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ANALYSIS OF MEASLES CASES IN A UNIVERSITY PEDIATRIC HOSPITAL DURING 1988 AND 1993 OUTBREAKS*

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SUMMARY: Oğuz F, Sıdal M, Uzel N, Özgeneci A. (Department of Pediatrics, İstanbul University Faculty of Medicine, Çapa-İstanbul, Turkey). Analysis of measles cases in a University Pediatric Hospital during 1988 and 1993 outbreaks. Turk J Pediatr 1995; 37: 83-92.

In Turkey, a mass measles immunization campaign was initiated in 1985, and the decision was made to administer the first of the measles vaccinations at nine months of age instead of 12-15 months. Following the campaign there was a decrease in the number of measles cases seen in the Outpatient Department of İstanbul University Children's Hospital in 1986 and 1987; however, after 1987 an increase was observed in measles cases, which continued until 1993.

In order to investigate the current measles epidemics, we reevaluated the measles cases seen in our Outpatient Department from 1986 to 1993. We also investigated the vaccination status and the hospitalization and mortality rates of measles cases in the epidemics of 1988 and 1993.

Since 1988 (except 1989) a significant increase (412-1375 percent) has been observed in measles cases, and between 1986 and 1993 more than half of all measles cases were in children older than four years of age. In 1988 and 1993 we found that most vaccinated measles cases were also in this age group, but the rate of complications and hospitalization among the vaccinated cases was lower compared to those who were not vaccinated. *Key words: measles, vaccinated measles cases, measles complication, measles mortality.*

Despite the fact that measles vaccination has become a standard public health procedure since 1963, the disease continues to be a major cause of morbidity and mortality[1-4]. WHO recommends that underdeveloped and developing countries start administering the measles vaccination at nine months of age[5, 6].

In Turkey, a mass measles immunization campaign was initiated in 1985, and the decision was made to administer the first measles vaccination in the series at nine months of age instead of 12-15 months.

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In 1983, before the campaign, measles vaccination rates were 12.3 percent for nine-to-12-month-old children, and 36.5 percent for children ≤ 5 years old. With the campaign this rate had been increased to 72.4 and 82.5 percent respectively[7].

After that campaign the number of measles cases seen in the Outpatient Department of Çapa Children's Hospital, University of İstanbul, declined in 1986 and 1987[8]. But there was an increase in 1988 which continued until 1993 (except in 1989 when no increase was noted).

In order to determine the characteristics of the measles cases that have been seen in the Outpatient Department from 1986 to 1993, their records were reevaluated.

Material and Methods

At the Outpatient Department of Çapa Children's Hospital, University of İstanbul, patients aged 0 to 16 years receive an examination card and acquire a file in the computer. Diseases that should be declared are reported to the National Notifiable Disease Surveillance System.

The number of measles cases diagnosed in the outpatient clinic from 1986 to 1993 was determined from the annual outpatient data and statistics. The characteristics of the measles cases that had been seen in 1988 and 1993 in the outpatient department were determined by a retrospective review of the records of measles patients. The diagnosis of measles was made when there was a generalized maculopapular rash lasting three days or more, fever of 38.3 °C or greater, and the presence of a cough, coryza or conjunctivitis[9]. The records of hospitalized measles cases in 1988 and 1993 were also reviewed retrospectively.

Frequency of disease, complications, hospitalization, mortality rates, vaccination situation and clinical data were assessed in two age groups, namely ≤ 4 years and ≥ 5 years of age. Monthly distribution of disease was also investigated. Chi-square test was used in the statistical analysis.

Results

Although the number of measles cases seen in the Outpatient Department decreased in 1986 and 1987 (0.08% of all clinical cases), a peak was observed in 1988 (0.75%). The significant decrease in number (0.08%) in 1989 was followed by a slight increase (0.41%) in 1990 and another peak (1.18%) in 1991. This increase continued slowly in 1992 (0.87%) and reached a rate in 1993 similar to that of 1991 (1.13%). Although the aim of the study was not epidemiological, our hospital findings are in concordance with the data of İstanbul Province Health Directorate (unpublished information), only on a smaller scale (Fig. 1).

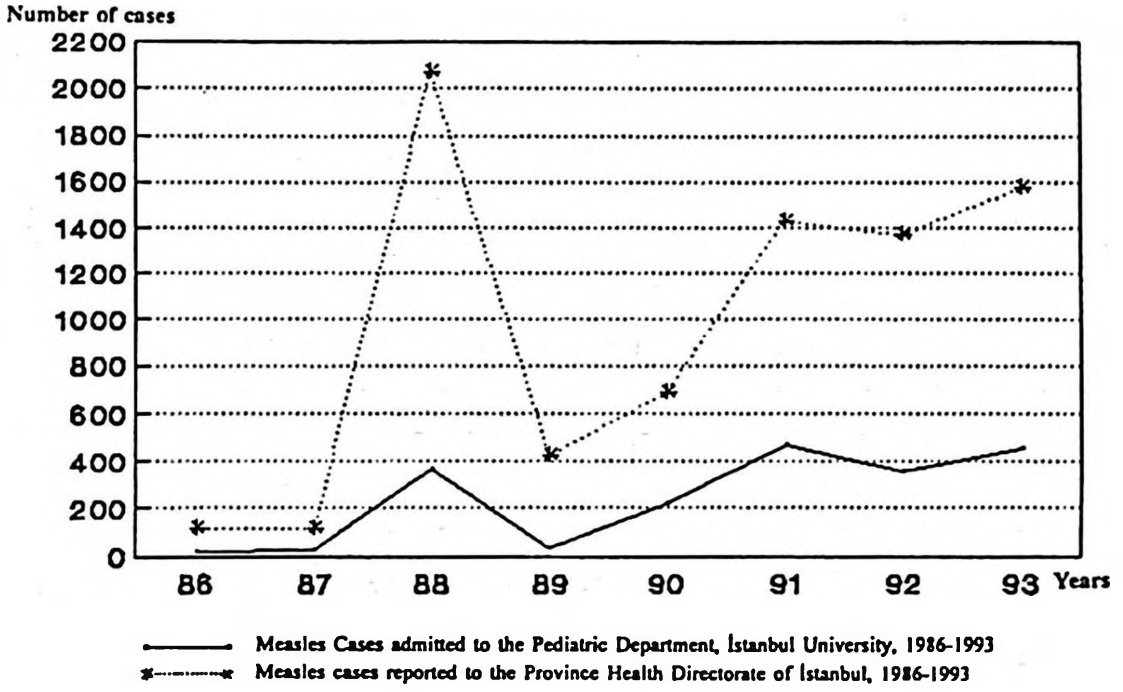

Fig. 1: The distribution of measles cases at outpatient clinics and at the Province Health Directorate of İstanbul.

Between 1986 and 1993, more than half of all measles cases were in children older than four years of age (Table I).

Table I: Age Distribution of Measles Cases, 1986 to 1993

	Number of Measles Cases		
Years	≤ 4 Years n (%)	≥ 5 Years n (%)	Total n
1986	8 (42.1)	11 (57.9)	19
1987	10 (41.7)	14 (58.3)	24
1988	154 (42.0)	213 (58.0)	367
1989	14 (40.0)	21 (60.0)	35
1990	96 (43.8)	123 (56.2)	219
1991	213 (45.9)	251 (54.1)	464
1992	151 (42.3)	206 (57.7)	357
1993	200 (44.1)	254 (55.9)	454

The decrease in measles epidemics observed in 1986 and 1987 was followed by an increase in 1988. Of the cases observed during the epidemics in 1988 and 1993, 42 percent of the cases in 1988 and 44 percent of the cases in 1993 were among younger children (≤ 4 yrs). Children in the 0-12-months-old group that were younger than nine months and consequently had not yet been vaccinated comprised 4.1 and 5.1 percent of the cases, respectively, in 1988 and 1993 (Table II). Two hundred sixtyfour (72%) of 367 measles cases in 1988, and 257 (56.6%) of 454 measles cases in 1993 had documentation of vaccination

against measles. In 1988, 44.7 percent of the 264 cases had a known history of vaccination and 57.3 percent of the 257 cases constituted vaccinated children in 1993. When these rates were compared, although high in both periods, the rates were found to be significantly higher in 1993 than in 1988 ($x^2 = 5.918224$, $p < 0.05$). The increase in 1993 was statistically significant only for the age group of ≤ 4 years ($x^2 = 5.189795$ $p < 0.05$) (Fig. 2).

Tablo II: Age Distribution of Measles Cases, 1988 and 1993

	Number of Measles Cases			
	1988		1993	
Age	n (%)		n (%)	
0-12 months	55[1] (15.0)		76[2] (16.7)	
		(42.0)		(44.0)
13 months-4 years	99 (27.0)		124 (27.3)	
≥ 5 years	213 (58.0)		254 (56.0)	
Total	367 (100.0)		454 (100.0)	

1.15 cases < 9 months.
2.23 cases < 9 months.

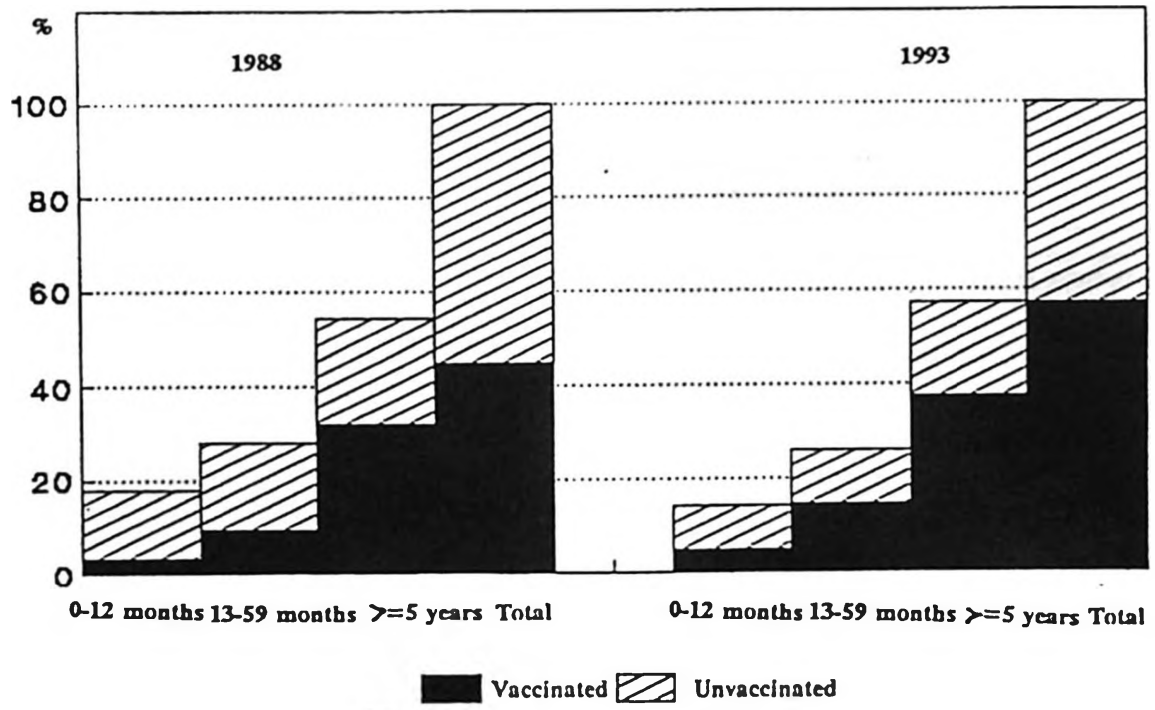

Fig. 2: Rates of vaccinated measles cases

The rate of the vaccinated measles cases correlated positively with the age of the children, and it was significantly higher in the ≥ 5 year-old children in 1988 ($x^2 = 23.67166$ $p < 0.001$) and 1993 ($x^2 = 16.12432$ $p < 0.001$). On the other hand, of measles cases with a known history of vaccination, 46.9 percent in 1988 and 45 percent in 1993 were children < 5 years of age (Fig. 2). Of unvaccinated

measles cases < 5 years old, 59.5 percent in 1988 and 37.5% in 1993 were of vaccine-eligible age (> 9 months).

