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Original Article

The Impact of Greenhouse Density on Cognitive Function in Primary School Children Using the WISC Method

Elham Mahdavian[1], Mohammad Hasan Ehrampoush[2], Sara Jambarsang[3], Fahimeh Teimouri[2*], Mohammad Javad Zare[4], Mohamad Nadi Sakhvidi[5]

[1]Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2]Environmental Sciences and Technology Research Center, Department of Environmental Health Engineering, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3]Center for Healthcare Data Modeling, Departments of Biostatistics and Epidemiology, School of public health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4]Occupational Health Research Center, Department of Occupational Health, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[5]Department of Psychiatry, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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***Corresponding author:**
Fahimeh Teimouri,
Email: f.teimouri@ssu.ac.ir

Abstract
Exposure to pesticides is associated with various health concerns and may also be related to impaired cognitive function. This study investigated the relationship between greenhouse density and cognitive function in primary school children using Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) and environmental sampling. This study was conducted on 128 children (6–9 years old) in Ebrahimabad village, Ashkezar, Yazd, Iran in 2019, and 10 students were excluded from the study after filling consent form. The WISC-IV measured children's cognitive function, and a flame ionization detector for gas chromatography (GC-FID) instrument was used to detect environmental exposure to pesticides. There was a significant inverse relationship between greenhouse density in children's homes and cognitive function scores with verbal thinking scores (*P* value = 0.003) and the total scale (*P* value = 0.0001) on the WISC. Further, the results showed that the obtained verbal thinking scores are significantly related to their fathers' education (*P* value = 0.008) and occupation (*P* value = 0.014). Moreover, the results of environmental exposure measurement confirmed the presence of malathion, ethion, chlorpyrifos, dieldrin, and oxadiazine with a maximum concentration of 183 μg/m^3 in response to malathion. The present study indicated that increasing the density of the greenhouse reduces children's cognitive functions. The results of ambient air analysis confirmed the environmental exposure to pesticides as well. Due to long-term chemical effects, management measures such as public education, substituting crop types, and the use of eco-friendly methods are unavoidable.
Keywords: Greenhouse density, Cognitive function, Environmental assessment, Pesticide exposure

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1. Introduction

Million tons of pesticides control various pests all around the world (1). Pesticides are a group of substances for destroying the population of multiple pests such as insects, weeds, pathogens, and the like (2). Modern pesticides are mostly organic chemicals and are classified into four main groups of organochlorines, organophosphates, carbamates, and pyrethroids (3). They are also used in most agricultural productions to prevent or reduce losses caused by pests. Pesticides can increase the nutritional value of food and sometimes maintain their safety (4).

The use of pesticides has significantly changed in the last decade. In addition to increasing the number of pesticides, farmers use other methods to control pests such as using more potent pesticides, increasing the number of pesticide programs, and increasingly combining several pesticides (5,6). Almost all these compounds cause poisoning in the nervous system of target organisms. Pesticides are not selective and can affect non-target organisms. In other words, they affect insect nerves, and simultaneously, they can have similar effects on human health. In this regard, the impact dosage, the level of contamination, and its duration determine the biological effects (7,8).

Both organophosphate and carbamate pesticides cause neurological effects by inhibiting acetylcholinesterase, which accumulates neurotransmitter acetylcholine and over-stimulates acetylcholine receptors. Pyrethroid pesticides are highly toxic, especially to insects and fish (9, 10). According to statistical reports, pesticide poisoning reaches more than five hundred thousand cases annually with twenty thousand deaths (11). It also influences human health through diseases such as carcinogenicity, neurological disorders, fertility disorders, immunology, and genotoxicity associated with dangerous pesticides (12). Some of the apparent benefits of pesticides for agricultural producers include increasing plant resistance to plant pests, improving the product quality, preserving beneficial insects and organisms, having flexibility toward product type, ability to combine with chemical compounds and toxins, lack of residues, investment return, sustainable technology, and added value at various levels from production to consumption (13,14).

