Role of environmental and genetic factors in autism spectrum disorder

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Abstract

The aim of this study was to find out the environmental as well as genetic factors responsible for increasing the number of autism spectrum disorder (ASD) patients in Bangladesh. A questionnaire was developed based on 12 environmental factors and genetic aspects. Sixty six patients of ASD and 66 non-ASD control were selected randomly. Among the environmental factors, the age of the mother, premature birth, air pollution, age of the father, hypoxia during childbirth and oral contraceptive came out as significant ($p<0.05$) factors for ASD incidence compared to the control. Association of multiple factors on an individual was found to be crucial to enhance the risk and exposure to five and six factors was statistically significant ($p<0.05$) for ASD development. Prospective parents should try to keep the number of risk factors as low as possible before 1-2 months of pregnancy, during pregnancy and 1-2 years after the child birth (for child only).

Introduction

Autism spectrum disorder (ASD) is a heterogeneous neuro-developmental disorder depicted by altered communication and social interaction and repetitive behaviors.[1] Neurological disorder shows abnormal EEG and MRI.[2] It can be classified into three subgroups: Autistic disorder (autism), Asperger disorder, and pervasive developmental disorder.[3] But the later version DSM-5 collapsed these three into one single broad diagnosis of ASD.[4]

ASD is considered as a major public health issue. At present estimated ASD incidence rate is significantly higher than pediatric cancer, HIV and heart disease collectively.[5] The global prevalence of autism has increased 20 to 30-fold since the earliest epidemiologic data were collected in the late 1960s and early 1970s. During that period, estimated prevalence from European studies were 1 in 2,500 children in the population and by the 2000s broader survey estimated about 1-2% of all children having ASD.[5]

The scenario of Bangladesh in this context is critical. Like most developing countries, there is the lack of data on the number of children or adults suffering from this lifelong incapacitating developmental neurological condition. As one of the densely populated countries in the world, proper epidemiological data derived information is urgent to address the multifaceted need of ASD-affected people in Bangladesh. For this reason, Bangladesh government launched Dhaka Declaration on autism and mental disorders in 2011[6] and later the resolution of the declaration was adopted in UN general assembly (67th session, agenda item 127). In 2013, a pilot study utilizing community health workers in Bangladesh has indicated a prevalence of 0.15% for ASD (3% in Dhaka city and 0.07% in rural area).[7] The number of the ASD children in Bangladesh is increasing at an alarming rate and it should be treated as a concerned issue. So, it is very urgent to find out the causes and factors responsible behind ASD in Bangladesh.

Both genetic and environmental factors seem to be responsible for ASD. Twin studies have shown a high concordance among identical twins (between 60 and 92%) which is much lower in fraternal twins (0 to 10%).[8] In the hunt of the specific gene responsible for ASD, it turned out that many genetic mutations in different chromosomes are symptomatic to ASD. However, it is very unlikely to find the specific gene for ASD manifestation as it seems to develop in combination of complex genetic factors and environment. From a number of array comparative genomic hybridization studies, even on large cohorts of patients, it was found that up to 10% of sporadic (non-inherited) ASD cases do show *de novo* copy number variants.[9] However, chromodomain helicase DNA binding protein 8 (CHD8) gene is the mostly proposed candidate for ASD so far. CHD8 may serve as a master regulator of a common ASD etiology.[10-12]

In addition to genetic influences on ASD etiology, environmental parameters can contribute

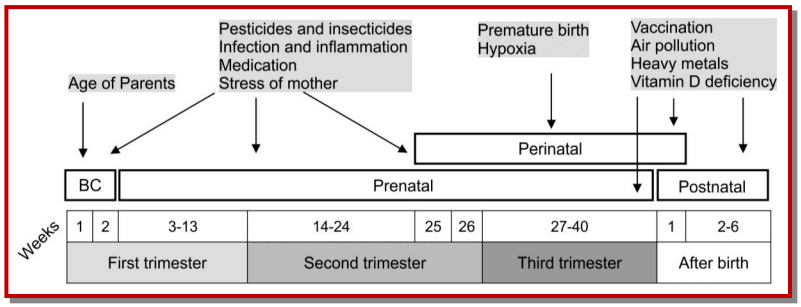

Figure 1: Different environmental factors responsible for ASD and their impact on gestation and child birth. BC=before conception

a major part. Environmental factors can be classified based on the affecting time period: Factors affecting at the time of conception, at the prenatal period (the time between conception and birth) and at the postnatal period (the time starts immediately after birth and extending for about 6 weeks) (Figure 1). But the most important period is the perinatal period. It is the time period immediately before and after birth (time starts at 22 completed weeks of gestation or at 25th week of the second trimester and ends after seven complete days after birth).