Complications were noted in 36 percent of the cases in 1988 and 44.9 percent of the cases in 1993. The elevation in 1993 was found to be statistically significant ($x^2 = 638325$ $p < 0.05$). Among the vaccinated cases, a slight increase in the number of complications was observed in 1993 compared to 1988, but the difference is not statistically significant ($x^2 = 2.2233695$, $p = 0.1$).

As for the distribution of the complicated cases according to age, the rates were found to be higher in children ≤ 4 years of age in both 1988 and 1993. The complication rate was significantly higher in unvaccinated children compared to vaccinated children in 1988 ($x^2 = 38.18582$ $p < 0.001$) and 1993 ($x^2 = 29.52505$ $p < 0.001$) (Table III). In both years, the complication noted in over two-thirds of the cases was bronchopneumonia.

Table III: Age-Specific Measles Complication Rates in Vaccinated and Unvaccinated Children, 1988 and 1993

	Complication Rates					
	1988			1993		
Age	Vaccinated n (%)	Unvaccinated n (%)	Total n (%)	Vaccinated n (%)	Unvaccinated n (%)	Total n (%)
0-12 monts	1 (11.1) (29.4)[1]	27 (71.0) (65.5)	29 (52.7) (51.9) [60.2][2]	3 (23.1) (42.0)[1]	29 (80.6) (77.3)	48 (63.2) (64.0) [62.7][2]
13 months - 4 years	9 (36.0)	30 (61.2)	51 (51.5)	18 (48.7)	22 (73.3)	80 (64.5)
≥ 5 years	12 (14.3)	26 (44.1)	52 (24.4) [39.4]	19 (19.6)	17 (38.6)	76 (29.9) [37.3]
Total	22 (18.6)	83 (56.8)	132 (36.0)	40 (27.2)	68 (61.8)	204 (44.9)

1. Measles complication rate in 0-4 years age-group.
2. Percentage of complication in certain age-groups among total complications.

The percentage of patients hospitalized and treated due to severe complications was similar in 1988 and 1993 (4.4%) (Table IV). In 62.5 percent of cases in 1988 and 80 percent of cases in 1993, the reason for hospitalization was bronchopneumonia; the rest had been hospitalized for encephalitis. Encephalitis was observed mainly in ≥ 5-year-old children. Of hospitalized cases in 1988 and 1993, 68.8 and 70 percent, respectively, were children ≤ 4 years old. None of the cases died in 1988, and only one patient died due to bronchopneumonia in 1993. The rate of hospitalization for vaccinated children was equal in both years and significantly lower than for those not vaccinated. (For 1988, $x^2 = 4.308483$ $p < 0.05$ and for 1993, $x^2 = 8.196599$ $p < 0.01$).

In conclusion, although the number of vaccinated measles patients and the rate of complications was higher in 1993, the distribution of the cases by age-group

and complication rate as well as the findings of the hospitalized cases were similar in both years. As expected, the rate of hospitalization and complications among the vaccinated cases was lower than in those not vaccinated. Another point in common was the monthly distribution of the cases. In both years, measles cases occurred with a very low incidence in January. A small increase in February and March and a peak in May were observed (Fig. 3). Cases diagnosed between April and June comprised 88.4 percent of all measles cases in 1988 and 67.9 percent of all measles cases in 1993.

Table IV: Age-Specific Measles Hospitalization Rates in Vaccinated and Unvaccinated Children, 1988 and 1993

	Hospitalization Rates					
	1988			1993		
Age	Vaccinated n (%)	Unvaccinated n (%)	Total n (%)	Vaccinated n (%)	Unvaccinated n (%)	Total n (%)
0-12 monts	0 (0)	3 (7.9)	3 (5.5) (18.8)[2]	0 (0)	6 (16.7)	6[1] (7.9) 30.0
	(2.9)	(9.2)	(7.8) (68.8)	(4.0)	(15.2)	(7.0) (70.0)
13 months 4 years	1 (4.0)	5 (10.2)	8 (8.1) (50.0)	2 (5.4)	4 (13.3)	8 (6.5) (40.0)
≥ 5 years	1 (1.2)	4 (6.7)	5 (2.3) (31.2)	2 (2.1)	4 (9.1)	6 (2.4) (30.0)
Total	2 (1.8)	12 (8.2)	16 (4.4)	4 (2.7)	14 (12.7)	20 (4.4)

1. 4 Cases are < 9 months old, and one of them died because of bronchopneumonia.
2. Percentage of hospitalization in certain age-groups among total hospitalization.

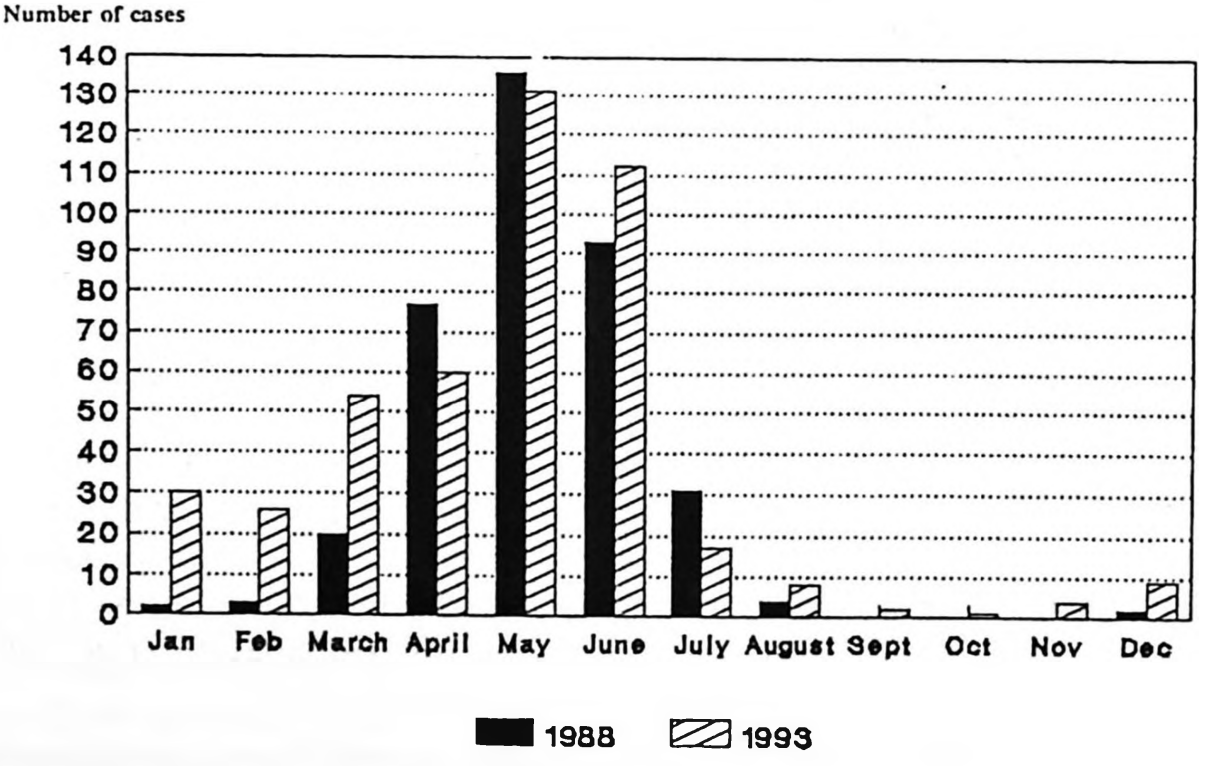

Fig. 3: Distribution of measles cases according to months (1988 and 1993).

Conclusion

The decrease of measles morbidity in our outpatient populations in 1986 and 1987 made us believe in the success of the campaign. During the epidemics of 1983, patients with measles comprised 3.5 percent of all patients in our clinic[10].

In 1986 and 1987 a remarkable decrease (97%) in this rate was observed. Since 1988 (except 1989), a significant increase (412% - 1375%) in measles cases has been seen, a rate which is in agreement with the data of İstanbul Province Health Directorate for the entire city of İstanbul. In spite of the increased rates after 1988, the rates were still lower (66%) than the 1983 rates, even in 1991 when the biggest peak was observed.

The vaccination rate (from 1986 to 1993) for ≤ 5 year-old children was 60-70 percent in İstanbul (data of İstanbul Province Health Directorate). Our data suggest that one of the factors that affected the increase in measles cases was failure to maintain the vaccination rates achieved during the campaign.

When we compared the age distribution of measles cases observed in the epidemics before and after 1985, we noted important differences. In 1983, 70 percent of all measles cases were in children ≤ 4 years of age[10], while after 1985 more than one-half of the measles cases occurred in ≥ 5 years old children. Two results are expected in a partially vaccinated population: a reduction in measles incidence greater than the level of vaccination coverage, and a shift in the age distribution of measles to older children[11-13]. These results are harmonious with our findings.

It is reported that immunization following vaccination after 15 months of age (Schwarz's type) is 95 percent, and the mean rate of primary inefficiency is 5 percent[14]. While only 5 percent or less of appropriately vaccinated children fail to respond, the high transmissibility of measles virus can lead to outbreaks[15-17]. As a result, the Practices Advisory Committee recommends that the second dose of vaccination be administered at the beginning of kindergarten or primary school[18], while the American Academy of Pediatrics recommends a second dose in secondary or high school[19]. When the vaccine is administered to nine-month-old children, the primary inefficiency rate rises to 20 percent[20, 21] and even 31 percent[22] or 37 percent[23]. If we consider Turkey, where vaccination is done routinely at nine months of age and the vaccination rate is around approximately 70 percent, the group at risk is larger and outbreaks can be easily observed. The rule of the cold-chain was not successful until 1985; no problems in the cold-chain were reported after this date[24]. In 1988, 44.7 percent of measles cases were among vaccinated children who were mostly five years old or older, We thought that this was primarily due to cold chain misutilization. In 1993, 57.2 percent of measles cases occurred in vaccinated children, and these cases also

accumulated in the ≥ 5 years group. The reason for this was considered to be the primary inefficiency of the nine-months-old vaccination. As a result, we believed that in both years primary vaccine failure was the cause of measles outbreaks, which mostly occurred in vaccinated children, and the low immunization coverage rates support these outbreaks. The fact that half of the cases observed during the epidemic in Etimesgut, Ankara, occurred among children vaccinated at nine months old[25] supports our thesis.

In the United States, measles epidemics occurring primarily in vaccinated children have been reported[15, 26-28]. During the epidemics of 1989-1990 in the United States, the efficiency of the vaccine was investigated and was found to be approximately 93-98 percent which was similar to rates obtained in previous years. It was also found that vaccine coverage rates among preschool-age children in some cities were 60 percent or lower. According to this data, it was believed that failure to vaccinate rather than vaccine failure was an important cause for the 1989 and 1990 measles epidemics. In this study, it was also demonstrated that 50 percent of the cases were in children under five years old, and 80 percent were unvaccinated although they were of vaccine-eligible age. In our cases, the vaccine efficiency was not investigated, but the rates of children in the < 5-years age-group that were unvaccinated but vaccine-eligible were lower when compared with the rates found in the United States. The incidence of the disease was lower for the unvaccinated younger age groups, and higher for the vaccinated school children in our country when compared with the USA where vaccination is performed routinely beginning at 15 months of age. These results show that vaccination at nine months is beneficial for the young age but becomes a disadvantage as the child grows older.

