrenia (Okazaki et al., 2018). However, the exact mechanism by which MIF elicits its function on depression is controversial (Moon et al., 2012). In this study, we found that ASD children had significantly elevated MIF levels. Additionally, higher circulating MIF levels are correlated with the severity of multiple ASD symptoms (Grigorenko et al., 2008). Our findings supported this conclusion.

ASD may be accompanied by an activation of the macrophages, and increased production of pro-inflammatory cytokines could play a role in the pathophysiology of ASD (Al-Ayadhi, 2005). MIF might contribute to the pathogenesis of ASD through regulates the expression of innate cytokines (Calandra and Roger, 2003). Pharmacologic inhibitors of MIF are presently in previous studies (Grigorenko et al., 2008), and therapies aimed specifically at MIF pathways in patients with ASD might be feasible.

There are several other mechanisms by which MIF might be associated with ASD. First, a strong relationship between polymorphisms within the MIF promoter and ASD related behavior has been observed (Grigorenko et al., 2008). Second, among the genes associated with ASD are mutations in CHD7 (Jiang et al., 2013). Interestingly, it has been suggested that expression of the CHD7 gene increases with the presence of MIF and that this augmentation is mediated by PAX6, known to be essential in early neurodevelopment of the mouse brain (Fukaya et al., 2016). These findings suggest that MIF might be involved in the early stages of neurodevelopment in cooperation with genes such as CHD7. Third, intricate association between MIF and the immune system of individuals suffering from ASD had been proposed. (Vargas et al. (2005)) have suggested that autistic phenotypes may have neuroglial and innate neuroimmune system activation in the brain and CSF. It is known that MIF is implied in immunomodulation processes (Calandra and Roger, 2003). Last, elevating MIF in neuronal cells inhibited the accumulation of misfolded superoxide dismutase (SOD) 1 and its association with the intracellular membranes and extended survival of mutant SOD1-expressing motor neurons (Israelson et al., 2015). (Wang et al. (2016)) shown that SOD play a role in the pathophysiology of ASD. Further study is warranted on the precise involvement of MIF in ASD, and the mechanism by which MIF contributes to ASD pathogenesis.

This study was accompanied by limitations. First, the number of children that were included in the study was rather low, and data from a single center. These initial findings will require further study in other samples of probands with ASD in order to determine their replicability. Second, in this study, serum concentration of MIF was measured only once at baseline of the study cohort. The fasting serum samples were collected at first morning at 8:00. Without serial measurement of the circulating MIF, this study yielded no data regarding when and how long MIF were changed in these patients. Interestingly, a previous study found that plasma MIF exhibits a significant circadian rhythm in normal human subjects, with a peak around 08:00 at a similar time to the peak of plasma cortisol (Petrovsky et al., 2003). Further study should be carried out to resolve this question. Third, polymorphisms within the MIF might play a role in the pathophysiology of ASD (Grigorenko et al., 2008). However, in this study we did not obtain this information. Lastly, our data do not imply any causal relationship between circulating MIF and development of ASD. In addition, no other human tissue except for peripheral blood was available.

6. Conclusions

These results identify high serum MIF levels are associated with severity of ASD. Further study is warranted on the precise involvement of MIF in ASD, and the mechanism by which MIF contributes to ASD pathogenesis. If this association is confirmed, therapies aimed specifically at MIF pathways in patients with ASD might be feasible.

Funding/Support

None.

Conflict of interest

All authors have no conflicts of interest to disclose.

Acknowledgement

We express our gratitude to all the children, the parents and physicians who participated in this study, and thereby made this work possible. We especially want to express our gratitude to those doctors who participated in the clinical data collection.