The age of the father and mother at the time of conception is found to be critical for their child to develop ASD.[13-18] Exposure to pesticide-insecticide-,[1, 8, 19, 20] infection driven inflammation,[21-23] medication (paracetamol, thalidomide)[1, 24] and stress of the mother[22] at the time of conception, the prenatal and perinatal period can enhance the risk to develop ASD for the child. Hypoxia during child birth[13] and premature birth (birth of the baby before 35 weeks of gestation)[13, 23, 25] are considered as risk factors for ASD. Air pollution,[13, 26-33] heavy metals,[34-37] and vitamin D deficiency[22, 38-40] during prenatal, perinatal or postnatal period can increase the risk for developing ASD for a child. Maternal and fetal inflammation,[22, 23] maternal diseases (diabetes mellitus, asthma),[22] maternal cigarette smoking,[22] use of oral contraceptive,[41] organophosphorus flame retardants,[8] prenatal and perinatal analgesic (paracetamol/acetaminophen)[1, 24] and valproic acid exposure[1, 22] etc. are suspected to be associated with ASD.

Table I

Characteristic profile of the participants

Participants	ASD (n=66)	Non-ASD (n=66)
Age range (years)	3-24	3-24
Gender		
Male	43	34
Female	23	32
School attendance	47	47
Siblings of ASD participants	Not applicable	25

The main objective of the study was to shed light on the important environmental factors responsible for ASD incidence in Bangladesh and the pattern how these factors might influence ASD development. It is very important to find out the major environmental risk factors associated with ASD to further investigate their mode of action and to take preventive measures to reduce the prevalence of ASD in Bangladesh.

Materials and Methods

Participants

The participants were ASD patients (n=66) and healthy non-ASD controls (n=66), randomly selected from Khulna and Comilla city of Bangladesh in 2016. Guardian of the participants was interviewed or they directly filled the structured questionnaires. The demographic data of participants of the study is presented in Table I. The age range of both ASD and non-ASD controls were between 3 to 24 years. Their gender ratio, educational and socio-economic status was not considered as the variable in this study. Some participants of the control group (n=25) was siblings of ASD patients. This was also a random choice and was not intended to influence any outcome of the study. ASD participants were selected both from specialized schools for ASD patients and also randomly from the neighborhood who does not attend specialized schools.

Measurement of data

The questionnaire was constructed with a focus on possible environmental risk factors that might be associated with the incidence of ASD in Bangladesh. After critical analysis of published literature total 12 environmental factors were considered to be analyzed. They were a) Age of the father, b) Age of the mother, c) Hypoxia during childbirth, d) Heavy metals, e) Air pollution, f) Infection, g) Medication, h) Maternal diseases, i) Premature birth, i) Mental stress of the mother, k) Pesticides and insecticides and l) Oral contraceptive. However, for few factors, more than one question was constructed. In such cases, positive response to any one question was considered positive for that particular factor (The questionnaire is in Supplementary data. The Bengali version of the questionnaire was used for the survey).

For environmental factors, after collecting the primary data, it was further processed in two different ways. First, factor-wise analysis was done. For each factor under study, a number of positive response(s) for both ASD and control group was counted and their percent exposure was measured. Second, person-wise association of factors was measured. To perform this, a number of factors (single or multiple factors) positively responded by a single individual was assessed. This was done for both ASD and the control group. Then the exposure distribution of single or multiple factors (for example, how many individuals are exposed to 2 factors or 5 factors) was converted to percent data for analysis.