On the other hand, in 1983 when vaccination was routine after 12-15 months, 27.5 percent of the outpatients and 44 percent of the inpatients were among 0-12-month-old children. After the vaccination age changed to nine months, these rates diminished to 15.1 and 18.8 percent, respectively, in 1988, and 16.7 and 30 percent, respectively, in 1993. During those years, a significant decrease was observed both in rates of mortality and hospitalization compared to 1983. For hospitalization, the rate was shown to be 90 percent reduced in both years, and the mortality rate was shown to be 100 percent reduced in 1988 and 91 percent reduced in 1993. It is obvious that vaccination at nine months diminishes the rate of mortality and morbidity, especially in infants. Although 63 percent immunization provided by vaccination at nine months old carries the benefit of being protective at least until 15 months, these children are under more risk (15%) after the 15th month compared with those vaccinated at 15 months of age.

As a result, and in confirmation of previous studies[15, 29-31] which showed that vaccination before 15 months is a risk factor, we found that it elevated the primary

vaccination inefficiency. Despite this disadvantage of early vaccination, the rates of both mortality and morbidity are diminished, especially in the ≤ 12-month-old age-group. On the other hand, if the vaccination rate is approximately 60-70 percent, an epidemic would not be avoided but its severity would be limited, and the hospitalization and mortality rates would be diminished. If the vaccination rate greater than 80 percent, the incidence decreases significantly. These findings are consistent with those of other researchers[26, 32].

With the information above, we recommend the following to achieve a decrease in epidemics in our country: continuing with the practice of vaccination at nine months, raising the vaccination rates for the first five years and particularly for the first two years, and giving a second opportunity to children who have not been vaccinated or have had primary vaccination inefficiency.

REFERENCES

1. Aaby P. Malnutrition and overcrowding intensive exposure in severe measles infection: review of community studies. Rev Infect Dis 1988; 10: 478-491.
2. Assaad F. Measles: summary of worldwide impact. Rev Infect Dis 1983; 5: 452-459.
3. Borgono JM. Current impact of measles in Latin America. Rev Infect Dis 1983; 5: 417-421.
4. Henderson RH, Keja J, Hayden G, Galazka A, Clements J, Chan C. Immunizing the children of the world: progress and prospects. Bull WHO 1988; 66: 535-543.
5. Ministry of Health of Kenya and World Health Organization. Measles immunity in the first year after birth and the optimum age for vaccination in Kenyan Children. Bull WHO 1977; 55: 21-31.
6. Heymann DL, Mayben GK, Murphy KR, Guyer G, Foster SO. Measles control in Yaounde: justification of a one dose, nine month minimum age vaccination policy in tropical Africa. Lancet 1983; 2: 1470-1472.
7. Tunçbilek E, Ulusoy M, Hancıoğlu A, et al. Hızlandırılmış aşı kampanyası sonrasında 0-5 yaşlarındaki çocukların aşılanma durumu ve kampanya öncesi ile karşılaştırılması. (Nüfusbilim Dergisi) Turk J Popul Stud 1986; 8: 5-35.
8. Uzel N, Sıdal M, Oğuz F, Neyzi O. İstanbul'da 1988 yılında görülen kızamık vakalarının özellikleri. XXXII. Türkiye Milli Pediatri kongresi. 26-28 Ekim, 1988, Ankara. Bildiri Özet Kitabı, 1988: 11.
9. Centers for Disease Control. Classification of measles cases and categorization of measles elimination programs. MMWR 1983; 31: 707-711.
10. Uzel N, Yaka M, Yazıcıoğlu M, Aydoğan Ü. Acil birimine başvuran kızamık vakalarının değerlendirilmesi. İstanbul Tıp Fakültesi VII. Kurultayı. Kurultay serbest bildiri özetleri p. 19, İstanbul, 1983.
11. Dabis F,.Sow A, Waldman RJ, et al. The epidemiology of measles in a partially vaccinated population in an African city: implications for immunization programs. Am J Epidemiol 1988; 127: 171-178.
12. Levy MH, Bridges-Webb C. "Just one shot" is not enough-measles control and eradication. Med J Aust 1990; 152: 489-491.
13. Taylor WR, Mambu RK, ma-Disu M, Weinman JM. Measles control efforts in urban Africa complicated by high incidence of measles in the first year of life. Am J Epidemiol 1988; 127: 788-794.

14. King GE, Markowitz LE, Patriarca PA, Dales LG. Clinical efficacy of measles vaccine during the 1990 measles epidemic. Pediatr Infect Dis J 1991; 10: 883-887.

15. Centers for Disease Control: Measles: United States, 1989 and first 20 weeks. MMWR 1990; 39: 361-363.

16. Brunell PA. Measles vaccine-one or two doses? Pediatrics 1988; 81: 722-724.

17. Gustafson TL, Lievens AW, Brunell PA, Moellenberg RG, Buttery CM, Sehulster LM. Measles outbreak in a fully immunized secondary-school population. N Engl J Med 1987; 316: 771-774.

18. Centers for Disease Control. Measles prevention: recommendations of Immunization Practices Advisory Committee (ACIP). MMWR 1989; 38 (Suppl 9): 1-18.

19. American Academy of Pediatrics. Commitee on Infectious Diseases. Measles: Reassessment of the current immunization policy. Pediatrics 1989; 84: 1110-1113.

20. Berry S, Hernandez H, Kanashiro R, et al: Comparison of high titer Edmonston-Zagreb, Biken-CAM and Schwarz measles vaccines in Peruvian infants. Pediatr Infect Dis J 1992; 11: 822-827.

21. Job JS, Halsey NA, Boulos R, et al. Successful immunization of infants at 6 months of age with high dose Edmonston-Zagreb measles vaccine. Pediatr Infect Dis J 1991; 10: 303-311.

22. Tidjani O, Grunitsky B, Guerin N, et al: Serological effects of Edmonston-Zagreb, Schwarz, and AIK-C measles vaccine strains given at ages 4-5 or 8-10 months. Lancet 1989; 2: 1357-1360.

23. Expanded Programme on Immunization seroconvertion after measles immunization. Wkly Epidemiol Rec 1981; 30: 234-237.

24. The Situation Analysis of Mothers and Children in Turkey, Goverment of Turkey-UNICEF Programme of cooperation. Country Programme, 1991-1995. April 1991; 167-175.

25. Ceyhan M, Kanra G, Vargel S, Işıkçelik Y. The evaluation of vaccination against measles at nine months of age: report of an epidemic. Turk J Pediatr 1992; 34: 127-133.

26. Schlenker TL, Bain C, Baughman AL, Hadler SC. Measles herd immunity: the association of attack rates with immunization rates in preschool children. JAMA 1992; 267: 823-826.

27. Markowitz LE, Preblud SR, Fine PE, Orenstein WA. Duration of live measles vaccine-induced immunity. Pediatr Infect Dis J 1990; 9: 101-110.

28. Grimes DE, Woolbert LF. Measles outbreaks: who are at risk and why. J Pediatr Health Care 1989; 3: 187-193.

29. Mast EE, Berg JL, Hanrahan LP, Wassell JT, Davis JP. Risk factors for measles in a previously vaccinated population and cost-effectiveness of revaccination strategies. JAMA 1990; 264: 2529-2533.

30. Chen RT, Goldbaum GM, Wassilak SG, Markowitz LE, Orenstein WA. An explosive point-source measles outbreak in a highly vaccinated population: modes of transmission and risk factors for disease. Am J Epidemiol 1989; 129: 173-182.

31. McCombie SC, Worrell RD, Porter BW, Browning JA, Ray GG. Risk for measles related to immunization status in two Tucson high schools. Public Health Rep 1988; 103: 162-166.

32. Centers for Disease Control. Progress toward achieving the national 1990 objectives for immunization. MMWR 1988; 37: 613-617.

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RESULTS OF VACCINATED INFANTS BORN TO HBsAg – POSITIVE MOTHERS WITH DIFFERENT HEPATITIS B VACCINES AND DOSES*

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*Zuhal Sağlam MD***, *Ahmet Alver MD***

SUMMARY: Kuru Ü, Turan Ö, Kuru N, Sağlam Z, Alver A. (Department of Pediatrics, Bakırköy Maternity and Children's Social Security Hospital, İstanbul, Turkey). Results of vaccinated infants born to HBsAg-positive mothers with different hepatitis B vaccines and doses. Turk J Pediatr 1995; 37: 93-102.

Seventy-eight infants born to HBsAg-positive women were randomly assigned to receive either the plasma-derived vaccine or 0.5 ml (10 µg HBsAg) yeast-derived recombinant hepatitis B vaccine within 24 hours of birth, simultaneously with hepatitis B hyperimmunoglobulin. In 67 infants who received the plasma-derived vaccine, one of the doses of 0.5 ml (2.5 µg HBsAg) was used randomly. In all of the infants, the second and third doses of both vaccines were given at one and two months of age, respectively. The booster doses were given at 12 month of age in all of the infants. These vaccinated infants were followed up until 13 months of age.

There were differences in the seroconversion rates with different vaccines and doses. The recipients of the half-dose of plasma-derived vaccine showed lower seroconversion rates than the others, and the newborns in this group showed more seronegativity (13.2%) than the others ($p < 0.05$).

The lowest anti-HBs geometric mean titers (GMTs) were obtained in newborns vaccinated with Hevac B 0.5 ml. Sixty percent of the anti-HBs GMTs in this group were under 100 mIU/ml.

There were statistically significant differences between males and females in anti-HBs seronegativity rates, with males having lower anti-HBs GMTs than females. The difference was particulary significant among male newborns vaccinated with a half-dose of plasma-drived vaccine. *Key words: hepatitis B virus, hepatitis B vaccine, immunization, reduced vaccination schedule, sex.*

Transmission of the hepatitis B virus (HBV) during the perinatal period leads to the most devastating consequences of HBV infection. Women who are positive for the surface antigen of HBV (HBsAg) at the time of delivery and who have hepatitis Be antigen (HBeAg) in their sera have a 70 percent chance or greater of transmitting the infection to their newborn infants. At least 90 percent of these infected infants become chronic carriers of HBV.These HBV-carrier children have

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a life-time risk of dying from primary hepatocellular carcinoma or cirrhosis, usually during adulthood[1]. In addition, they serve as a reservoir of HBV infection for their families and communities[2,3].

Fortunately, treatment of these newborns shortly after birth with a combination of hepatitis B immune globulin (HBIg) and hepatitis B vaccine is 85-95 percent effective in preventing development of the HBV-chronic carrier state[4]. Both of the hepatitis B (HB) vaccines, plasma-derived vaccine and recombinant vaccine, are available in Turkey. When these vaccines are used by physicians in indicated cases, the dose and vaccination schedule which are advised by the manufacturer must be applied. However, in Turkey both of these HB vaccines are used in newborns of HBV-carrier-mothers at a dose of 0.5 ml, usually at zero, one and six months of age. However, the plasma-derived vaccine, Hevac B, is recommended by the manufacturer to be used at a dose of 1 ml (5 µg) and in a schedule of zero, one, two and 12 months of age for newborns of HBsAg-positive carriers.

Our study was conducted to determine the long-term effect of immunization against HBV infection with a one-half-dose of the plasma-derived vaccine, as well as to examine any differences in response to the hepatitis B vaccines between males and females.

Material and Methods

Hepatitis B Immunoglobulin and Passive Immunization:

Immuneglobulin containing 200 IU/ml of the antibody to HBsAg (Anti-HBs) (Hepuman Berna, Switzerland) was used within 24 hours of birth. HBIg was applied intramuscularly to the anterolateral thigh at a dose of 0.5 ml.

