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Role of rubella in congenital malformations in India

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SUMMARY

In the present study rubella HI antibodies were determined in cases of congenital malformation and bad obstetric history to determine the role of rubella in such cases in India, as it has been reported to be rare in Japan in contrast to Western countries. The incidence of antibodies was statistically significant in cases of congenital eye, C.N.S., visceral and miscellaneous malformations and cases of spontaneous abortion and still-birth compared with controls of matching age groups. This is further supported by the demonstration of IgM antibodies in seven cases of congenital malformations and ten cases of spontaneous abortion. Our findings show that congenital rubella is not rare in India.

INTRODUCTION

If acquired in early pregnancy rubella may damage the fetus and result in spontaneous abortion or the delivery of malformed infants. Thus, the extensive epidemic in the U.S.A. in 1963–4 resulted in the birth of some 10,000–20,000 malformed infants (Cooper & Krugman, 1966). During non-epidemic years it is estimated that about 200 cases of congenitally acquired rubella occur annually in the U.K., although only about 25% are actually reported (Best, Banatvala & Bowen, 1974). Less is known of the role of rubella virus as a cause of congenital malformations in India, although previous investigations have shown that some 15–20% of women of child-bearing age in Lucknow do not have rubella antibodies (Mathur, Chaturvedi & Mehrotra, 1974), a proportion which is similar to that reported among women of child-bearing age living in urban communities in other parts of the world (Rawls *et al.* 1967). However, as far as we are aware, there have been no rubella epidemics in Lucknow during the last 15 years. This paper describes our attempts to assess the role of rubella virus as a cause of congenital malformations among children, and determine the pattern of rubella-caused congenital defects.

MATERIALS AND METHODS

Cases

The present study was carried out on cases of different congenital malformations and bad obstetric history who came to the out-patient clinics or were admitted to the wards of this hospital. They came from different parts of Northern India, most

Table 1. *Cases included in the study*

Congenital defects	Total No.	Diagnosis
Heart	30	Atrial septal defect, ventricular septal defect, patent ductus arteriosus, pulmonary stenosis, Fallot's tetralogy and dextrocardia
Eye	16	Cataract, optic atrophy, pthisis bulbi, ptosis, coloboma iris, cryptophthalmos, glaucoma, anophthalmos and micro-ophthalmos
Ear	23	Deafness with mutism, and defect of pinna with partial deafness
CNS	34	Mental retardation with or without microcephaly, mental retardation with cerebral palsy, meningocele, hydrocephalus, cranial defects and spinal defects
Musculo-skeletal	69	Defect in upper limb, lower limb, congenital dislocations, osteo-genesis imperfecta congenita, cleftlip and palate, dental defects, multiple malformations, hypospadias, anorectovaginal defect, congenital hernia and muscular dystrophies
Visceral	16	Neonatal jaundice, visceroptosis, congenital hepatomegaly and renal defect of duplication
Miscellaneous	9	Purpura, teratoma, cystic hygroma, retinoblastoma, general growth retardation, and congenital malformations
Obstetric cases	144	Spontaneous abortion, still-birth, premature delivery and delivery of malformed babies
Healthy controls	114	—

of them being from places around Lucknow. Sera from 197 cases of congenital defects were collected, aged between 8 months and 10 years except the cases of neonatal jaundice and two others. The congenital defects encountered during the course of study are shown in Table 1. Six cases with multiple defects were seen but have been grouped according to the predominant defect. The 144 cases with bad obstetric history included those of spontaneous abortion, still-birth and delivery of premature or malformed babies. The clinical diagnosis of each case was established by the clinicians in charge after thorough examination using standard methods and tests where indicated. For control, sera from 114 healthy individuals of matching sex and age groups were collected from the general population and from children attending clinics for vaccination.

Haemagglutination inhibition test

The sera were tested for the presence of rubella haemagglutination inhibiting antibodies using the micro-technique of Halonen, Ryan & Stewart (1967). The details of the procedure, reagents and the antigen have been reported earlier (Mathur *et al.* 1974). Doubling dilutions of serum were used starting from 1/10.