For data related to the genetic association, the question about the family history (possible inherence) and the presence of other genetic diseases was investigated. However, this was only limited to ASD patients and was not performed in control

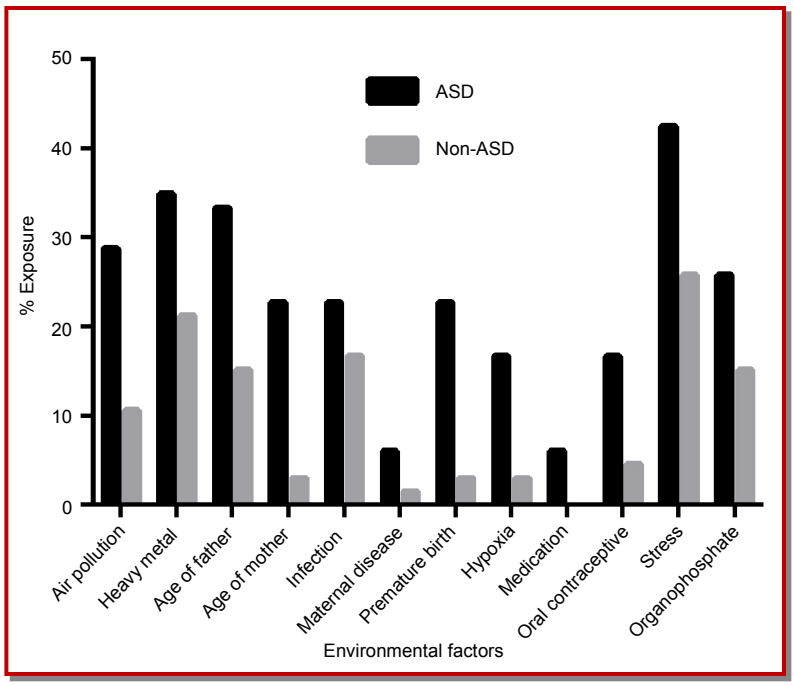

Figure 2: Comparative analysis of the incidence of environmental factors in ASD and non-ASD individuals. There were significant differences between ASD and non-ASD groups for age of mother (p=0.0012) and premature birth (p=0.0012). There were also significant differences for air pollution (p=0.015), age of father (p=0.0246), hypoxia (p=0.0164) and oral contraceptive (p=0.0447). Heavy metals, infection, maternal disease, medication, stress and organophosphate pesticides and insecticides were found non-significant. Statistical analysis was done in GraphPad Prism V.6.0.

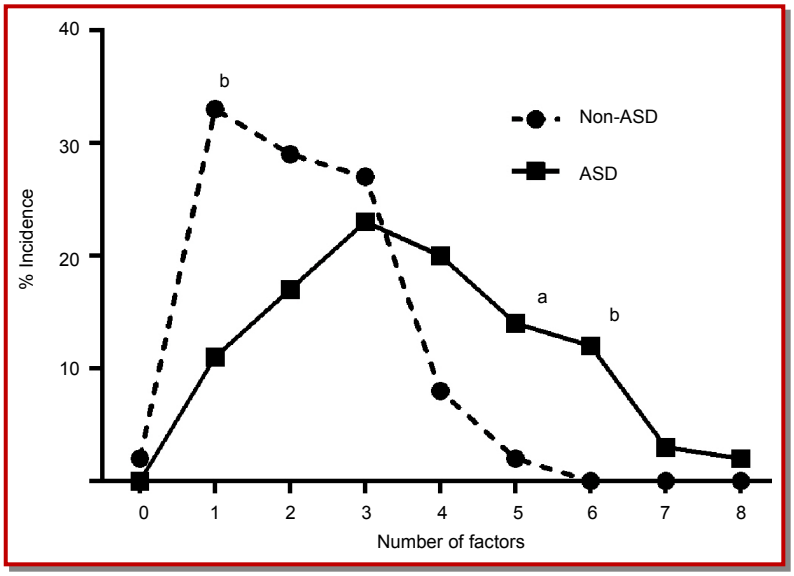

Figure 3: Comparative analysis of multiple risk factors on ASD and non-ASD individuals. The numbers of factors are plotted on the x axis. On the y axis, the % incidence is plotted. The incidence was higher up to 3 factors for non-ASD cases. But after 3 factors, the incidence became higher for the ASD group. In the cases of 5 factors (^{a}p=0.017) and 6 factors (^{b}p=0.0062), incidences were statistically significant in ASD group compared to non-ASD group

group for two reasons. First, parents of healthy individuals are not aware of other genetic diseases and the tests are not regularly done in Bangladeshi clinical settings. Second, in the control group, 25 participants were siblings of ASD patients, so their family history could not be considered and thus could not be compared with ASD group.