Vaccination and Active Immunization Schedule:

Active immunization was accomplished with a total of four injections of two different vaccines. Plasma-derived (Hevac B, Pasteur Institute) and recombinant vaccine (Engerix B, Smith Kline Biologicals) were used in the study. The plasma-derived vaccine was composed of 5 µg/ml of HBsAg, and the recombinant vaccine consisted of 20 µg/ml of HBsAg. Two doses of plasma-derived vaccine were used randomly: 0.5 ml (2.5 µg HBsAg) and 1 ml (5 µg HBsAg). The dose of recombinant vaccine which we used was 0.5 ml (10 µg HBsAg). These vaccines and doses were used in newborns randomly. The manufacturer of yeast-derived recombinant vaccine has marketed this vaccine only recently in Turkey; thus the number of cases vaccinated with this vaccine was lower than that of plasma-derived vaccine. The first doses were given within 24 hours after birth, as was HBIg. The second dose was administered at one month of age, and the third at two months of age. The booster doses were given at twelve months of age.

Mothers and Infants:

A total of 5366 pregnant Turkish women living in İstanbul and attending antenatal clinics of Bakırköy Maternity and Children's Social Security Hospital between February 1, 1991, and December 1, 1991, were screened for HBsAg by Elisa. The prevalence of HBV infection among the pregnant women was 4.2 percent.

Two hundred and twenty-five pregnant women with HBsAg-positive tests were informed of their status and of the risk of infection for their infants. Seventy-eight healthy full-term infants (33 males and 45 females) born to HBsAg-positive mothers were included in the study. All of these infants were vaccinated with one of the vaccines and doses, and HBIg was administered in all cases with 24 hours after birth. All of these infants were followed up for 13 months. Seven of the 78 mothers were both HBsAg and HBeAg-positive, and 71 of 78 were positive for HBsAg and the antibody to hepatitis Be antigen (anti-HBe).

Of the 78, twenty-five infants were vaccinated with Hevac B 0.5 ml, 42 infants with Hevac B 1 ml, and 11 infants with Engerix B 0.5 ml.

Laboratory Tests:

All of the infants were followed up until 13 months of age and were revisited at one, two, three, six, nine, 12 and 13 months of age. At the time of their follow-up visits, their blood samples were obtained by venipuncture. Serum was separated from clot within one hour of collection and stored at -30 °C until assayed and studied within 15 days. All of the HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe) and the antibody to hepatitis B core antigen [anti-HBc]) were evaluated. Anti-HBs values were expressed in geometrical mean titer (GMT) as milli-international units per millilitre (mIU/ml) Anti-HBs values were found by diluting the serum samples between 1/2 and 1/4096 according to the case. World Health Organization (WHO) standard anti-HBs serum which contained 10 mIU/ml anti-HBs was used as a reference. The absorbance value of the final positive dilution was multiplied by a dilution factor, and anti-HBs was calculated as mIU/ml.

It was accepted that there was seroconversion after the hepatitis B vaccination when the anti-HBs titer was greater than or equal to 10 mIU/ml.

Statistics:

Data were analyzed with statistical software (Extatix and StatView^{Tm}) by MacIntosh LC computer. Student's t test, Chi-Square, Kruskal Wallis and Mann Whitney U tests were used according to the situation. A p value of < 0.05 was considered to be significant.

Results

The seroconversion rates with different vaccines and different doses are presented in Table I. There was a statistical difference in seronegativeness and vaccination, with Hevac B 0.5 ml having the lowest seroconversion rate overall ($p < 0.05$).

Table I: Seroconversion Rates Which are Obtained with Different Hepatitis B Vaccinations

Vaccine	Dose ml	Total Test Number	Anti-HBs < 10 mIU/ml n	%	Anti-HBs ≥ 10 mIU/ml n	%
Hevac B	0.5	135	20	14.8	115	85.2
Hevac B	1	269	15	5.6	254	94.4
Engerix B	0.5	70	5	7.1	65	92.9

($p < 0.05$, chi-square)

Protective levels of anti-HBs with different vaccines and different doses at different time intervals are shown in Table II. One month after the booster dose, seroconversion rates were 97-100 percent in all groups. However, the seroconversion rates in the group vaccinated with Hevac B 0.5 ml were lower than the other groups in the first three months, and the difference in the first month was statistically significant ($p < 0.05$).

Table II: Seroconversions at Different Time Intervals with Different HB Vaccinations

Vaccine	Dose ml (µg)	n	Anti-HBs > 10 mIU/ml (%) Months 1	2	3	6	9	12	13
Hevac B	0.5 (2.5)	25	17/24 (70.8)	19/22 (86.4)	19/22 (86.4)	22/23 (95.7)	18/21 (85.7)	20/23 (87)	25/25 (100)
Hevac B	1 (5)	42	36/38 (94.7)	38/39 (97.4)	36/39 (92.3)	35/36 (97.2)	33/34 (97.1)	25/31 (85.4)	41/42 (97.6)
Engerix B	0.5 (10)	11	10/11 (91.9)	11/11 (100)	10/10 (100)	5/6 (83.3)	8/10 (80)	10/11 (91.9)	11/11 (100)

Table III shows the distributions of GMTs of anti-HBs at different time intervals. The lowest GMTs were obtained in the group of infants who were vaccinated with Hevac B 0.5 ml, but it was not statistically significant ($p > 0.05$). When we compared the anti-HBs GMTs obtained by different vaccines and doses according to months, there were statisticaly significant differences at one and six months ($p \leq 0.05$). As shown in Table IV, 60 percent of anti-HBs titers were less than 100 mIU/ml with Hevac B 0.5 ml usage. However, there was no statistical difference between the groups.

Table III: Anti-HBs GMTs at Different Time Intervals with Different Hepatitis B Vaccine and Dose

Vaccine	Dose ml	GMTs (mIU/ml) Months 1	2	3	6	9	12	13
Hevac B	0.5	19.9	30.4	23.4	51.2	113	119	1882
Hevac B	1	68	49.2	51	216	290	131	2997
Engerix B	0.5	50	35.7	68.2	43.5	108.5	122	7698

Table IV: Anti-HBs GMTs According to the Vaccines and Doses

Vaccine	Dose ml (µg)	n*	GMTs (mIU/ml) (%)				
			< 10	10-99	100-999	1000-10000	> 10000
Hevac B	0.5 (2.5)	160	20/160 (12.5)	76/160 (47.5)	40/160 (25)	19/160 (11.9)	5/1600 (3.1)
	1 (5)	269	15/269 (5.5)	108/269 (40)	88/269 (32.7)	44/269 (16.3)	15/269 (5.5)
Engerix B	0.5	70	5/70	31/70	18/70	11/70	5/70
	10		(7.1)	(44.4)	(25.7)	(15.7)	(7.1)

* n: shows total test numbers ($p > 0.05$), Chi-square).

Table V shows seroconversion rates according to sex. Of 215 tests done during a period of 13 months, 25 seronegative cases were found (13.2%) in the male newborn group. But among female newborns, the seronegative rate was 4.94 percent (14/283) and there was a statistically significant difference between sexes ($p < 0.01$). We concluded that male newborns showed more seronegativeness than females.

Table V: Seroconversion Rates According to Sex

Sex	n	Anti-HBs > 10 mIU/ml (%) Months						
		1	2	3	6	9	12	13
Male	33	26/32 (81.3)	30/31 (96.8)	31/33 (94)	25/27 (92.6)	21/27 (77.8)	24/32 (75)	33/33 (100)
Female	45	41/41 (100)	36/40 (90)	36/38 (94.7)	34/38 (89.5)	37/38 (97.4)	41/43 (95.3)	44/45 (97.8)

As for the GMTs according to sex, male newborns showed lower anti-HBs titers than female newborns; these results were statistically significant until 12 months ($p \leq 0.05$), but after the booster doses the difference was not significant ($p > 0.05$). Female newborns showed higher anti-HBs GMTs than males (Table VI).

Table VI: Anti-HBs GMTs According to Sex at Different Time Intervals

Sex	n	Anti-HBs GMTs mIU/ml (n) Months						
		1	2	3	6	9	12	13
Male	33	31.6 (32)	40.1 (31)	32.5 (33)	66.7 (27)	57.3 (27)	46.6 (32)	2261 (33)
Female	45	54.2 (41)	44.4 (40)	51.9 (38)	163.6 (38)	421 (38)	264 (43)	3504 (45)

Table VII shows anti-HBs GMTs at different times according to sex, vaccine and dose. There was a statistical difference between males who were vaccinated with Hevac B 0.5 ml and female newborns who were vaccinated with 1 ml Hevac B before the booster dose ($p < 0.05$). After the booster doses there was no significant difference.

Table VII: Anti-HBs GMTs Which were Obtained with Different Vaccines and Doses and Changes According to Sex

				GMTs (mIU/ml)						
				Months						
Sex	Vaccine	Dose (ml)	n	1	2	3	6	9	12	13
Male	Hevac B	0.5	10	10	36.6	23.6	55.1	36.6	52.1	1160
		1	17	62.3	49.7	32.7	117	133	42.5	2806
	Engerix B	0.5	6	42.7	27.7	54.4	13.5	14.2	49.6	3720
Female	Hevac B	0.5	15	32.7	23.2	23.2	48.8	264.2	223	2610
		1	25	72.5	61	81.8	364	469	270	3018
	Engerix B	0.5	5	62.4	48.5	96.8	104	822	350	18410

The same significant difference occurred when comparing male newborns vaccinated with Hevac B 0.5 ml and female newborns vaccinated with Engerix B 0.5 ml ($p < 0.05$). However, there was no significant difference between female newborns vaccinated with Hevac B 0.5 ml and male newborns vaccinated with Hevac B 1 ml ($p > 0.05$). The male newborns vaccinated with Hevac B 0.5 ml showed lower anti-HBs GMTs than male newborns vaccinated with Hevac B 1 ml, but there was no statistically significant difference between the two groups ($p > 0.05$). The female newborns vaccinated with Hevac B 0.5 ml had lower anti-HBs GMTs than those vaccinated with Hevac B 1 ml, and there was a significant difference between the two groups before the booster doses ($p < 0.05$).

Discussion

HBV-related liver diseases continue to be important health-care problems in developing countries. In areas of hyperendemicity, HBV is transmitted from asymptomatic carrier-mothers to their babies, especially when mothers are seropositive for hepatitis Be antigen. Almost all of such babies become persistent HBsAg carriers and constitute a reservoir of HBV for further spreading the virus in their families and the community. Also, chronic carriers of HBsAg are at high risk of developing chronic liver diseases such as cirrhosis and primary hepatocellular carcinoma later in life.

Vaccination with the hepatitis B vaccine and HBIg at birth is a safe and effective way of preventing HBV infection in endemic areas[5-7]. In order to eradicate HBV,

measures must be taken not only to interrupt mother-to-baby transmission in the perinatal period, but also to prevent horizontal transmission in later life[8, 9]. To date, approximately 50,000,000 doses of plasma-derived hepatitis B vaccine have been administered around the world. Although the vaccine is prepared from material obtained from the blood of HBsAg carriers, there have been no cases of acquired immune deficiency syndrome (AIDS) in plasma-derived hepatitis B vaccine recipients[9]. It has also been shown that recombinant-hepatitis B vaccines are effective and safe vaccines to prevent HBV infections[10].

The major factor affecting the immunogenicity will be the structure and source of antigen. HBsAg is a glycosylated protein composed of pre-S and s gene encoded regions. Vaccines differ in mode of purification and inactivation. In the process of isolation and purification from plasma, the structure of native HBsAg could be affected. Therefore recommendations from manifacturers as to the optimal doses and vaccination schedule of different vaccines which will produce 90 to 95 percent seroconversion, as well as suitable geometric mean titers at the end of immunization, can differ. Between two required criteria of HB vaccine for effective immunization, the dose which produces a 90-95 percent seroconversion rate is reasonable and universally accepted. The criteria for high titers of anti-HBs is more controversial. Some authors believe that once immunized above the so-called protective level of 10 mU/ml, subjects retain immunologic memory and sufficient immunity to be resistant to carrier-state infections even in the absence of detectable anti-HBs titers[11, 12]. But there are some conflicting data about this conclusion.