Pesticides can be more harmful to children than to adults as children take in more air, food, and drink (per kilogram of body weight) compared to adults. One of the most important effects is their influence on the brain and its function (15). Pesticides may lead to learning disabilities, hyperactivity, attention deficit disorder, autism, stunted growth, and emotional and behavioral problems in children (16). Since children's nervous system grows and develops rapidly in the early years of life, they are much more likely to develop neurological disorders resulting from pesticide exposure (15). Soil, air, and water are not the only potentially dangerous ways through which pesticides enter children's bodies. Pesticides may also enter the body during pregnancy, through breastfeeding, and when working on farms; moreover, these chemicals can get into children's bodies through skin contact or inhalation of polluted air, depending on the type of pesticides (17).

Iran is one of the unique regions for greenhouse production due to its geographical latitude and climatic conditions. According to the Ministry of Agriculture Jihad statistics, the area under cultivation of greenhouse vegetables was about 1500 and 2000 hectares in 2001 and 2003, respectively. By the end of 2009, this area has reached 5,258 hectares, showing an increase of more than three times in 8 years. However, due to recent droughts and water shortages, the cultivation of these crops is possible with less water and is economically profitable. Greenhouse density means the accumulation and compactness of greenhouses, which plays a role in management decisions. The exposure index (previous greenhouse density) is a measure of proximity and turnover of the nearest greenhouse; therefore, it considers the proximity, area, and potential pesticide application in the greenhouse. Due to the greenhouse expansion in Iran, it is necessary to investigate the use of pesticides and their effects on humans and other living organisms' health (18). Similar studies have been conducted on the residential proximity to greenhouses and its effect on children's mental function, but the current study aimed to investigate the density of greenhouses on the mental function of children using the Wechsler Intelligence Scale for Children-Fourth Edition (WISC- IV). The primary purpose of this study was to examine and determine the relationship between greenhouse density and the cognitive function of children aged 6-9 years using the WISC and environmental exposure monitoring.

2. Methods

2.1. Study Area and Demographic Characteristics

This study was performed on children aged 6-9 years old (n = 128 children) in Ashkezar county, Yazd, Iran, in 2019. Two checklists were used to collect information on children, their families, and the greenhouse, and children's cognitive function was obtained using the WISC-IV.

To be included in this study, students need to be 6-9 years old, have at least one year of residence in this location, and have five healthy senses. Students with conditions such as congenital disease, taking a particular medicine, divorced parents, and parents' death were excluded from the study. At the beginning of the study, demographic and social characteristics (e.g., parents' occupations, parents' education, characteristics of the home, chemical compounds exposure, residence history, children's lifestyle, and medical history) were provided to the interviewer.

2.2. Cognitive Function Test

The WISC- IV developed by Kamkari was used to assess children's cognitive function. This scale is sensitive to diagnosing neuropsychological damage associated with exposure to low doses of pesticides.

The WISC-IV consists of 15 subtests indicating scores in verbal comprehension, perceptual reasoning, working memory, and processing speed areas. In addition, the total WISC includes verbal comprehension (i.e., vocabulary, similarities, and comprehension), working memory (i.e., digit span task and letter and digit ordering), perceptual reasoning (i.e., image concepts, block design, and matrix reasoning), and processing speed (i.e., coding, symbol, and animal search). Finally, the WISC's total score was obtained based on the four domains' scores (18, 19). As explained in the WISC-IV (18,19), standard intelligence quotient IQ scores are expressed as very excellent (> 130), perfect (120-129), above average (110-119), average (90-109), below average (80-89), boundary (70-79), and dimwitted (below 69).

2.3. Environmental Exposure Analysis

Ambient air samples were collected within a 50-meter radius from the greenhouse and analyzed based on the instruction method by the Environment Protection Authority. A single sampling pump of Gillian brand LFS 113 with a flow rate of 0.5 L/min was active for 53 minutes to perform the sampling, and pesticides were collected by passing the air deliberately. The sample's environmental conditions included airflow velocity of less than 0.2 m/s, a humidity of 36.1%, and an average dry ambient temperature of 32.7°C. Pesticides were trapped through the filter and adsorbed into a solid sorbent tube (OVS-

2 tube: 13 mm quartz filter; XAD-2, 270 mg/140 mg). Having transferred the samples to the laboratory, they were analyzed using a gas chromatographic instrument (Agilent 6890) equipped with a flame ionization detector (FID) as presented in Table 1 (20).

Finally, the concentration of pesticides was obtained using equation (1):

$$C\left(\frac{mg}{m^3}\right) = \frac{\left(W_f + W_b - B_f - B_b\right)}{V} \quad (1)$$

where W_f denotes respective analytic found in the sample front, W_b indicates back sorbent sections, B_f is media blank front, and B_b indicates back sorbent sections (21).