Statistical analysis

All statistical analysis was performed in GraphPad Prism v. 6.0. The contingency table was constructed for each factor and Fisher's exact test was used to analyze the statistical significance. p value of ≤0.05 was considered as significant when compared with control group.

Results

Environmental factors highly associated with ASD

By analyzing the survey data, among 12 environmental factors, the age of mother (p=0.0012) and premature birth (p=0.0012) were found as significant environmental factors for ASD incidence compared to the control group (Figure 2). There were also significant exposure differences between the ASD and non-ASD groups for air pollution (p=0.015), the age of father (p=0.0246), hypoxia during childbirth (p=0.0164) and oral contraceptive (p=0.0447). Other factors e.g. heavy metals, infection, maternal diseases, medication, the mental stress of mother and pesticides and insecticides were found to be non-significant in the current study.

Genetic factors

The analysis of the genetic factors was performed only for the ASD samples. Among the ASD participants (n=66), 15.1% had a record of other ASD individuals in their family. In the case of presence of related genetic disease, a large portion (69.7%) was not aware of the related genetic diseases. Only 10.6% participants confirmed that they had related genetic diseases (Table II).

Multifactor exposure to single individual can pose risk for ASD

An exciting and interesting observation was found by analyzing the number of factors exposed by an individual. For both ASD and the control group, percent data of exposure from 'no factor' towards increasing number of factors were plotted (Figure 3). Up to three factors, percent incidence was much higher in non-ASD group compared to ASD group. Especially for 'one factor', the difference was statistically significant. This trend, however, started to change in opposite direction, when the number of factors was over three. Percent incidence of

Table II

Distribution of genetic aspects among ASD patients

Genetic aspects	Number of individuals with ASD (n=66)		
	Yes	No	Don't know
Related genetic diseases	7	13	46
Any other ASD patient in the family/ancestors	10	56	

association with more than three factors was found to be higher in ASD group compared to the control. In the cases of five factors (p=0.017) and six factors (p=0.0062), the difference in incidence was statistically significant. It was also observed that for a higher number of factors, there was no incidence observed in healthy control at all. From these, it might be concluded that many ASD patients showed multiple factor association at a time (e. g. 5, 6 or more factors). This may increase the risk of ASD development which is not evident in non-ASD control.

Discussion

In our study, we aimed to know the important factors behind ASD in Bangladesh. Air pollution, the age of parents, premature birth, hypoxia during childbirth and oral contraceptive were found as significant risk factors. Physical and developmental alteration on the fetus had been reported where mothers were exposed to air pollution during pregnancy. In recent years, several researchers investigated the effect of air pollution exposure on ASD.[13, 26-33] In California Volk et al examined the relationship between air pollution due to traffic and autism in two population based case-control studies from the Childhood Autism Risks from Genetics and the Environment (CHARGE) study. The first study reported increased risk among those living within 309 m (~1010 feet) of a major freeway; especially during the third trimester.[26] The second research revealed the correlation of increased autism risk with exposure to model indicators of traffic-related air pollutants and regional level of NO_2, fine particulate matter PM2.5 and PM10.[27]

Some other researchers found that living in areas with higher levels of styrene and chromium in the air was associated with increased risk of ASD.[13] Additionally, autism has been associated with estimated regional concentrations of hazardous air pollutants, including arsenic and nickel, and with diesel PM exposure in early childhood. Toxicological studies suggested a biologically plausible role of air pollution in disrupting brain development and function during critical time points in gestation and early life. Diesel exhaust particles present in traffic-related pollution can disrupt endocrine activity and affect sexual differentiation transplacentally and alter cognitive function in mice.[26] From a recent study conducted on mice, researchers suggested air pollution as a risk factor not only for ASD but also for other neuro-developmental disorders.[32] As Bangladesh is a highly populated country with numerous industries and huge traffic in the major cities, lack strict monitoring of air pollution, people residing in the city vicinity are at high-risk to develop ASD.