In our study, 78 infants born to HBsAg-positive mothers underwent active-passive immunization starting in the newborn period. In all groups, 97-100 percent of vaccinated infants showed protective antibody titers at 13 months of age. These high rates of seroconversion, which were obtained by hepatitis B vaccines, were also found in other studies[13-15]. Although the number of yeast-derived vaccines was lower than plasma-derived vaccines in our study, the vaccines elicited similiar immune responses. We also saw no side effects in the infants after the vaccination.

Although hepatitis B vaccine produces reliable immunity to infection by HBV, the high cost of the vaccine diminishes common use of it. Recombinant hepatitis B vaccines decreased this high cost, but did not solve the problem. The doses and the numbers of vaccinations were reduced to diminish the costs and were shown effective in several studies[16-18]. The intradermal route (id) was thought to be an alternative way to immunize against hepatitis B infection. Several studies have indicated that the less expensive id route will induce or boost immunity[19-20]. Some investigators have found that immunity following id vaccination was delayed and resulted in lower serum concentrations of anti-HBs than those observed following intramuscular vaccination[21]. Lee et al[22]. have demonstrated that infants

vaccinated with low-dose hepatitis B vaccine were more likely produce the HBV carrier state than those vaccinated with the full dose of vaccine. They concluded that low doses of hepatitis B vaccine and the id route were not recommended in newborns.

In our study, the low GMTs of anti-HBs and the high percentages of seronegativity were obtained after administration of the half-dose of plasma-derived vaccine. This can be explained by low presentation of HBsAg to the newborn and/or deficiency in their immune systems in this period. The low anti-HBs titers and high rates of seronegativity after low-dose hepatitis B vaccination were obtained in the first three months of age. We know that these infants are more dependent on their HBsAg-positive mothers in these first months than in other months of their lives. Despite hepatitis B vaccination, infants may be at risk of hepatitis B infection because of low immune response. We have planned to follow up the infants in our study for five years. Most of the infants are three years old now. HBsAg positivity was determined in one infant at two years of age. He was vaccinated with the half-dose plasma-derived vaccine and showed seropositivity at the 13-month follow-up. However, he was a low responder. Therefore, although some authors concluded that high anti-HBs titers were not important, and nor were high seroconversion rates at the end of hepatitis B vaccination as in our low-dose vaccinated infant pattern, HBV can in fact be obtained later by vaccinated infants who show seroconversion but have low anti-HBs titers. In our study, the vaccination schedule was chosen to be at birth and at one, two and 12 months of age. If a half-dose of plasma-derived vaccine were administered in three doses of hepatitis B vaccine, which is the schedule usually used in our country (at birth and at one and six months of age), then lower seroconversion rates and anti-HBs titers would be obtained. This means that more infants would be at risk of HBV infection by horizontal transmission.

It has been demonstrated by some investigators that female hepatitis B vaccine recipients had better seroconversion rates and higher anti-HBs titers than male recipients[23-25]. We too have found higher seroconversion rates and anti-HBs titers in female than male infants. There was a statistically significant lower response in male infants who were vaccinated with the half-dose plasma-derived vaccine.

Both plasma-derived and recombinant hepatitis B vaccine are available in Turkey. Both are effective and safe vaccines. There are some differences in the doses and vaccination schedules of different hepatitis B vaccines. We conclude that all newborns of HBsAg-positive mothers must be vaccinated at the dose and schedule which is advised by the manufacturer of that vaccine.

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REFERENCES

1. Balistreri WF. Viral hepatitis. Pediatr Clin North Am 1988; 35: 375-401.
2. Kuru Ü, Turan O, Ceylan Y, et al. Gebelerde HBsAg taşıyıcılığı sıklığı. Klimik Dergisi 1992; 5: 19-22.
3. Editorial. Routine prenatal screening for hepatitis B surface antigen. JAMA 1988; 259: 408-409.
4. West DJ, Calandra GB, Ellis RW. Vaccination of infants and children against hepatitis B. Pediatr Clin North Am 1990; 37: 585-601.
5. Soulie JC, Devilier P, Santarelli J, et al. Immunogenicity and safety in newborns of a recombinant hepatitis B new vaccine containing S and pre-S2 antigens. Vaccine 1991; 9: 545-548.
6. The Gambia Hepatitis Study Group. Hepatitis B vaccine in the expanded programme of immunisation. The Gambian experience. Lancet 1989; ii: 1057-1059.
7. Tsai YT, Lo KJ, Lee SD, Wang JY, Wu TC. Immunoprophylaxis against hepatitis B virus infection: a controlled trial in infants born to HBeAg-negative HBsAg carrier mothers in Taiwan. Chin J Gastroenterol 1984; 1: 181-185.
8. Committee on Infectious Diseases. Prevention of hepatitis B virus infections. Pediatrics 1985; 75: 362-364.
9. Prince AM. Control of hepatitis B virus infection in third-world countries. Transfusion Medicine Reviews 1990; 4: 187-190.
10 Yeoh EK, Chang WK, Ip P, Chan KH, Chan E, Fung C. Efficacy and safety of recombinant hepatitis B vaccine in infants born to HBsAg-positive mothers. J Infec 1986; 13 (suppl A): 15-18.
11 Hadler SC, Francis DP, Maynard JE, et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986; 315: 209-214.
12. Berger R, Just M, Andre F, Safary A. Boosting against hepatitis B: must it be done when titres disappear? in Zuckerman AJ (ed). Viral hepatitis and Liver Diseases. New York: Alan R. Liss Inc; 1988: 1006-1008.
13. Coursaget P, Yvonnet B, Chotard J, et al. Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 1986; ii: 1143-1144.
14. Beasley RP, Hwang LY, Lee GCY, et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983; ii: 1099-1102.
15. Maupas P, Chiron JP, Barin F, Coursaget P, Goudeau A. Efficacy of hepatitis B vaccine in prevention of early HBsAg carrier state in children. Lancet 1981; i: 289-292.
16. Piazza M. Hepatitis B immunisation with a reduced number of doses in newborn and children. Hepatitis B and Vaccination Symposium. Bruxelles, March 19, 1986.
17. King JW, Taylor EM, Crow SD, et al. Comparison of the immunogenicity of hepatitis B vaccine administered intradermally and intramuscularly. Rev Infec Dis 1990; 12: 1035-1043.
18. Theppisai U, Thanuntaseth C, Chiewsilp P, Siripoonya P. Prevention of hepatitis B infection in infants born to hepatitis B carrier mothers: low dosage vaccination. Int J Gynaecol Obstet 1990; 32: 353-357.
19. Redfield RR, Innis BL, Scott RM, Cannon HG, Bancroft WH. Clinical evaluation of low-dose intradermally administered hepatitis B virus vaccine. A cost reduction strategy. JAMA 1985; 254: 3203-3206.

20. Horowitz MM, Ershler WB, McKinney WP, Battiola RJ. Duration of immunity after hepatitis B vaccination: efficacy of low-dose booster vaccine. Ann Intern Med 1988; 108: 185-189.

21. Zoulek G, Borbeer B, Jilg W, Deinhardt F. Evaluation of a reduced dose of hepatitis B vaccine administered intradermally. J Med Virol 1984; 14: 27-32.

22. Lee CY, Hwang LY, Beasley RP. Low-dose hepatitis B vaccine. Lancet 1989; ii: 860-861.

23. Pongpipat D, Suvatte V, Assateerawatts A, Bhethraratt S. Active pre-exposure immunisation against hepatitis B virus immunogenicity of hepatitis B vaccine in healthy Thai adults and children. Asian Pacific J Allerg and Immunol 1987; 5: 63-65.

24. Szmuness W, Stevens CE, Harley EJ, et al. The immune response of healthy adults to a reduced dose of hepatitis B vaccine. J Med Virol 1981; 8: 123-129.

25. Delage G, Prince SR, Montplaisir S. Combined active-passive immunization against the hepatitis B virus: five-year-follow-up of children born to hepatitis B surface antigen-positive mothers. Pediatr Infect Dis J 1993; 12: 126-130.

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TRANSCATHETER CLOSURE OF THE DUCTUS ARTERIOSUS IN CHILDREN AND YOUNG ADULTS*

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SUMMARY: Aydoğan Ü, Dindar A, Cantez T, Ertuğrul T, Tanman B, Ayhan Yİ, Alpay T. (Cardiology Unit, Department of Pediatrics, İstanbul University Faculty of Medicine, Çapa-İstanbul, Turkey). Transcatheter closure of the ductus arteriosus in children and young adults. Turk J Pediatr 1995; 37: 103-109.

Transcatheter occlusion of persistent patent ductus arteriosus (PDA) was attempted in 32 patients (22 female and 10 male, mean age 5.12 ± 3.98 years, range 9 months to 19.2 years) using Rashkind's occluder device (USCI). Implantation of a second occluder device was attempted in three of the patients. Device embolization to a pulmonary artery occurred in three patients, all with the 12 mm occluder device; two of these devices were retrieved by grabber catheter and the last with thoracotomy without adverse sequelae. Embolization to the right atrium occurred in another patient during a second device implantation attempt because of fluoroscopy problems; this patient required open-heart surgery with sequala of 2 (+) tricuspid insufficiency. In another patient with a significant shunt after the implantation of a 17 mm occluder device, mechanical hemolysis developed, but surgical intervention was not required. The overall complication rate was five out of 35 implantation procedures (14.3%). Besides these, sublingual nifedipine was required for two patients whose systolic blood pressure exceeded 160 mmHg just after the implantation procedure.

Sixteen 12 mm and fifteen 17 mm occluder devices were successfully and uneventfully implanted in the first procedure, except for two patients in whom a 17 mm occluder device was implanted after retrieval of an embolized 12 mm occluder. Overall early and mid-term complete occlusion was achieved in 24 patients (75%). Complete occlusion of PDA in the first days after the procedure was achieved in all patients, with the narrowest ductal diameter of less-than 3 mm with the 12 mm occluder device, and less than 6 mm with the 17 mm occluder device. Catheter occlusion of the PDA using Rashkind's umbrella appears to be a safe and effective method of nonsurgical management. However, good fluoroscopy, an experienced team, careful monitoring of systemic blood pressure, a good patient selection and a preference for the 17 mm occluder device when possible is highly recommended. *Key words: interventional cardiology, patent ductus arteriosus congenital heart disease.*

Successful transcatheter occlusion of persistent patent ductus arteriosus (PDA) offers a number of advantages over surgical intervention. It avoids general anesthesia, thoracotomy pain, a chest scar, extended hospitalization, a prolonged

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convalescence period, psychological trauma, the possible need for blood products and injury to the recurrent laryngeal nerve. The early transarterial approaches suffered from a relatively high rate of complications[1], but the development of the double umbrella occlusion device and the application of a transvenous technique for its placement have stimulated renewed interest in transcatheter occlusion[2,3]. We outline our 2.5 year experience with 32 patients who underwent occlusion procedures using the Rashkind device.

Material and Methods

Patients: Between August 1991 and March 1994, parents of patients diagnosed clinically by two-dimensional and color Doppler examination to have persistent patent ductus arteriosus appropriate for a transcatheter occlusion procedure were offered the options of surgical division or transcatheter occlusion with the Rashking PDA occlusion device. Patients whose parents preferred the surgical approach underwent elective division of the PDA without a preceding cardiac catheterization. Others were admitted electively for diagnostic cardiac catheterization in order to identify the ductal anatomy and to measure the narrowest diameter of the ductus.

Thirty-two patients, 22 female and 10 male, ages nine months to 19.2 years (median 4.44 years, mean ± standard deviation 5.12 ± 3.98 years) with weights of 8.6 to 46.0 kilograms (median 14.7 kg, mean ± standard deviation 16.24 ± 8.04 kg) participated in the study. One of the patients had Down's syndrome, while another had a bicuspid aorta with moderate aortic regurgitation as the associated anomalies.

