Maternal hormonal interventions as a risk factor for Autism Spectrum Disorder: An epidemiological assessment from India

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Globalization and women empowerment have led to stressful life among Indian women. This stress impairs women's hormonal makeup and menstrual cycle, leading to infertility. National Family Health Survey-3 (NFHS-3) reports a decline in fertility status in India, indicating a rise in various infertility treatments involving hormonal interventions. No studies are available from India on the risk association link between maternal hormonal treatments and ASD. Hence, this study explores the association of maternal hormonal interventions with risk for ASD. Parents of 942 children (471 ASD and 471 controls) across 9 cities in India participated in the questionnaire-based study. The questionnaire was pilot tested and validated for its content and reliability as a psychometric instrument. Data collection was done at 70 centres through direct interaction with parents and with the help of trained staff. Statistical analysis of data was carried out using SAS 9.1.3. Out of the 471 ASD cases analysed, 58 mothers had undergone hormonal interventions (12.3%) while there were only 22 mothers among controls who underwent hormonal interventions (4.6%). According to logistic regression analysis maternal hormonal intervention (OR=2.24) was a significant risk factor for ASD.

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1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction, communication, repetitive stereotyped behaviours and narrow interests (DSM IV). ASD is expected to be on rise in India, with nearly 2 million affected individuals (Karande 2006). India is a multifaceted society with various regional, religious, social and economic groups. Even today, bearing a child remains an important factor in the socio-economic wellbeing of most Indian women (Kishor *et al*. 1995). According to National Family Health Survey-3 (NFHS-3), fertility is on a decline in Indian population (NFHS-3 2006), and WHO estimates the incidence of infertility to be in the range of 13% to 19% in India. This has led to an increase in the use of various infertility treatments across the country.

Infertility treatments involve administration of ovulation-inducing drugs such as Clomifene Citrate, which is an

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estrogen receptor modulator, human menopausal gonadotropin (hMG), which contains follicle-stimulating hormone (FSH) and luteinizing hormone (LH), human chorionic gonadotrophin (hCG) and gonadotrophin-releasing hormone agonists (GnRH) (Glud *et al*. 1998). Also, natural or synthetic progesterone is administered to maintain the luteal phase. These hormones are administered depending on the cause of infertility and the protocol used (Glud *et al*. 1998). Thus, these treatments involve regulation of the maternal hormonal environment.

Maternal hormonal and nutrient environment has been implicated to impact the developing foetus, which in turn influences susceptibility to a wide range of neurodevelopmental, metabolic and psychiatric diseases in later life (Troisi *et al*. 2003; Baron-Cohen *et al*. 2004; Zeltser and Leibel 2011). Hormonal disturbances are also reported to have direct link to obstetric complications, which is reported to influence the risk of ASD in children (Brimacombe *et al*. 2007; Baron-Cohen *et al*. 2009, 2011; Auyeung *et al*. 2010). Medical, obstetric and psychological events during pregnancy are reported to influence the health status of an infant at birth and are anticipated to be the determinants of long-term health and quality of life (O'Keane and Scott 2005). Therefore, maternal hormonal disturbances are hypothesized to affect the growth of developing foetus, contributing to neurodevelopmental abnormalities like ASD.

ASD is thought to involve both heritable and environmental component in its aetiology (Newschaffer *et al*. 2007). Environmental risk factors are implicated via complex gene–environment interactions, but no specific link between exposures and significant population effects are reported (Newschaffer *et al*. 2007). So, analysis of risk factors for their association with ASD could shed light on these links and help in exploring the pathways affected due to these risk-aiding environments. The estimated increased prevalence coupled with the country's contemporary growth makes the Indian population a compelling model for such studies. Maternal conditions during gestation affect fetal development and can be a probable risk area that needs to be explored for their association with ASD. Thus, we aim to explore the association between hormonal interventions in mothers, with the aetiology of ASD in children. In a country like India, with a large population, changing lifestyles and decreased fertility rates, it becomes paramount to look at the effect of maternal hormonal treatments on the risk for ASD.

2. Methods

Code of human research ethics (ICMR 2006) was adhered to in conducting our research. The objective was clearly explained to the parents and questions were clearly defined in the questionnaire, informed consent was collected prior to the data collection and care was taken to protect the confidentiality of the individual involved. The validated questionnaire also had the approval of Institutional Human Ethics Committee (IHEC), and was used for data collection.