2.4. Greenhouse Density and Children's Residential Location

This study determined the location of the greenhouse and children's homes using a global positioning system device. Then, the coordinates of the designated points were entered into geographic information systems software (2020) and analyzed. The density of the greenhouse (22) was calculated by equation (2):

Greenhouse Density = Pesticide concentration × Number of cultivation months × number of pesticide spray-ing × (area/distance) (2)

2.5. Data Analysis

The data were analyzed using SPSS software version 24. The chi-square test, mean comparison, and the Mann-Whitney U test (for normal distribution) were used. The linear regression model was also employed to investigate the relationships between children's demographic characteristics and their families by the WISC. The significance level in all tests was set at 0.05.

3. Results and Discussion

This study was performed on primary school children aged 6-9 years old in Ashkezar, Yazd, Iran, in 2019 who constituted 128 children. After obtaining their parents' consent, 118 informed consent forms were signed and submitted by their parents or guardians.

3.1. Distance Distribution of Children's Homes and Greenhouse as Well as Demographic Characteristics

The present study investigated the mental function of children living in the Ebrahimabad Rustaq village in Ashkezar county located in Yazd province, Iran. One of the most important reasons for choosing this area was the density of greenhouses, their number, and their proximity to residential areas. It should be noted that this village is located 35 km north of Yazd. Fig. 1 shows the distribution of children's homes and neighborhood greenhouses.

Characteristics of greenhouse (area, the number of pesticides, and others) are presented in Table 2.

As observed in Table 2, the average area of the greenhouse is 28 700 m^2. Cucumber and tomato are the main greenhouse products, and pyrethroid and organophosphate pesticides are used more frequently.

The results of demographic characteristics of this region indicate that 70% of the residential homes are more than 500 m away from the greenhouse location (Fig. 1). There is also a strong tendency to overuse pesticides in this area, so the average chemical consumption is 125-1300 mL/spray of pesticide, and in each crop cultivation period (twice a year), the number of consumed pesticides is estimated to be between 4-70.2 L. Correspondingly, the highest frequency in this area is related to organophosphate and pyrethroid pesticides (Table 2). The results of this study are consistent with results of studies performed by Hajjar (23) and Saeed et al (24) on the excessive use of pesticides by farmers.

3.2. Environmental Exposure Sampling

Fig. 2 and Table 3 depict the results of the analysis performed by the GC-FID instrument and pesticide measurement.

As can be seen, malathion as one of the organophosphate pesticides has the highest concentration in the air samples of the studied region.

The results of environmental sampling (Fig. 2 and

Table 1. Working Conditions for GC-FID Instrument

Injected volume	1-2 µL
Injection temperature	240°C
Detector temperature	180°C to 215°C
Carrier gas	Heat 15 psi (104 kPa)
Column type	Fused silica capillary column

Note. GC-FID: Flame ionization detector for gas chromatography; psi: Pounds per square inch; kPa: Kilopascal. Source: (19).

Fig. 1. Locations Related to Homes and Greenhouse of the Study Area. *Note*. Pink Point: Greenhouse; Blue point: Children's homes.

Table 2. Characteristics of Greenhouse in Ebrahimabad Village in Ashkezar

Greenhouse characteristics		Number of Greenhouse	Percent	Mean	Standard Deviation
Number and area of greenhouse (m^2)		62	-	28701.97	2613.54
Product type	Tomato	62	100%	-	-
	Cucumber	62	100%	-	-
Daily pesticide content (mL)	Maximum	62	-	323.70	361.69
	Minimum	62	-	229.83	249.17
Greenhouse function (y)		62	-	6.14	3.65
Number of monthly sprays	Maximum	62	-	8.87	327.67
	Minimum	62	-	4.02	260.35
Usage of pesticide type	Organophosphate	62	100%	-	-
	Organochlorine	Yes (18)	29%	-	-
		No (44)	71%	-	-
	Pyrethroid	62	100%	-	-

Table 3. Main Results for Environmental Sampling

Pesticides	Concentration (μg/m^3)
Malathion	183
Ethion	23
Chlorpyrifos	14
Dieldrin	16
Oxadiazon	12