Occlusion Technique: All patients were sedated one hour before the intervention with the combination of chlorpromazine 0.3 mg/kg, pheniramine 0.5 mg/kg and pethidine 1 mg/kg. Ketamine 0.5-1.0 mg/kg was also given intravenously before the start of the implantation procedure when it was needed. The procedure was performed from the right femoral vein by using 8 or 11-F sheath (for the 12 mm and 17 mm occlusion devices, respectively) and by following previously described techniques[3-5]. Of note, for the arterial approach, a 4-F pig tail catheter was introduced through a 5-F sheath in patients weighing more than 12.0 kg in order to facilitate continuous monitoring of arterial pressure from the side port during the implantation procedure. In patients weighing less than 12.0 kg a 4-F sheath and pig tail catheter were used; thus, pressure monitoring was interrupted during the implantation procedure.

Echocardiographic-Doppler Examination: All patients underwent a complete two-dimensional echocardiogram and color-flow Doppler examination within the first three days of occlusion and at six-month intervals during follow-up. Studies were performed using previously described techniques[6] on a Hewlett-Packard SONOS 1000 system, using either a 3.5 or 5.0 MHz transducer.

Results

12 mm Occluder Device: The 12 mm occluder device was used in 19 patients (60%) with the narrowest diameters ranging from 1.5 to 5.0 mm. The patients' ages ranged from nine months to 10.3 years (median 4.56 years, mean ± 2.4 years), and their weights ranged from 8.6 to 22.0 kg (median 14.4 kg, mean ± standard deviation 14.44 ± 4.33 kg). Successful implantation of the occluder device without any complication was achieved on the first attempt in 15 patients (79%). Of these, an anterograde approach with a femoral vein-femoral artery wire loop technique was required in the two patients with the narrowest diameters < 2 mm[7].

In the remaining four patients, the proximal arms of the occluder device did not open completely although all of them were in the appropriate position and gentle traction had been maintained on the safety wire with advancement of the sheath against the proximal legs after the prosthesis was extruded from the sheath. As a result, the tip of the safety wire became entangled in the foam covering after release. Dislodgement was successful in one of these four patients by performing the technique described above[4]. Embolization of the occluder device to the pulmonary artery occurred in the remaining three patients during the trial of dislodgement procedure, two to the right and one to the left. In the first two patients it was retrieved uneventfully from the pulmonary artery with the use of a grabber catheter (multipurpose grasping forceps; Microvasive, Boston Scientific Corp., Watertown, Mass) and was withdrawn into a larger sheath in the pulmonary artery; the sheath was then withdrawn. One of these patients received another 17 mm device during the same procedure, and the other at a later date.

In the third patient, weighing 8.6 kg, the occluder device embolized to the proximal left pulmonary artery, This patient required surgical PDA division and retrieval of the device by thoracotomy; surgery was uneventful.

As a result, solely the 12 mm occluder device implantation was used in 17 patients (50.3%) with the narrowest ductal diameters ranging from 1.5 to 5.0 mm. Among them, one patient (6.25%) required surgical PDA ligation and retrieval of the embolization device by thoracotomy. In the remaining 16 patients, the narrowest duct diameter was less than 3 mm in five, 3 mm in five, 4 mm in four, and 5 mm in two patients. Among these, complete occlusion of the ductus in the first three days of the post-occlusion period was achieved in all patients, with the narrowest duct diameter being less than 3 mm. In the patients with 4 mm ductal diameter, complete occlusion in the early post-occlusion period was observed in one patient and during follow-up in another. Residual leakage with only systolic murmur still persisted in the other two patients at one and 12 months follow-up, respectively. In both of the 5 mm ductal diameter patients, residual shunting persisted in the early post-occlusion period. Complete occlusion

occurred in one during follow-up, but a second 12 mm occluder device with a femoral vein-femoral artery loop technique was required for the second patient six months later to achieve complete occlusion.

17 mm Occluder Device : A 17 mm occluder device was used in the remaining 15 patients, including the two patients in whom the 12 mm occluder embolized to the right pulmonary artery (40.67% + 6.25% = 46.92%) with the narrowest ductal diameters ranging from 4 to 11 mm; the patients' ages ranged from 1.24 to 19.24 years (median 3.0 years, mean + standard deviation 5.68 ± 5.28 years), and their weights ranged from 9.3 to 46.0 kg (median 12.0 kg, mean ± standard deviation 17.71 ± 10.85 kg). Three patients weighed less than 10.0 kg, and eight weighed less than 12.0 kg. Successful implantation of the occluder device was achieved without any complication in all of the patients in the initial procedure.

Then narrowest duct diameter in these patients was 4 and 5 mm in three each 6, 7 and 8 mm in two each; and 9, 10 and 11 mm in one each, respectively. Among these, complete occlusion of the PDA in the first three days of the post-occlusion period was achieved in all patients whose narrowest ductal diameter less than 6 mm, and in another patient whose narrowest ductal diameter was 7 mm. The remaining six patients (40%) had residual leakages or significant shunts after the initial implantation procedure.

The patient with a 9 mm duct needed a second 12 mm occluder in order to achieve complete occlusion. In another patient with a ductal diameter of 10 mm, a second 17 mm occluder device implantation was also attempted; however, fluoroscopic visualization of the landmarks and occluder was inadequate to ensure proper placement, so retrieval of the occluder device was attempted after both the distal and proximal arms of the occluder were opened. Withdrawing it into the Mullins sheath was unsuccessful, and it became entangled in the chordae of the tricuspid valve and required open-heart surgical removal. A 2 (+) tricuspid insufficiency was found on color Doppler examination during follow-up of this patient.

In another patient whose narrowest ductal diameter was 11 mm, significant mechanical hemolysis developed eight hours after the implantation and continued for 26 days, requiring six blood transfusions. This patient did not come for follow-up after discharge. The remaining three patients in the 17 mm occluder device group still have leakages rather than a significant shunt and are followed by color Doppler examinations.

Overall Results : The number of completely occluded patients in the first three days after the transcatheter PDA occlusion procedure with a single device implantation, including the two patients in whom a 17 mm occluder was implanted after the retrieval of the 12 mm occluder device, was 20/32 (62.5%).

This number increased to 24 (75%) with the addition of two patients in whom complete occlusion had occurred during mid-term follow-up and another two with a second occluder device implantation.

Occluder device embolizations occurred in four patients (12.5%, three with 12 mm occluder and one with a second 17 mm occluder). Transcatheter retrieval was uneventful in two; but one of the others needed uneventful thoracotomy and the last open-heart surgery with a sequala of 2 (+) tricuspid insufficiency. In another patient, significant mechanical hemolysis developed after the implantation procedure. The overall complication rate was five in 35 implantations (14.3%).

Besides the above-mentioned complications, sublingual nifedipine administration was needed for two completely occluded patients just after the implantation procedure due to systolic blood pressure increases to 176 and 161 mmHg.

Discussion

Rashkind et al[2] recommended the 12 mm occluder for PDA of less than 5 mm in diameter. The overall incidence of residual shunting was 8 percent in their group. However, they did not specify in their study whether this result was from the first day or from later follow-up. On the other hand, the incidence of residual shunting was reported to be 32% percent on the day of the procedure in another study in which the 12 mm occluder was utilized for PDA up to 5 mm[6]. The incidence of ductal shunting was also high in the series in which an upper limit of 4 mm ductal diameter had been selected for the 12 mm occluder[4,8]. On the contrary, Latson[8] and Wessel[4] in two separate series with the upper limit of 3 mm, reported residual shunting of 16 and 4 percent, respectively. Complete occlusion also was unsuccessful in our two patients in whom the 12 mm occluder device was implanted with a 4 mm ductal diameter. In these two patients, residual leakage persisted after mid-term follow-up. Although it has been shown that ductal shunting may disappear as late as 40 months after the occlusion procedure[6], we recommend the 17 mm occluder device for PDA larger than 3 mm if the weight of the patient is permissible (> 10 kg). As a general rule, after inserting an 8-F catheter through the ductus, if aortography shows a significant left-to-right shunt around the catheter, then a 17 mm occluder should be selected for the occlusion. On the other hand, the 17 mm occluder device has relatively stronger springs on the proximal arms and is more stable, so that the arms can open widely after the occluder is extruded from the sheath and entanglement of the safety wire in the foam covering after release may be avoided.

We could not accomplish complete occlusion in any PDA larger than 7 mm with a single 17 mm Rashkind occluder. This result supports Hosking's[6] findings that one may risk residual shunting and require a second occluder implant if an attempt

is made to occlude a PDA larger than 7 mm with the Rashkind device. The report of the European Registry on transcatheter occlusion of persistent arterial duct[10] and Hosking's[6] study showed a higher proportion of patients with residual flow when the larger device (17 mm) was used. This finding may be related to the relatively stronger springs on the arms of the occluder device that may change the shape of the long, tubular ductus, or the ductus with a long, narrow aortic end other than to the size of the duct as the authors of the European Registry[10] emphasized. Besides these, changing the rectangular configuration of the 17 mm occluder device to a cricle or polygon may be of further benefit in complete occlusion of the larger ducts.

Mechanical hemolysis in incompletely occluded patients is an uncommon complication of transcatheter PDA occlusion. Ladusans et al.[11] performed surgical intervention for such a complication, and Grifka et al.12 developed a technique for retrieving the leaking occluder. In our series, it was speculated that progressive clot formation around the device would eventually cover the rough surface of the occluder and surgical intervention could be avoided. The rate of hemolysis did decrease by the fifth day after the procedure and completely ceased by the twenty-sixth day. However, multiple transfusions were required. Multiple blood transfusions are associated with some major risks such as contamination with hepatitis-B, AIDS and other infections, and precise serological controls are necessary.

Systemic hypertension crisis just after the occlusion procedure may be the natural result of abrupt cessation of vacsular failure related to left-to-right shunting through PDA and still circulating, high levels of angiotensin[13] other than volume overload and ketamine anesthesia. The case of the patient who developed transient hemiparesis during surgical PDA ligation without ketamine anesthesia in O'LAughin et al'.s report[14] supports this hypothesis.

We conclude that transcatheter PDA occlusion with the Rashking occluder device is a safe and effective method of nonsurgical management, but good fluoroscopy, an experienced team, careful monitoring of systemic blood pressure, good patient selection and a preference for the 17 mm occluder device when possible will result in favorable results.

REFERENCES

1. Porstman W, Wierny L, Warnke H, et al. Catheter closure of patent ductus arteriosus: 62 cases treated without thoracotomy. Radiol Clin North Am 1971; 9: 203-218.
2. Rashkind WJ, Mullins CE, Hellenbrand WE, Tait MA. Nonsurgical closure of patent ductus arteriosus: clinical application of the Rashkind PDA Occluder System. Circulation 1987; 75: 583-592.
3. Bash SE, Mullins CE. Insertion of patent ductus occluder by transvenous approach: a new technique. Circulation 1984; 70 (Suppl II): 285.

4. Wessel DL, Keane JF, Parness I, Lock JE. Outpatient closure of the patent ductus arteriosus. Circulation 1988; 77: 1068-1071.

5. Dyck JD, Benson LN, Smallhorn JF, McLaughlin PR, Freedon RM, Rowe RD. Catheter occlusion of the persistently patent ductus arteriosus. Am J Cardiol 1988; 62: 1089-1092.

6. Hosking MC, Benson LN, Musewe N, Dyck JD, Freedom RM. Transcatheter occlusion of the persistently patent ductus arteriosus; forty-month follow-up and prevalence of residual shunting. Circulation 1991; 84: 2313-2317.

7. Benson LN, Dyck J, Hecht B. Technique for closure of the small patent ductus arteriosus using the Rashkind occluder. Cathet Cardiovasc Diagn 1988; 14: 82-84.