Detection of IgM

The presence of immunoglobulin M (IgM) in the sera was detected by the technique of Banatvala *et al.* (1967). Sera which were positive for rubella antibodies on preliminary screening were treated with 1/10 volume of 0·5 M

Table 2. *Rubella antibodies in cases of congenital malformations of different age groups*

Congenital defects	Age (years) 0–1 No.	0–1 +ve	1–3 No.	1–3 +ve	4–6 No.	4–6 +ve	7–10 No.	7–10 +ve	Total No.	Total +ve	%
Heart	6	2	6	1	11	3	7	4	30	10	33
Eye	8	4	3	3	2	1	3	3	16	11	69
Ear	—	—	4	1	8	2	11	4	23	7	30
CNS	12	4	10	4	3	1	9	6	34	15	45
Musculo-skeletal	28	6	13	2	10	2	18	7	69	17	25
Visceral	15	14	—	—	—	—	1	1	16	15	94
Miscellaneous	5	4	2	1	1	1	1	0	9	6	67
Control	16	2	13	1	17	7	18	8	64	18	28

2-mercaptoethanol for 1 hr. at 37° C. The HI test was repeated with 2-mercaptoethanol treated sera, and untreated sera to which 1/10 volume of PBS was added as controls.

RESULTS

Cases of congenital malformations

The incidence of HI antibodies in different groups of malformations is shown in Table 2. It was observed that 33% of cases of cardiovascular anomalies had rubella antibodies while 69% of cases of ocular and 94% of visceral manifestation were seropositive. The cases of congenital ear malformation, mental retardation with CNS involvement, musculo-skeletal cases and miscellaneous cases had antibodies in 30%, 45%, 25% and 67% respectively. All the six cases who had multiple deformities were positive for rubella antibodies. Among 64 controls of matching age groups antibodies were present in 28%.

The incidence of rubella antibodies in different conditions of congenital defects in children aged below 3 years has been presented in Fig. 1. Among 29 controls of this group antibodies were present in 10%. Maximum incidence of 93% was seen in cases of visceral defects, 64% in eye defects and 71% in the miscellaneous group; 36% showed antibodies out of 22 cases of C.N.S. involvement. The difference between the controls and eye, C.N.S., visceral and miscellaneous defects group was found to be statistically significant by the chi-square test using Yates correction, at 5% level of probability.

The incidence in children aged between 4 and 10 years is shown in Fig. 2. In this group the incidence of antibodies among 35 controls was 43%. The maximum incidence of antibodies noted in this group was 80% in cases of eye defects. The difference between the controls and the diseased group was not statistically significant in this age group.

An attempt was made to study the incidence of antibodies in total cases of congenital defects compared with controls in different age groups (Fig. 3). Among 74 patients aged less than 1 year 34 (46%) had antibodies while in the corresponding controls they were found in 12%. Similarly in the age group 1–3 years the

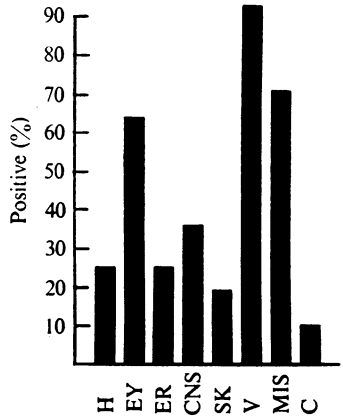

Fig. 1

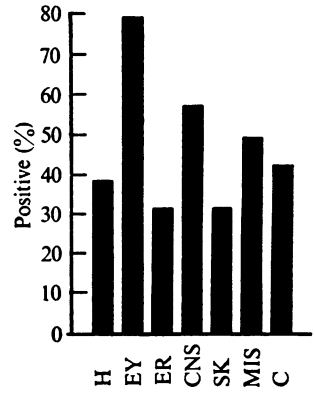

Fig. 2

Fig. 1. The incidence of rubella antibodies in children aged 0–3 years in different congenital malformations. H, Heart; EY, eye; ER, ear; CNS, central nervous system; SK, skeletal tissue; V, visceral; MIS, miscellaneous; C, controls.

Fig. 2. The incidence of rubella antibodies in children aged 4–10 years in different congenital malformations. H, Heart; EY, eye; ER, ear; CNS, central nervous system; SK, skeletal tissue; MIS, miscellaneous; C, controls.