Advanced paternal and/or maternal age can be a risk factor for ASD[13-18] and has emerged in our present study too. In 2015, a population-based study spanning five countries (Denmark, Israel, Norway, Sweden and Western Australia) with 57,66,794 children including 30,902 cases of ASD, researchers found that autism risk was associated with parental age and increasing age difference between parents.[16] In another study on 2,53,347 participants, researchers found that both maternal and paternal age were independently associated with autism.[15] Croen et al. found that risk of ASD was increased significantly with each 10 years increase in maternal and paternal age.[14] According to some researchers, the father aged over 35 years[42] and mother aged over 30 or 35 years[1, 21] were suspected as a high-risk condition. This delaying childbearing trend contributes around 4.6% increase in autism over the decade.[20]

Some researchers found autism risk across generations epigenetically. A statistically significant nonlinear monotonic association was found between grand-paternal advanced age at the time of birth of the parent and autism risk in their grandchildren. The study was conducted on 5,936 cases and 30,923 controls and it was found that men who became the father at age over 50, were 1.79 times more likely to have a grandchild with autism, compared with who became the father at age 20 to 24 years.[43] DNA repair genes are down-regulated as males' ages, and oxidative DNA damage level increases three times in spermatozoa when a man is over 35 compared to that of young aged men. Thus, paternal aging was known to be linked with several adverse clinical conditions including ASD.[42] The study of Lampi et al supports premature birth as a significant risk factor for ASD.[25] In Bangladesh around 13 to 25% babies are reported to born preterm,[44, 45] which is quite high and supports our finding that neurodevelopmental disorder may arise from preterm birth leading ASD. Some researchers suggested hypoxia during pregnancy[13] and use of oral contraceptive[41] as risk factors for ASD which also turned out as significant risk factors in our study.

Heavy metal poisoning can be triggered from different sources including chemical products, fertilizers, industrial paint, building materials, fish with a high level of mercury, silver containing

dental fillings, nasal sprays and mercury-containing preservatives (thiomersal) in vaccines. Mohamed et al analyzing hair minerals (mercury, lead, and aluminum) of 100 autistic and 100 healthy children found that the levels were significantly higher among cases than controls. To track the sources of the metals they concluded that mercury, lead, and aluminum levels were positively correlated with maternal fish intake, residence near gasoline stations, and use of aluminum pans, respectively.[34] Another study conducted on 60 children (ASD=30 and controls=30), researchers got strong association and significant difference in the lead, mercury and cadmium concentrations between ASD and control.[35] Yassa, 2014 conducted a research on blood and hair samples obtained from 45 children with autism and 45 children served as controls between the ages of 2 and 10 years to find out exposure variation to lead and/or mercury as heavy metals. The results showed significant difference among the two groups, kids with autism had high level of mercury and lead.[36] In a study on 18 ASD participants, Geier et al., 2012 found that increasing hair Hg concentrations were significantly correlated with increased ASD severity.[37]

Children being unable to differentiate between edible and non-edible item, may develop toxic lead levels as they get the poisoning from old houses with old painted wall or plumbing with cracks and dust which can be swallowed.[36, 46] Mercury is found in many foods, including high fructose corn syrup. Dietary zinc is important in the metabolic processes to eliminate mercury; few artificial food color additives had shown to confer zinc deficiency. Dietary deficiencies of iron, zinc, iodine, selenium, copper, manganese, fluoride, chromium and molybdenum are linked with mild to considerable alterations in brain function resulting underdeveloped health, behavior and learning.[1] However, some researchers claimed a negative association between heavy metals and ASD [47-49] which are reflected in our findings. This could be due to the less frequent use of canned or packed food and fish product in Bangladesh and comparatively less industrial exposure compared to developed countries.