8. Ali Khan MA, Mullins CE, Nihill MR, et al. Percutaneous catheter closure of the ductus arteriosus in children and young adults. Am J Cardiol 1989; 64: 218-221.

9. Latson LA, Hofschire PJ, Kugler JD, Cheatham JP, Gumbiner CH, Danford DA. Transcatheter closure of patent ductus arteriosus in pediatric patients. J Pediatr 1989; 115: 549-553.

10. Transcatheter occlusion of persistent arterial duct. Report of the European Registry. Lancet 1992; 340: 1062-1066.

11. Ladusans EJ, Murdoch I, Franciosi J. Severe haemolysis after percutaneous closure of a ductus arteriosus (arterial duct). Br Heart J 1989; 61: 548-550.

12. Grifka RG, O'Laughin MP, Mullins CE. Late transcatheter removal of a Rashkind PDA occlusion device for persistent hemolysis using a modified transseptal sheath. Cathet Cardiovasc Diagn 1992; 25: 140-143.

13. Aydoğan Ü, Cantez T, Ertuğrul E, et al. Effect of angiotensin-I levels on systemic blood pressure in transcatheter occlusion of patent ductus arteriosus. Arch Turkish Society of Cardiol 1993; 21: 352-354.

14. O'Laughlin MP, Nihill MR, Mullins CE. Patent ductus arteriosus occlusion-results in 205 procedures. Circulation 1990; 82 (Suppl-III): 582.

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PHARMACOKINETICS OF AMIKACIN, NETILMICIN AND TOBRAMYCIN IN CHILDREN WITH CHRONIC RENAL FAILURE*

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SUMMARY: Nayır A, Şirin A, Emre S, Eti Ş, Yeşilbek T. (Nephrology Unit, Department of Pediatrics, İstanbul University Faculty of Medicine, Çapa-İstanbul, Turkey). Pharmacokinetics of amikacin, netilmicin and tobramycin in children with chronic renal failure. Turk J Pediatr 1995; 37: 111-116.

The single-dose pharmacokinetics of amikacin, netilmicin and tobramycin administered intramuscularly at doses of 7.5 mg/kg amikacin, 2.5 mg/kg netilmicin or 3 mg/kg tobramycin were studied in 30 children with chronic renal failure. Serum amikacin, netilmicin and tobramycin levels were measured by a radioimmunoassay method. The correlation between serum creatinine levels and the half-life of the antibiotics was found to be statistically significant. There were interpatient differences in serum aminoglycoside levels among those with the same serum creatinine levels. Thus, monitoring of serum creatinine and aminoglycoside levels is recommended, especially for those with renal failure, in order to maintain an optimal dosage between toxic and noneffective serum aminoglycoside levels. *Key words: chronic renal failure, pharmacokinetics, amikacin, netilmicin, tobramycin.*

Pediatric patients experience unique differences from adults in pharmacokinetic parameters[1]. Medications used in pediatric medicine often lack dosage guidelines for this group. Most drugs and their metabolites are excreted fully or partially by the kidney; therefore accumulation and toxicity can develop rapidly if dosages are not adjusted in patients with impaired renal function[2-4]. The margin between the therapeutic and toxic concentrations for any aminoglycoside antibiotic is narrow. The dosage of aminoglycosides should be adjusted in patients with renal failure to avoid adverse effects and to ensure efficacy[5, 6].

In pediatric chronic renal patients, the use of aminoglycosides carries important risks, such as different pharmacokinetic properties in the patient, the toxic effect of aminoglycosides and drug modification difficulties in renal failure. To our knowledge, pharmacokinetic studies of aminoglycosides have not been reported in the literature on pediatric patients with chronic renal failure (CRF).

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This study was conducted in order to investigate the pharmacokinetics of amikacin, tobramycin and netilmicin in children with CRF.

Material and Methods

Thirty children between two and 13 years of age (median age seven) with CRF were recruited into six groups of five (1a, 1b, 2a, 2b, 3a, 3b) according to their serum creatinine (Scr) levels. Fifteen other children with normal renal function between the ages of two and 15 years undergoing aminoglycoside therapy were chosen at random to comprise the control groups (1c, 2c, 3c) (Table I). A dosage of 7.5 mg/kg of amikacin was administered by intramuscular (IM) injection to group 1a (Scr greater than 4 mg/dl), group 1b (Scr 3-4 mg/dl) and group 1c. A dosage of 2.5 mg/kg netilmicin was administered by intramuscular injection to group 2a (Scr 2-3 mg/dl), group 2b (Scr 1-2 mg/dl) and group 2c. A dosage of 3 mg/kg of tobramycin was administered IM to group 3a (Scr 2-3 mg/dl), group 3b (Scr 1-2 mg/kg) and group 3c.

Serum samples were collected at zero, one, four, and 24 hours postinjection from patients with normal renal function; zero, one, four, eight, 24 and 48 hours postinjection in patients with Scr between 1-3 mg/dl; and at zero, one, four, 24, 48 and 72 hours postinjection in patients with Scr greater than 4 mg/dl.

Serum amikacin, netilmicin and tobramycin were measured by radioimmunoassay method (Coat-A-Count Amikacin, Coat-A Count Netilmicin and Coat-A-Count Tobramycin kits from Diagnostic Products Corporation). Biochemical analyses were performed by standard methods.

Kinetics: Kinetic parameters of the amikacin, netilmicin and tobramycin groups were calculated by linear regression analysis[6]. Serum kinetic parameters were derived after a single dose of amikacin, netilmicin and tobramycin. Calculations were done as follows: the area under curve (AUC) by trapezoidal rule; the elimination rate constant (k_e) by regression analysis of the terminal phase; elimination half-life ($t_{1/2}$) from the reciprocal of the terminal slope (B) time log2; volume of distribution (Vd) by dose/C_0 where C_0 intercepts the elimination slope at time 0; and total body clearance by (Vd.B).

Statistical analyses: values are expressed as means ± SD. To compare the significance between measurements, the Mann-Whitney U test for unpaired data was used. Linear regression analysis was performed to study the relationship between quantitative variables.

Table I: The Characteristics of the Groups

	Group 1 a Amikacin Scr > 4 mg	Group 1b Amikacin Scr: 3-4 mg	Group 1c Amikacin Scr < 1 mg (control)	Group 2a Netilmicin Scr: 2-3 mg	Group 2b Netilmicin Scr: 1-2 mg	Group 2c Netilmicin Scr < 1 mg (control)	Group 3a Tobramycin Scr: 2-3 mg	Group 3b Tobramycin Scr: 1-2 mg	Group 3c Tobramycin Scr < 1 mg (control)
Age (year)	11.2 ± 1.3	9.8 ± 2.8	6.1 ± 3.9	5.5 ± 2.4	6.1 ± 1.8	9.3 ± 4.9	7.2 ± 2.4	9.9 ± 2.8	6.4 ± 2.6
Sex (F/M)	3/2	2/3	2/3	4/1	3/2	4/1	3/2	2/3	3/2
Weight (kg)	26.9 ± 4.5	21 ± 5.6	20.1 ± 8	17.3 ± 5.2	18.3 ± 4	31.8 ± 17.8	17.3 ± 3.9	22.3 ± 5.6	21.2 ± 5.8
Urea (mg/dl)	19.3 ± 16	151.2 ± 18.4	23.6 ± 3.4	74.8 ± 14	58.2 ± 5.4	24.4 ± 3.2	81.6 ± 7.8	59.8 ± 3	22.4 ± 3.1
Creatinine (mg/dl)	4.8 ± 0.5	3.32 ± 02	0.57 ± 0.2	2.38 ± 0.3	1.44 ± 0.2	0.69 ± 0.2	2.5 ± 0.2	1.57 ± 0.1	0.6 ± 0.2
Creatinine clearance ($ml/min/1.73m^2$)	14.41 ± 0.8	20.1 ± 3	109.2 ± 30	24.74 ± 4.2	42.4 ± 8.9	100.4 ± 9.7	23.6 ± 2.5	36 ± 5.6	102.3 ± 30.1

Results

There was substantial interpatient variation in drug elimination and clearance values in each group (Table II). Peak serum levels (first hour) for amikacin in group 1a ranged from 27 to 60 mg/L, in group 2a for netilmicin from 8.8 to 12 mg/L, and in group 3a for tobramycin from 9.7 to 10.05 mg/L. There was a linear correlation between the serum creatinine and serum half-life of amikacin, netilmicin and tobramycin (r values were 0.82, 0.86 and 0.78, respectively). The serum half-life time values ($t_{1/2}$) of aminoglycosides in each group were found to be as follows: for amikacin 1a = 31h, 1b = 18.7h, 1c = 4.4h; for netilmicin 2a = 13.9h, 2b = 7.3h, 2c = 7.6h; and for tobramycin 3a = 8.2h, 3b = 6.6h, 3c = 3.7h. The linear correlation (r) between serum drug concentration and Scr for the 24th hour was found to be statistically significant for each group; r values were as follows: amikacin 0.79 ($p < 0.05$), netilmicin 0.9 ($p < 0.005$) and tobramycin 0.61 ($p < 0.05$). Other pharmacokinetic parameters are given in Table III.

Table II: Serum Drug Levels (µg/ml) of The Groups

			Hours						
Antibiotic	Groups		0	1.	4.	8.	24.	48.	72.
Amikacin	1a	mean ± SD	0	44 ± 11.9	44 ± 13.1		17.4 ± 9.3	13.9 ± 6.5	9.3 ± 5.8
		range		(27-60)	(32-58)		(14-31)	(7.6-23.5)	(3.5-16)
	1b	mean ± SD	0	36.2 ± 5.4	31.6 ± 2.4		14.2 ± 5.2	6.8 ± 2.8	2.2 ± 1.9
		range		(31-43)	(25-39)		(9-23)	(4-11)	(0.5-5)
	1c	mean ± SD	0	30.3 ± 7.6	6.7 ± 2.3		0.5 ± 0.8		
		range		(19-42)	(3-10)		(0-1.9)		
Netilmicin	2a	mean ± SD	0	9.9 ± 1.2	5 ± 0.18	3.06 ± 0.48	0.96 ± 0.17	0.48 ± 0.3	
		range		(8.8-12)	(4.8-5.2)	(2.5-3.8)	(0.8-1.2)	(0.12-0.8)	
	2b	mean ± SD	0	7.94 ± 1.4	4.14 ± 0.7	2.0 ± 0.23	0.21 ± 0.04	0.08 ± 0.05	
		range		(6.4-9.8)	(3.2-5.1)	(1.7-2.2)	(0.18-0.28)	(0-0.12)	
	2c	mean ± SD	0	9.12 ± 1.08	2.98 ± 0.49	0.9 ± 0.49	0.11 ± 0.07		
		range		(7.6-10.2)	(2.5-3.8)	(0.4-1.12)	(0-0.2)		
Tobramycin	3a	mean ± SD	0	11.31 ± 2.01	6.25 ± 2.45	5.74 ± 2.74	2.35 ± 2.09	0.16 ± 0.08	
		range		(9.7-10.05)	(4.4-10)	(2.7-8.7)	(0.82-5.2)	(0.1-0.26)	
	3b	mean ± SD	0	10.06 ± 0.4	4.56 ± 0.16	2.81 ± 0.89	0.5 ± 0.21	0.06 ± 0.07	
		range		(9.6-10.6)	(4.4-4.8)	(1.85-3.9)	(0.34-0.8)	(0,0.14)	
	3c	mean ± SD	0	6.98 ± 2.64	2.29 ± 0.75	0.58 ± 0.35	0.06 ± 0.1		
		range		(3.8-10.5)	(1.15-3.1)	(0.17-0.9)	(0-0.23)		

Table III: Pharmacokinetic Parameters of the Groups

Antibiotic	Groups	k_e (h^{-1})	$t_{1/2}$ (h)	Vd (l/kg)	AUC (µg.h/ml)	Cl (ml/kg.h)	C_0 (mg/l)
	1a	0.022	31	0.19	1534	4.9	39.8
Amikacin	1b	0.037	18.7	0.2	990.3	7.6	37.1
	1c	0.16	4.4	0.34	131.6	57	22
	2a	0.05	13.8	0.39	97.5	25.4	6.47
Metilmicin	2b	0.095	7.25	0.47	51.8	48.1	5.26
	2c	0.095	7.26	0.37	35.1	71.1	6.75
	3a	0.08	8.1	0.3	148.2	20.2	11
Tobramycin	3b	0.1	6.6	0.39	70.3	42	7.8
	3c	0.18	3.7	0.59	25.3	117.9	5.1

k_e : Elimination rate constant. $t_{1/2}$: Elimination half-life time.
Vd : Volume of distribution. AUC : Area under the curve.
Cl : Drug clearance. C_0 : the value which intercepts the elimination slope at time 0.