Table 3. *Antibody titres in cases of congenital malformations*

Congenital defects	Total No.	Positive	Reciprocal antibody titres 10	40	80	160	320	640
Heart	30	10	3	5	—	—	2	—
Eye	16	11	3	5	1	2	—	—
Ear	23	7	5	1	—	—	1	—
CNS	34	15	5	7	—	1	2	—
Musculo-skeletal	69	18	7	6	1	4	—	—
Visceral	16	15	4	5	2	3	—	1
Miscellaneous	9	6	1	4	1	—	—	—
Control	64	18	6	4	2	5	1	—

incidence of antibodies in patients was 32% in contrast to 8% seen in controls. In the 4–6 years group antibodies were present in 28% of cases and 41% of controls, while in age group 7 to 10 years antibodies were present in 50% of cases and 44% of controls. Because of the similarity of the data the findings of children below 3 years and those above 4 years were pooled. By the chi-square test using Yates correction the incidence of antibody in cases aged below 3 years was significantly different from that in matching controls at 5% level of probability. On the other hand in children of the higher age group (4–10) the difference was not significant.

An analysis of the antibody titres in these sera is shown in Table 3. In 61 out of 82 (74%) seropositive cases the titre was 40 or less. Titres of 320 were recorded in 2 cases of heart disease, 2 cases of central nervous system defects and 1 case of ear defect. A single case of neonatal hepatitis had a titre of 640. In 10 cases titres were 160. Among the controls 10 out of 18 (55%) sera had titres of 40 or less and 5 out of 18 (27·5%) sera had a titre of 160; only 1 case had a titre of 320.

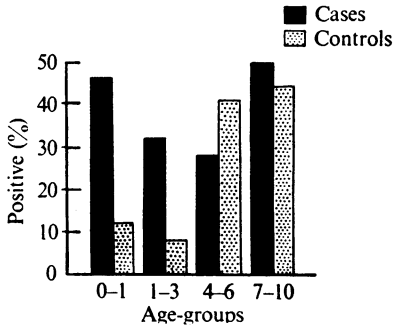

Fig. 3

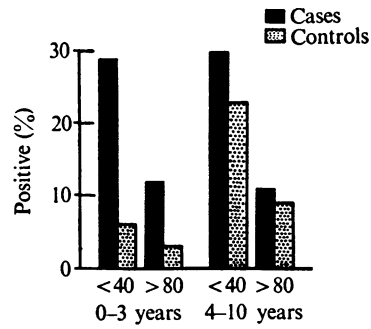

Fig. 4

Fig. 3. The incidence of rubella antibodies in total cases of congenital malformation compared with controls in different age groups.

Fig. 4. The incidence of antibody titres in children aged up to 3 years and 4–10 years.

Table 4. *Rubella antibodies in cases of bad obstetric history*

	Age groups (years)										
	16–20		21–25		26–30		>30		Total		
Cases	No.	+ve	No.	+ve	No.	+ve	No.	+ve	No.	+ve	%+ve
Spontaneous abortion	13	11	25	22	20	19	5	4	63	56	88·8
Still-birth	7	5	16	13	17	17	2	2	42	37	88·1
Malformed babies	1	1	20	18	8	6	3	3	32	28	87·5
Premature delivery	0	0	3	3	3	2	1	1	7	6	85·9
Controls	11	8	19	16	14	12	6	5	50	41	82·0

The findings of the antibody titres in patients with congenital anomalies and corresponding controls were analysed with relation to age groups. It was observed that 12 out of 34 positive sera of children below 1 year had a titre of 80 or more, 1 had a titre of 640, whereas the 2 positive control sera of this age group had titres of 40 and 10. Among the children aged 6–10 years, 8 out of 25 (32%) positive sera had titres of 80 or above with 5 having a value of 320. Among the controls one case each had titres of 80, 160 and 320. Fig. 4 shows that the difference between the controls and diseased groups in children aged below 3 years was much greater than this difference in higher age groups.

Cases of bad obstetric history

Out of 144 cases of bad obstetric history 127 (88%) showed antibodies while among the 50 controls with normal obstetric history 82% were seropositive (Table 4). There was not much difference in the incidence of antibodies in cases of still birth, spontaneous abortion, premature and malformed delivery. Antibody titres in these cases and controls were studied (Table 5). It was observed that 91

Table 5. *Rubella antibody titres in cases of bad obstetric history*

Cases	Reciprocal antibody titres				
	10	40	80	160	320
Spontaneous abortion	24	17	8	7	—
Still-birth	19	10	2	5	1
Malformed babies	5	11	5	7	—
Premature delivery	1	4	1	—	—
Controls	15	15	4	6	1

out of 127 (72%) positive sera showed titres $\leqslant 40$. A similar pattern of 73% of positive was noted in controls.