It is critical to identify the more vulnerable period of the fetus in terms of immunity. But infection driven inflammation can increase the risk of ASD.[21, 22] In a study on 407 cases and 2,075 controls, no overall association was reported between any maternal infection during pregnancy and ASD in the child. But they found women with bacterial infections, were at increased risk of delivering a child with ASD.[23] Atladóttir et al. conducted a study on children in Denmark from 1980, through 2005.[50] They found no association between maternal infection and ASDs, but viral infection and bacterial infection were found to be connected to ASD in the offspring in the first trimester and in the second trimester respectively. They also reported urinary tract infections as the most common bacterial infection during hospitalization.[50] Maternal fever during pregnancy has also been linked to increased risk of ASD.[51] However, in our study we found maternal infection as a non-significant risk factor for ASD.

Although carefully designed, surveyed and analyzed, the limitation of this preliminary study was small sample size; and a larger sample from multiple cities is needed to improve the statistical power of our findings. The participants were mainly from urban or semi-urban areas, due to the fact that specialized school was situated in cities and this restricted the participation of ASD patients mainly from urban areas. This should be noted that environmental risk factors may vary in villages and can have a different impact on ASD due to comparatively less urbanization in rural areas. To answer some questions, respondent had to answer based on memory, and depending on the age of their child the information might be slightly inaccurate as they had difficulties to remember exact information after several years.

Conclusion

The age of the mother, premature birth, air pollution, the age of the father, hypoxia during childbirth and oral contraceptive were found as significant risk factors for ASD in Bangladesh compared to control group. In addition, exposure to five and six factors on an individual was found to be statistically significant risk factors for ASD development and progression.

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Supplementary Data

Like other countries of the world, the number of the ASD children has been increasing in Bangladesh. Based on the existing identified causes of ASD, this Questionnaire has been made based on the perspective of Bangladesh. Our aim is to identify the important factors responsible for increasing ASD children in Bangladesh. Your co-operation can help us in this important perspective.

Environmental aspects:

Factor 1: Age of father, Factor 2: Age of mother

Name of the autistic child: Age (year):

Name of the mother: Age (year):

Name of the father: Age (year):

Factor 3: Hypoxia during child birth

Did the mother face hypoxia during the child birth?

☐ Yes ☐ No

Factor 4: Heavy metals

Was the house being painted where the mother stayed during pregnancy?

☐ Yes ☐ No

Did the child eat any painted piece of wall before he/she reaching 1 (year)?

☐ Yes ☐ No

Did the mother stay in a very old building (where dust of painted wall was available) during pregnancy?

☐ Yes ☐ No

Did the mother filling her teeth (with amalgam) during or before pregnancy?

☐ Yes ☐ No

Which type of water[1] did the mother drink during pregnancy?

☐ tap water ☐ filtered water

☐ tube well water ☐ pond water

Did the mother eat excessive artificial color contained food during pregnancy?

☐ Yes ☐ No

Factor 5: Air pollution

What was the distance[2] of the house where the mother lived during pregnancy/before the child reaching 1 year?

☐ less than 1010 feet ☐ more than 1010 feet

Was it an industrial area where the mother lived during pregnancy/before the child reaching 1 year?

☐ Yes ☐ No

Factor 6: Infection

Did the mother have any urinary infection before the pregnancy period?

☐ Yes ☐ No

Was the mother be hospitalized for any kind of infection during pregnancy?

☐ Yes ☐ No

Factor 7: Medication

Did you eat excessive paracetamol during pregnancy?

☐ Yes ☐ No

Factor 8: Maternal diseases

Did the mother have diabetes mellitus/asthma before conceive?

☐ Yes ☐ No

Factor 9: Premature birth

Was the child a premature baby (birth of a baby before 35 weeks of pregnancy)?

☐ Yes ☐ No

Factor 10: Mental stress of the mother

Did the mother face excessive mental strain during pregnancy?

☐ Yes ☐ No

Factor 11: Pesticides and insecticides

If any of the option from below situated near the house where the mother lived during pregnancy, kindly put tick mark on that option-

☐ pesticide manufacturing industry

☐ farm land (where chemical fertilizer was used)

Factor 12: Oral contraceptive

Did you take oral contraceptive just before 2 months of pregnancy?

☐ Yes ☐ No

[1]tap water or pond water can contain metals
[2]air of 1010 feet from the highway is considered as polluted area