2.1 *Case*

Our study population consisted of children between 2 and 10 years of age across India, recruited between September 2010 and December 2012. As the first signs of the disorder are normally seen even before the child is 3 years old (Boyd *et al*. 2010; Mefford *et al*. 2012) and concrete diagnosis is usually available between 3–4 years of age, and certain conditions like the Asperger's syndrome may have a delayed diagnosis at around 5–6 years (CDC 2012), we restricted our age group between 2 and 10 years. Internationally accepted standards like Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV) or International Classification of Diseases 10 (ICD 10) or Indian Scale for Assessment of ASD (ISAA) were used by trained physician at child psychiatry departments of various hospitals for ASD diagnosis. A total of 500 children were initially enrolled for the study. Out of them, 20 children were excluded. The excluded group consisted of children who were under the suspicion for ASD or presented with only autistic traits but did not have a formal diagnosis or had cerebral palsy or Down's syndrome. Thus, 480 cases were the initial enrolment number and out of them 9 cases were eliminated for missing data on hormonal interventions. Hence, the total numbers of participants included were 471.

2.2 *Control*

The control populations were identified randomly across India between September 2010 and December 2012. The children were between 2 and 10 years of age and were gender-matched with ASD cases. The enrolment was done parallel to the recruitment of children with ASD. Intellectual progress of the child in main stream educational systems, as per the teacher's report as well as parental report, was taken as added confirmation for control recruitment.

2.3 *Collaborations*

Due to limited availability of registered medical records, various independent organizations were approached for data collection from parents of children with ASD across India. Collaborations were established with a total of 70 centres

Table 1. Gender characteristics of children born to mothers who have undergone hormonal treatment regimens

	ASD			Control		
	N	Boys (%)	Girls (%)	N	Boys (%)	Girls (%)
Maternal Hormonal treatments	58	46	12	22	17	5
Hormonal Injections	23	18	5	4	3	1
Oral hormone drugs	19	15	4	5	3	2
Both injections and oral drugs of hormones	16	13	3	13	10	3
Hormonal treatment for clinical-condition-induced infertility						
PCOS mothers who underwent hormonal treatment for infertility	12	9	3	1	1	0

dealing with children with ASD covering 9 cities. The details about the centres were provided by The National Trust, psychiatric departments of various hospitals across 9 cities (Hyderabad, Chennai, Mumbai, Bangalore, New Delhi, Mysore, Ahmedabad, Guntur, Nagpur), institutes like National Institute for the Mentally Handicapped (NIMH), various autism centres and thorough Internet search. For control population recruitment, collaborations were established with schools (regular, government-run and private school) and care was taken to have representations from all sections of children from varied socio-economic background. Also, data was collected through personal visits to houses having kids in the age group across the cities. This further ensured the representation of all sections of society.

2.4 *Questionnaire*

A psychometric instrument in the form of questionnaire was designed based on the probable risk factors of ASDs from existing literature. The questionnaire was initially pilot tested with a convenient sample followed by other procedures for its validation like content (percentage agreement and content validity index) and reliability testing (Internal consistency and test-retest). The results obtained were statistically significant, and we concluded that it is a good instrument for conducting the research. These results are communicated for publication elsewhere. The same questionnaires were uniformly distributed at all centres after IHEC approval.

2.5 *Data collection*

Informed consent was obtained from parents of the participating children (both cases and controls) prior to the study. Data collection was carried out by personal interaction with parents or with the help of teachers at various organizations dealing with children with ASD. The teachers were trained with respect to the details of the questionnaire and they assisted parents to complete the questionnaire by translating the content to their respective dialects and clarified any doubts as well. 70% of the children (cases and controls) were below 7 years of age. We had cooperation from 90% of the participating parents for data collection. Also we performed a quality check with the medical records of 20% of the participating mothers. Thus, adequate care was taken to reduce discrepancies arising out of memory recall bias.

2.6 *Statistical analysis*

Out of 480 cases enrolled, 9 questionnaires (2%) were excluded due to missing data for hormonal intervention. Thus, a total of 471 ASD-affected children and an equal number of controls were enrolled for the study. The male to female ratio in both cases and controls was 4:1. Statistical analysis was done using SAS 9.1.3 (SAS Institute, Inc, Cary NC) version. An unadjusted and adjusted (child's age, gender and maternal age) univariate analysis was performed to calculate odds ratios and 95% confidence intervals (CIs) at 5% α.

Table 2. Percentage of mothers undergoing various kinds of hormonal treatment

	ASD		Control	
	N	%	N	%
Hormonal treatments	58	12.3	22	4.6
Hormonal Injections	23	4.8	4	0.8
Oral hormone drugs	19	4.0	5	1.0
Both injections and oral drugs of hormones	16	3.3	13	2.7
Hormonal treatment for clinical-condition-induced infertility				
PCOS mothers who underwent hormonal treatment for infertility	12	2.5	1	0.2

3. Results

The average age of mothers (both cases and control) was 27 years with 70% of the mothers being ≤30 years of age, while of fathers (both cases and control) was 32 years old with 60% of them being ≥30 years of age. Hence, in our study group, parental age of the cases and controls recruited was found to be comparable. Upon analysis of the gender characteristics of children born to mothers who underwent hormonal treatment, the male to female ratio in both cases and controls among was found to be 4:1 (table 1).