The statistical analysis of the incidence of seropositive cases both in controls and patients with different bad obstetric histories was done using 'large sample proportion test'. The P value was 2·83 when total incidence of positive sera in this group was compared with the total controls. The P value was 2·3 in both the groups of mothers who had spontaneous abortion and still-birth, while in mothers who delivered a malformed baby or had premature delivery it was 1·4, which is less than the standard value of 1·96. Therefore, it was concluded that the difference in the proportion of the positive sera in mothers with spontaneous abortion and still-birth when compared with the matching controls, is significant.

Studies on immunoglobulin M

A definite reduction in HI titre after treatment with 2-ME was noted in 17 cases. They included 5 cases of neonatal jaundice, 1 case each of anophthalmos and microcephaly and 10 cases of spontaneous abortion. It was observed that all the cases of congenital malformation who had a fall in titre were under 11 months of age. All the 10 cases of abortion who showed a reduction in titre had aborted within the first trimester of pregnancy.

DISCUSSION

Findings of the present study demonstrate that among children aged below 3 years the incidence of rubella antibodies in the eyes, C.N.S., visceral and miscellaneous groups was 64, 36, 93 and 71% respectively compared with 10% in the control group. This difference was statistically significant. Dudgeon (1967) and Gumpel, Hayes & Dudgeon (1971) have shown that presence of antibody in infants between 6 months and 4 years of age is strongly suggestive of intrauterine rubella. Thus our findings indicate the role of rubella virus in these cases. Such significant differences were not observed in cases of ear, heart and skeletal defects. This could be due to smaller sample size and a variety of clinical conditions included in each group.

It was further observed that in malformed children aged above 4 years there was no statistically significant difference in antibodies compared with controls. This may be due to infection with rubella virus which occurs in our population between 5 to 10 years of age (Mathur *et al.* 1974). When all the cases of congenital defects

were pooled in different age groups and compared with controls it was observed that the incidence of antibody in the former was statistically significant in age groups 0–1 year and 1–3 years (Fig. 3). This was not seen in older children. Another significant observation was the presence of higher titres in a larger number of cases of congenital defects than in the controls (Fig. 4). These findings are in line with those of Dudgeon (1967) and Gumpel *et al.* (1971). The role of rubella in the etiology of congenital malformations was further confirmed by demonstration of 2-mercaptoethanol-sensitive (IgM) antibodies in five cases of neonatal hepatitis and one case each of anophthalmos and microcephaly. It has been shown that 2-mercaptoethanol treatment of sera for detecting rubella specific IgM is a relatively insensitive method (Best, Banatvala & Watson, 1969). If more sensitive methods for detecting rubella-specific IgM had been available, an even higher proportion of infants with serological evidence of intra-uterine infection by rubella might have been detected.

In cases with bad obstetric histories the incidence of antibodies was statistically significant in cases of spontaneous abortion and still-birth. This was further confirmed by demonstration of IgM in 10 cases of spontaneous abortion. We are not aware of any rubella epidemic occurring here during the last 15 years. In some of the cases a history suggestive of rubella infection in mothers was available but in a number of cases the rubella infection was subclinical. It is quite likely that some cases of congenital rubella go unrecognized in India as no attempt is made to confirm the diagnosis virologically. In a previous study we noted that about 15–20% of females in Lucknow do not have rubella antibodies (Mathur *et al.* 1974). The findings of this study show that in some of these potential cases rubella infection did occur during pregnancy resulting in spontaneous abortion (at least in ten cases) or congenital malformations.

Congenital rubella was rarely noted in Japan even after extensive epidemics between 1966 and 1970 (Kono, Hayakawa, Hibi & Ishii, 1969; Potter, Banatvala & Best, 1973). This could be because the Japanese strain did not infect the conceptus (Kono *et al.* 1969) or it may have a greater capacity to produce interferon (Potter *et al.* 1973). Best *et al.* (1974) have discussed evidence both for and against the Japanese virus being non-teratogenic and have suggested that the biological response of Japanese women to rubella virus may be different. It is interesting to note that increased incidence of HL-A antigens 1 and 8 have been reported recently in cases of congenital rubella (Honeyman & Menser, 1974). They have further shown that these antigens are rarely encountered among Japanese while they are fairly common in India. This shows that, genetically, Indians are more prone to congenital rubella. This makes out a strong case for starting rubella vaccination in India also.

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