References

Al-Ayadhi, L.Y., 2005. Pro-inflammatory cytokines in autistic children in central Saudi Arabia [J]. Neurosciences 10 (2), 155–158.
Anon, 2014. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years – Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2010. Developmental Disabilities Monitoring Network Surveillance Year 2010. Principal Investigators: Centers for Disease Control and Prevention (CDC). MMWR Surveill Summ, Atlanta, pp. 63.
Assis, D.N., Leng, L., Du, X., et al., 2014. The role of macrophage migration inhibitory factor in autoimmune liver disease. Hepatology 59 (2), 580–591.
Baugh, J.A., Bucala, R., 2002. Macrophage migration inhibitory factor. Crit. Care Med. 30 (1), S27–S35.
Brown, A.S., Sourander, A., Hinkka-Yli-Salomäki, S., et al., 2014. Elevated maternal C-reactive protein and autism in a national birth cohort. Mol. Psychiatry 19 (2), 259.
Calandra, T., Roger, T., 2003. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat. Rev. Immunol. 3 (10), 791–800.
Esnafoglu, E., Ayyıldız, S.N., 2017. Decreased levels of serum fibroblast growth factor-2 in children with autism spectrum disorder. Psychiatry Res. 257, 79–83.
First, M.B., 2013. Diagnostic and statistical manual of mental disorders, 5th edition, and clinical utility. J. Nerv. Ment. Dis. 201, 727–729.
Flaster, H., Bernhagen, J., Calandra, T., et al., 2007. The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity. Mol. Endocrinol. 21 (6), 1267–1280.
Fukaya, R., Ohta, S., Yaguchi, T., Matsuzaki, Y., Sugihara, E., Okano, H., Saya, H., Kawakami, Y., Kawase, T., Yoshida, K., Toda, M., 2016. MIF maintains the tumorigenic capacity of brain tumor-initiating cells by directly inhibiting p53. Cancer Res. 76, 2813–2823.
Funamizu, N., Hu, C., Lacy, C., et al., 2013. Macrophage migration inhibitory factor induces epithelial to mesenchymal transition, enhances tumor aggressiveness and predicts clinical outcome in resected pancreatic ductal adenocarcinoma. Int. J. Cancer 132 (4), 785–794.
Grigorenko, E.L., Han, S.S., Yrigollen, C.M., et al., 2008. Macrophage migration inhibitory factor and autism spectrum disorders. Pediatrics 122 (2), e438–e445.
Israelson, A., Ditsworth, D., Sun, S., Song, S., Liang, J., Hruska-Plochan, M., McAlonisDownes, M., Abu-Hamad, S., Zoltsman, G., Shani, T., Maldonado, M., Bui, A., Navarro, M., Zhou, H., Marsala, M., Kaspar, B.K., Da Cruz, S., Cleveland, D.W., 2015. Macrophage migration inhibitory factor as a chaperone inhibiting accumulation of misfolded SOD1. Neuron 86, 218–232.
Jiang, Y.H., Yuen, R.K., Jin, X., Wang, M., Chen, N., Wu, X., Ju, J., Mei, J., Shi, Y., He, M., Wang, G., Liang, J., Wang, Z., Cao, D., Carter, M.T., Chrysler, C., Drmic, I.E., Howe, J.L., Lau, L., Marshall, C.R., Merico, D., Nalpathamkalam, T., Thiruvahindrapuram, B., Thompson, A., Uddin, M., Walker, S., Luo, J., Anagnostou, E., Zwaigenbaum, L., Ring, R.H., Wang, J., Lajonchere, C., Wang, J., Shih, A., Szatmari, P., Yang, H., Dawson, G., Li, Y., Scherer, S.W., 2013. Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. Am. J. Hum. Genet. 93, 249–263.
Leyton-Jaimes, M.F., Kahn, J., Israelson, A., 2018. Macrophage migration inhibitory factor: a multifaceted cytokine implicated in multiple neurological diseases. Exp. Neurol. 301, 83–91.
Moon, H.Y., Kim, S.H., Yang, Y.R., Song, P., Yu, H.S., Park, H.G., Hwang, O., Lee-Kwon, W., Seo, J.K., Hwang, D., Choi, J.H., Bucala, R., Ryu, S.H., Kim, Y.S., Suh, P.-G., 2012. Macrophage migration inhibitory factor mediates the antidepressant actions of voluntary exercise. Proc. Natl. Acad. Sci. U. S. A. 109, 13094–13099.
Musil, R., Schwarz, M.J., Riedel, M., Dehning, S., Cerovecki, A., Spellmann, I., Arolt, V., Müller, N., 2011. Elevated macrophage migration inhibitory factor and decreased transforming growth factor-beta levels in major depression — no influence of celecoxib treatment. J. Affect. Disord. 134, 217–225.
Nobre, C.C.G., de Araújo, J.M.G., de Medeiros Fernandes, T.A.A., et al., 2017. Macrophage migration inhibitory factor (MIF): biological activities and relation with cancer. Pathol. Oncol. Res. 23 (2), 235–244.
Okazaki, S., Hishimoto, A., Otsuka, I., et al., 2018. Increased serum levels and promoter polymorphisms of macrophage migration inhibitory factor in schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 83, 33–41.
Pardo, C.A., Vargas, D.L., Zimmerman, A.W., 2005. Immunity, neuroglia and neuroinflammation in autism. Int. Rev. Psychiatry 17, 485–495.
Petrovsky, N., Socha, L., Silva, D., et al., 2003. Macrophage migration inhibitory factor exhibits a pronounced circadian rhythm relevant to its role as a glucocorticoid counter-regulator. Immunol. Cell Biol. 81 (2), 137.
Tordjman, S., Somogyi, E., Coulon, N., et al., 2014. Gene× Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. Front. Psychiatry 5, 53.
Tu, W., Yin, C., Guo, Y., et al., 2013. Serum homocysteine concentrations in Chinese children with autism[J]. Clin. Chem. Lab. Med. 51 (2), e19–e22.
Twu, O., Dessí, D., Vu, A., et al., 2014. Trichomonas vaginalis homolog of macrophage migration inhibitory factor induces prostate cell growth, invasiveness, and inflammatory responses. Proc. Natl. Acad. Sci. 111 (22), 8179–8184.
Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann. Neurol. 57, 67–81.
Wang, L., Jia, J., Zhang, J., et al., 2016. Serum levels of SOD and risk of autism spectrum disorder: a case-control study. Int. J. Dev. Neurosci. 51, 12–16.
Wu, D.M., Wen, X., Han, X.R., et al., 2018. Relationship between neonatal vitamin d at birth and risk of autism spectrum disorders: the NBSIB study. J. Bone Miner. Res. 33, 458–466.
Yang, C.J., Liu, C.L., Sang, B., et al., 2015. The combined role of serotonin and interleukin-6 as biomarker for autism. Neuroscience 284, 290–296.
Zernecke, A., Bernhagen, J., Weber, C., 2008. Macrophage migration inhibitory factor in cardiovascular disease. Circulation 117 (12), 1594–1602.
Zhang, Q., Jiang, L., Lu, Y.J., 2014. Serum brain-derived neurotrophic factor levels in Chinese children with autism spectrum disorders: a pilot study[J]. Int. J. Dev. Neurosci. 37, 65–68.
Zimmerman, A.W., Jyonouchi, H., Comi, A.M., et al., 2005. Cerebrospinal fluid and serum markers of inflammation in autism. Pediatr. Neurol. 33, 195–201.