Hormones like estrogens, FSH, progesterones and gonadotropins are extensively administered during treatment of reproductive impairments (Brown 2011). The mothers in our study group were administered the following treatment options:

- Hormonal injections – estrogens or progesterones
- Hormone oral drugs – gonadotropins or hMG or Clomifene or GnRH

Considering the total data, in the ASD population, 58 (12.3%) (table 2) mothers underwent hormonal treatment regimens (23 [4.8%] had hormonal injections only, 19 [4%] had hormone oral drugs only and 16 [3.3%] in the form of both injections and oral drugs) for fertility. In the control population, a total of 21 (4.4%) (table 2) mothers underwent hormonal treatment regimens (4 [0.8%] had hormonal injections only and 5 [1%] had hormone oral drug and 13 [2.7%] in the form of both injections and oral drugs) for fertility. Out of the 58 mothers of children with ASD, 12 had undergone infertility treatment regimens due to polycystic ovary syndrome (PCOS) (table 2). These results points out that the hormonal interventions were higher among mothers who had children with ASD than control population. To further validate the results, logistic regression analysis (table 3) was performed and the results indicated a significant association of maternal hormonal interventions with ASD (OR=2.375 [CI=1.396–4.039] P=0.0014). Even after adjusting the variables to child's birth year, gender and maternal age, the value of the maternal hormonal interventions remained significant (OR=2.240 [CI = 1.309–3.835] P=0.0033).

4. Discussion

Maternal hormonal and nutrient environment is reported to impact the developing foetus, especially organization and activation of the brain (Bale *et al*. 2010; Fernandez and Ozanne 2010; Zeltser and Leibel 2011), and any disturbances in the foetal environment could have consequences in the foetal development (Brown 2011). Also, estrogens are administered as part of infertility treatments and estrogen exposure is already implicated in pathology of neurodevelopmental disorders like schizophrenia either from excessive dose, timing and/or duration of estrogen exposure or modification of estrogen receptor function (McEwen 1987). Maternal reproductive hormone disturbances are reported to cause obstetric complications which have already been implicated as a risk for ASD (Newschaffer *et al*. 2007).

Apart from the above-mentioned reports, limited studies are available for analysing the association of assisted reproduction in mother as a risk factor for ASD (Maimburg and Vaeth 2007; Hvidtjorn *et al*. 2011; Lyall *et al*. 2011, 2012; Zachor and Itzchak 2011; Lehti *et al*. 2013). Results from our study in India are similar to those of other studies (Maimburg and Vaeth 2007; Hvidtjorn *et al*. 2011) reporting an increased risk of ASD in children born to mothers who underwent hormonal treatment regimens. But since our results are based on epidemiological study, quantitative assessment is required to further investigate the role of maternal hormonal interventions as a risk for ASD.

Our study had a number of strengths, including a large sample size, detailed validated questionnaire, maximum participation of the mothers, first report from a developing country like India, etc. Nonetheless, it was limited due to the absence of published report on the incidence and prevalence. Absence of the detailed treatment regimens with respect to dosage and frequency of the hormonal intervention is also one of the limitations of our study. We also do not rule out the memory recall bias, but a quality check with medical records of 20% mothers minimizes the memory bias. Genetic predisposition contributing to increased risk for ASD or for the exposures under study has not been validated as part of this study and needs to be addressed separately.

Table 3. Analysis of maternal hormonal treatment for the aetiology of ASD

	Cases		Controls		Unadjusted analysis				Adjusted analysis#			
	N	(%)	N	(%)	OR+	95% CI+		P+	OR+	95% CI+		P+
Hormonal treatment	58	12.3	22	4.6	2.375	1.396	4.039	0.0014*	2.240	1.309	3.835	0.0033*

#, The adjusted analysis was performed by adjusting child's birth year, gender and maternal age; +, OR – odds ratio; CI – confidence interval; P – probability value; *, significant value.

5. Conclusion

This epidemiological study from the Indian population sheds light on the association of maternal hormonal interventions as a risk factor and ASD. Statistically, significant risk association was found for hormonal interventions in mother and the aetiology of ASD in child, but more extensive studies on quantitative assessment in terms of dosage, treatment regime and its outcome along with genetic predisposition are recommended.

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