Table 3) reveal that malathion, ethion, chlorpyrifos, dieldrin, and oxadiazon are predominant in the ambient air of Ebrahimabad region greenhouse. The latter results confirmed the necessity of pesticide impacts on public health. These findings are consistent with the results obtained by Rowe et al (25), Fiedler et al (26), Muñoz-Quezada et al (27), Rauh et al (28), and Kartini et al (29) on pesticides' effectiveness. Lah found that agricultural pesticides improve human health by controlling pest-transmitted diseases; however, the likelihood of exposure to these chemicals has increased over the past few decades. Moreover, long-term and indiscriminate use of pesticides has severe effects on human health, especially on infants and children (30). A study by Hashemi (31) revealed that greenhouse owners use more pesticides due to existing concerns, regardless of pesticide user's health and environmental risks, especially in the event of severe pests. The belief that chemical pesticides are necessary to achieve high efficiency has played a key role in the overuse of pesticides; thus, the strong desire to achieve high yields may have led them to overuse pesticides (32). Further, some people (e.g., infants, young children, farmworkers, and pesticide sellers) are more exposed to the toxic effects of pesticides compared to others. Although the human body has mechanisms for excreting toxins, in some cases, they are kept in the circulatory system through absorption. It can normally take months or years for their toxic effects to be manifested. Hence, their effects will be chronic and long-term (33).

In this study, given that most of the pesticides were from the organophosphate group, it can be concluded that these pesticides can affect the functioning of the mental system in the long run by inhibiting and disrupting the function of the acetylcholinesterase enzyme. Accordingly, this effect will be more noticeable among children due to the slow development of children's nervous system and its gradual development.

3.3. Demographic Characteristics of Children and Their Families and the Mean Scores of the WISC-IV

Table 4 presents the relationship between children's demographic characteristics and their families by the WISC scores.

As illustrated in Table 4, there is no significant relationship between the demographic characteristics of children and their families (*P* value > 0.05). It should be noted that the scores of all four areas (verbal thinking, perceptual reasoning, working memory, and processing speed) were calculated according to standard scores, and the total score was obtained from the full standard scores of all four areas of WISC. Further, in this study, standard IQ scores are considered excellent, above average, average, and below average (results not shown).

The findings from the mean scores obtained from the WISC exhibited no significant difference in gender,

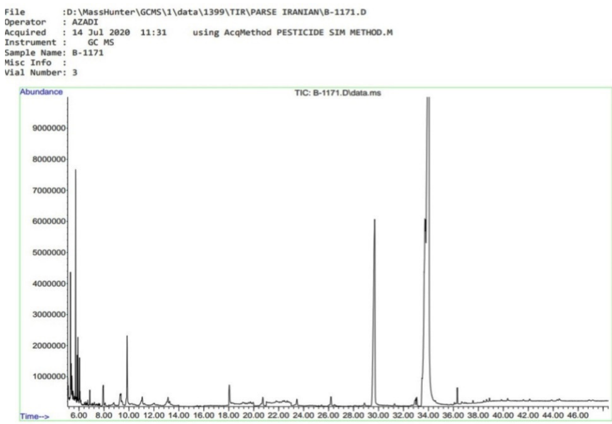

Fig. 2. Diagram of Environmental Samples by GC-FID Instrument. *Note.* GC-FID: Flame ionization detector for gas chromatography.

Table 4. Descriptive Statistics of Children Demographic Characteristics and Their families and WISC

Demographic Characteristics		Frequency (%)	Lower Than Average	Medium	Above Average	Excellent	*P* value
Gender	Girl	56 (55.1)	6 (10.7)	46 (82.1)	3 (5.4)	1 (1.8)	0.72
	Boy	45 (44.6)	7 (15.6)	36 (80)	2 (4.4)	0 (0)	
Age	6-7	29 (28.7)	0 (0)	26 (89.7)	2 (6.9)	1 (3.4)	0.11
	7-8	27 (26.7)	7 (25.9)	20 (74.1)	0 (0)	0 (0)	
	8-9	45 (44.6)	6 (13.3)	36 (80)	3 (6.7)	0 (0)	
BMI	Under weight*	22 (21.8)	0 (0)	22 (100)	0 (0)		0.089
	Normal weight	79 (78.2)	13 (16.5)	60 (75.9)	5 (6.3)	1 (1.3)	
Father's education	High school	36 (35.6)	4 (11.1)	31 (86.1)	1 (2.8)	0 (0)	0.44
	Diploma	44 (43.6)	7 (15.9)	35 (79.9)	2 (4.5)	0 (0)	
	Academic	21 (20.8)	2 (9.5)	16 (76.2)	2 (9.5)	0 (0)	
Mother's education	High school	29 (28.7)	7 (24.1)	22 (75.9)	0	0 (0)	0.28
	Diploma	26 (25.8)	4 (8.7)	38 (82.6)	3 (6.5)	1 (2.2)	
	Academic	46 (45.5)	2 (7.7)	22 (84.6)	2 (7.7)	0 (0)	
Mother's occupation	Employee	11 (10.9)	2 (18.2)	9 (81.8)	0 (0)	0 (0)	0.8
	Housewife	90 (89.1)	11 (12.2)	73 (81.1)	5 (5.6)	1 (1.1)	
Father's occupation	Employee	22 (21.8)	2 (9.1)	17 (77.3)	2 (9.1)	1 (4.5)	0.23
	Worker	50 (49.5)	5 (10)	44 (88)	1 (2)	0 (0)	
	Other	29 (28.7)	6 (20.7)	21 (72.4)	2 (6.9)	0 (0)	
Parent smoking	Yes	19 (18.8)	2 (10.5)	17 (89.5)	0 (0)	0 (0)	0.64
	No	82 (812)	11 (13.4)	65 (79.3)	5 (6.1)	1 (1.2)	
Breastfeeding	Yes	83 (82.2)	11 (13.3)	66 (79.5)	5 (6)	1 (1.2)	0.68
	No	18 (17.8)	2 (11.1)	16 (88.9)	0 (0)	0 (0)	
Birth order	1-2	80 (79.2)	9 (11.3)	65 (81.3)	5 (6.3)	1 (1.3%)	0.5
	>3	21 (20.8)	4 (19)	17 (81)	0 (0)	0 (0)	

Note. WISC: Wechsler intelligence scale for children; BMI: Body mass index.
*Based on the World Health Organization child growth standard.

body mass index (BMI), smoking, breastfeeding, and birth order of children aged 6-9 years (Table 4). Marks et al (34) indicated that 5-year-old boys who were in the clinical border for attention problems and hyperactivity symptoms gained higher scores compared with girls. However, a study by Staller et al (35) and another study by Biederman et al (36) indicated some evidence of the effect of the child's gender; in other words, reflections on the clinical manifestations of child dysfunction were reported to be different considering child's gender. Girls showed more in-attention problems, while boys exhibited more hyperactive-impulsive behaviors. However, the results of this study were not in agreement with the findings of recent studies.

Observations of gender differences in various studies may be related to differences in pesticide exposure and endocrine gland-disrupting compounds that interfere with sex hormone or steroid receptors. These compounds can induce sexual deformities in brain development and structure, causing neurological and behavioral differences between men and women (37-39). Another study by Gunstad et al (40) on BMI and cognitive function in healthy children and adolescents demonstrated no significant relationship between BMI and cognitive function in healthy children and adolescents. Regarding the BMI, data from long-term and longitudinal studies are required to assess individuals from childhood to adulthood. Taras et al (41) and Li et al (42) reported an inverse relationship between school-age weight (6-19 years) and cognitive function, which was associated with mental ability in children.

The present study found no significant relationship between parent smoking and children's cognitive function scores. A survey by Almomani et al (43) showed no difference between children's cognitive function and smoking father. This result can be explained by the fact that women's smoking in Jordanian/Arabic cultures is not as common as in Western cultures, and most men smoke outside the home (at work, on the balcony, or on the street). However, many other studies revealed a significant relationship between parent smoking and cognitive function areas (44-46). For example, Yolton et al indicated an inverse relationship between tobacco exposure and children's cognitive function and academic achievement (47).

Moreover, the relationship between the demographic characteristics of the studied children and the average scores in each area was examined separately. The results showed a significant relationship between the obtained verbal thinking scores of children and their father's education (*P* value = 0.008) and occupation (*P* value = 0.014). In other words, the average scores obtained in verbal thinking were the same in all cases except for the father's education and occupation. Castillo et al (48) examined the relationship between parental education and occupation levels and cognitive function in Spanish adolescents, concluding that parents' education and occupation groups were positively correlated with cognitive abilities and the overall scale. In a study, Parisi et al (49) examined the relationship between children's obesity, gender, and their parents' education with regard to the areas of cognitive function, finding a direct relationship between parents' education and verbal thinking scores in WISC. They also explored the correlation between children's demographic characteristics and the average scores obtained from the two sections of processing speed (*P* value = 0.0001) and working memory (*P* value = 0.014) in the WISC. The results showed a significant relationship with respect to age. In other words, the percentage of children with average processing speed in this area was higher. Furthers, at a distance of 500 meters, the WISC total scores were significantly higher at the intermediate level.

3.4. Greenhouse Density at Children's Home Location

The relationship between greenhouse density and the mean scores of children's cognitive function is presented in Table 5.

After adjusting data based on children age and father's occupation (which were significant in univariate analysis), the results indicated that the relationship between the greenhouse density and the dimensions of the Wechsler test is significant in all areas (Table 5). The increase in children's chances at the intermediate level decreased with increasing density. As indicated, there is a significant inverse relationship between greenhouse density in children's homes with verbal thinking scores (*P* value = 0.003) and the total scale (*P* value = 0.0001) on the WISC.

Table 5 also indicates that there is a significant inverse relationship between the density of greenhouse and verbal thinking scores (*P* value = 0.003) and the total score (*P* value = 0.0001) of the WISC. It means that the higher density of greenhouses near the children's homes, the lower the mean scores of children's cognitive function will be in the area of verbal thinking and the total score of the WISC. In some studies, a significant relationship was observed between pesticide exposure and individuals' neurological and behavioral function, which is consistent with results of the current study. In the study by González-Alzaga et al (12) which explored the proximity of residential homes to farms and exposure to pesticides, an inverse relationship was found between farms' density near children's homes and their total IQ, verbal thinking, and processing speed in children aged 6 - 11 years. Hence, the higher density of agricultural fields near residential houses, the more children are exposed to pesticides, and as a result, children's cognitive function was lower in verbal thinking, processing speed, and the total scale.

4. Conclusion

This study aimed to investigate the relationship between greenhouse density and cognitive function of children aged 6-9 years old in Ebrahimabad region of Ashkezar, Ahvaz, using environmental exposure measurement and the WISC. The results revealed a significant inverse relationship between greenhouse density in children's homes and verbal thinking scores and the total score of the WISC. Further, results showed that the relationship between their fathers' education and occupation and the obtained verbal thinking scores is significant. In other words, the average obtained scores in verbal thinking were the same in all cases except for the father's education and occupation. Environmental samples confirmed the exposure to pesticides. According to the results of the present study, measures should be taken to maintain human health, especially in children, and education is needed to improve people's awareness. Further studies are also needed using the WISC in similar communities to

Table 5. Relationship Between Greenhouse Density in Children's Homes and Cognitive Function Scores by WISC

Area of WISC	Category	Number of People	Greenhouse Density			OR (*P* Value)	Adjusted OR (*P* Value)*
			Mean	Median	Range		
Verbal thinking	Under average	17	3143.02	2104.12	1016.07- 13291.73	0.04 (0.0001)	0.044 (0.0001)
	Medium	84	1748.05	1464.26	989.25- 8974.43		
Perceptual reasoning	Under average	2	2970.018	2970.01	2255.35- 3684.68	0.38 (0.051)	0.26 (0.016)
	Medium	99	1962.90	1512.68	989.25- 1329.73		
Processing speed	Under average	43	2375.05	1726.22	989.25- 13291.73	0.188 (0.008)	0.124 (0.008)
	Medium	58	1692.07	1511.12	1065.31- 5503.48		
Working memory	Below average	9	3768.97	2155.33	1065.31- 13291.73	0.23 (0.142)	0.22 (0.172)
	Medium	92	1808.11	1505.13	989.25- 8974.43		
Total scale	Under average	13	3918.91	3684.68	1512.69- 13291.73	0.17 (0.003)	0.159 (0.002)
	Medium	88	1696.83	1457.32	989.25- 8974.43		

Note. WISC: Wechsler intelligence scale for children; OR: Odd ration; *Adjusted *P*-value on age of children and father's occupation based on logistic regression model.

confirm the findings.

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Competing Interests
The authors declared that there is no conflict of interests in the present study.

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