In patients with CRF, drug peak values were higher than those of the control group, but the differences were not statistically significant, No toxicity was observed after the single dose of antibiotics.

Discussion

The present study shows that amikacin, netilmicin and tobramycin have in common a prolonged half-life (proportional to the serum creatinine level) in pediatric patients with reduced renal function. Pediatric patients with normal serum creatinine levels demonstrated a significantly enhanced clearance and a shorter half-life compared to those patients with impaired renal function. Although there is a good correlation between serum creatinine and the half-life of aminoglycoside antibiotics, it was not absolute. Indeed the clinician can use the serum creatinine level for pediatric patients as a guideline for dosage modification if other pharmacokinetic dosage calculations are not available[7-10].

All patients in the study received the usual intial dose for a patient with normal renal function, since in practice the same loading dose is used for both groups. The drug peak values were higher in patients with CRF than in the control groups, but the difference was statistically insignificant. In the amikacin group, one patient with a creatinine level greater than 4 mg/dl had a peak serum level of 60 µg/ml. In conventional aminoglycoside treatment with three times daily dosing, therapeutic peak serum levels between 6 and 12 µg/ml generally recommended for both tobramycin and netilmicin. The corresponding value for amikacin is 24 to 48 µg/ml[11]. This study suggests that in patients with severe renal impairment, peak serum levels may be higher than those in the study group and reduction of the loading dose could be necessary. This result should be interpreted with caution because our sample groups were small. The data requires confirmation by further studies involving more patients.

Results showed that the adjustment of dose intervals in patients with renal failure should be the mode of choice. This usage is associated with improved antibacterial activity and reduced risk of adverse effects[12].

Large inter-individual variations of the pharmacokinetics and serum concentrations were observed in both children with normal renal function and those with chronic renal failure. Drug dosages needed for optimal therapeutic effects differ widely among individuals[13, 14]. The "usual dose" of most potent drugs can accomplish little in some individuals it can cause serious toxicity in some, and is fully satisfactory in few. Obviously large individual differences exist regarding the various half-lives of aminoglycosides. The habitual administration of the conventional dose can be satisfactory only when a drug's therapeutic margin is very large and when its full therapeutic potential is not required. On the contrary,

the therapeutic margin of aminoglycosides is very narrow, and a certain level is necessary for antibacterial effect[15]. Therefore the standard dose schedule of the aminoglycosides is to be considered only as a clinical guideline and should be supplemented with serum concentration measurements whenever possible. Although drug level monitoring may provide a more precise tool to ensure therapeutic efficacy and safety, this service is expensive and not available in every hospital.

In conclusion, dose schedules suggested for adults can also be used for pediatric patients. But they should only serve as crude clinical gudelines and should be supplemented with serum concentration measurements whenever possible[2]. In pediatric patients with CRF, this drug monitoring is especially mandatory for therapy with aminoglycosides.

REFERENCES

1. Skaer TL. Dosing considerations in the pediatric patient. Clin Ther 1991; 13: 526-544.
2. Swan SK, Bennett WM. Drug dosing guidelines in patients with renal failure. West J Med 1992; 156: 633-638.
3. Bennett WM. Guide to drug dosage in renal failure. Clin Pharmacokinet 1988; 15: 326-354.
4. Aronoff GR. Practical therapeutics in patients with renal impairment (editorial). West J Med 1992; 156: 664.
5. Kaloyanides GJ. Renal pharmacology of aminoglycoside antibiotics. Contrib Nephrol 1984; 42: 148-167.
6. Kaloyanides GJ, Pastoriza-Munoz E. Aminoglycoside nephrotoxicity. Kidney Int 1980; 18: 571-582.
7. Sarubbi FA Jr, Hull JH. Amikacin serum concentrations: prediction of levels and dosage guidelines. Ann Intern Med 1978; 89: 612-618.
8. Rowland M, Tozer TN. Clinical Pharmacokinetics. Philadelphia: Lea and Febiger; 1989: 113-177.
9. Dahlgren JG, Anderson ET, Hewitt WL. Gentamicin blood levels: a guide to nephrotoxicity. Antimicrob Agents Chemother 1974; 8: 58-68.
10. Lesar TS, Rotschafer JC, Strang LM, Solem LD, Zaske DE. Gentamicin dosing errors with four commonly used nomograms. JAMA 1982; 248: 1190-1193.
11. Hickling K, Begg E, Moore ML. A prospective randomised trial comparing individualised pharmacokinetic dosage prediction for aminoglycosides with prediction based on estimated creatinine clearance in critically ill patients. Intensive Care Med 1989; 5: 233-237.
12. Craig WA, Vogelman B. The postantibiotic effect. Ann Intern Med 1987; 106: 900-902.
13. Schentag JJ, Jusko WJ. Renal clearance and tissue accumulation of gentamicin. Clin Pharmacol Ther 1977; 22: 364-370.
14. Verpooten GA, Giuliano RA, Verbist L, Eestermans G, De Broe ME. Once-daily dosing decreases renal accumulation of gentamicin and netilmicin. Clin Pharmacol Ther 1989; 45: 22-27.
15. Brogard JM, Comte F, Spack MO. Nephrotoxicity and aminoglycosides: effects on pharmacokinetics and prevention. Contrib Nephrol 1984; 42: 182-195.

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SKIN INVOLVEMENT IN CHILDREN WITH NON – HODGKIN'S LYMPHOMA*

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SUMMARY: Büyükpamukçu M, İlhan İ, Çağlar M, Akyüz C, Berberoğlu S, Kotiloğlu E. (Oncology and Pathology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Skin involvement in children with non-Hodgkin's lymphoma. Turk J Pediatr 1995; 37: 117-123.

This study was performed to present our clinical experience with patients with cutaneous malignant lymphoma. Eight of 856 (1%) patients admitted to Hacettepe University Pediatric Oncology Department were diagnosed with skin involvement of non-Hodgkin's lymphoma between November 1971 and December 1992. At the time of diagnosis, the mean patient age was 9.5 years (range 4-15). The male-to-female ratio was 1.7:1. Three of the eight cases had primary cutaneous lymphoma, four had non-Hodgkin's lymphoma (NHL) with skin involvement and one case cutaneous lymphoma as a part of advanced stage NHL. According to Murphy's clinical classification, three cases with primary cutaneous lymphoma were in stage I E, two of the remaining five patients were in stage III and three patients with organ involvement were in stage IV. All eight patients' skin lesions were 6 to 10 cm in diameter, hyperemic, firm and nodular. The skin of the head and neck region, especially the right cheek, was the most involved area. In primary cutaneous lymphomas, the duration between involvement and diagnosis was two to six months. All but two patients received the LSA2 L2 protocol. The other two were treated with the COP protocol and a modified COMP protocol. Three patients in stage I E are now living disease-free. One of the two patients in stage III is disease-free, and the other is in the fifth month of therapy with very advanced disease and is lost to follow-up. Among the three patients in stage IV, one was living disease-free for 38 months after diagnosis, while the other two patients are still under therapy without disease. In our opinion, patients with cutaneous NHL who have different clinical properties need special treatment modalities and advanced immunohistologic studies. *Key words: skin involvement, non-Hodgkin's lymphoma, childhood.*

In large series of Non-Hodgkin's lymphoma (NHL), cutaneous involvement occurs in one to five percent of cases, a rate which is similar in adults and children[1,2]. Patients with cutaneous malignant lymphoma are divided into three groups:

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1. Only skin is involved (primary skin lymphoma); 2. Other organs besides skin are also infiltrated; and 3. No skin involvement at first, but at the advanced stages, the disease spreads to skin[3].

Because of low case numbers and variations of case selection criteria, histopathologic classifications, staging systems and treatment methods, comparison of NHL cases with skin involvement is difficult[1,4].

The aim of this report is to present eight cases of NHL with skin involvement and to introduce our experience with treatment and prognosis of this condition.

Material and Methods

Of 856 patients admitted to Hacettepe University's Pediatric Oncology Department, eight patients (1%) were diagnosed histopathologically with cutaneous NHL between November 1971 and December 1992. In all patients, clinical history, physical examination, whole blood counts, blood chemistry, bone marrow aspiration and/or biopsy, chest x-ray and abdominal ultrasonography were performed for clinical evaluation. Patients were classified according to Murphy's classification[5], and histopathologic evaluation was performed on the basis of the Working Formulation[6].

Results

In our study, the age of the patients at the time of diagnosis was 4-15 years, with a mean age of 9.5 years. Three out of eight cases had primary skin lymphoma, four non-Hodgkin's lymphoma with skin involvement, and the other was a patient with NHL in whom skin involvement occurred during dissemination of the disease.

According to Murphy's Clinical Classification, three cases with primary cutaneous lymphoma were in stage I E, two of the other five patients were in stage III and three patients with organ involvement were in stage IV. One of these three patients had bone marrow infiltration. Skin lesions were approximately 6-10 cm in diameter, hyperemic, firm and nodular. In patients with skin and other organ involvement, the diagnosis was made by histopathological study of the skin obtained together with regional lymph nodes. The hyperemic subcutaneous nodule with infiltration and edema which was palpated on the abdomen of the stage IV patient was considered to be dissemination of disease; therefore a biopsy was not obtained.

The sites most commonly involved were the skin of the head and neck. It is of interest that in all three primary cutaneous lymphoma cases and one stage IV case, the site of involvement was the skin of the cheek (Figs. 1-3). Abdominal skin was the second-most involved area.

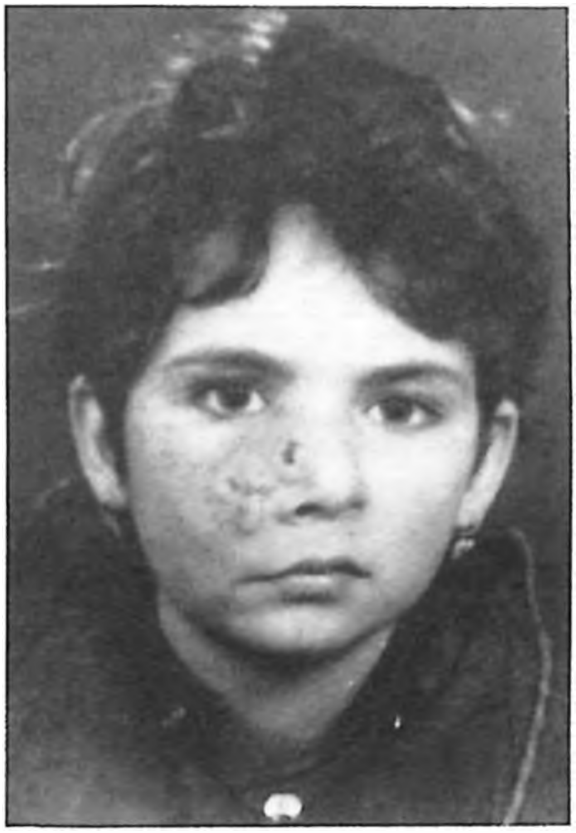
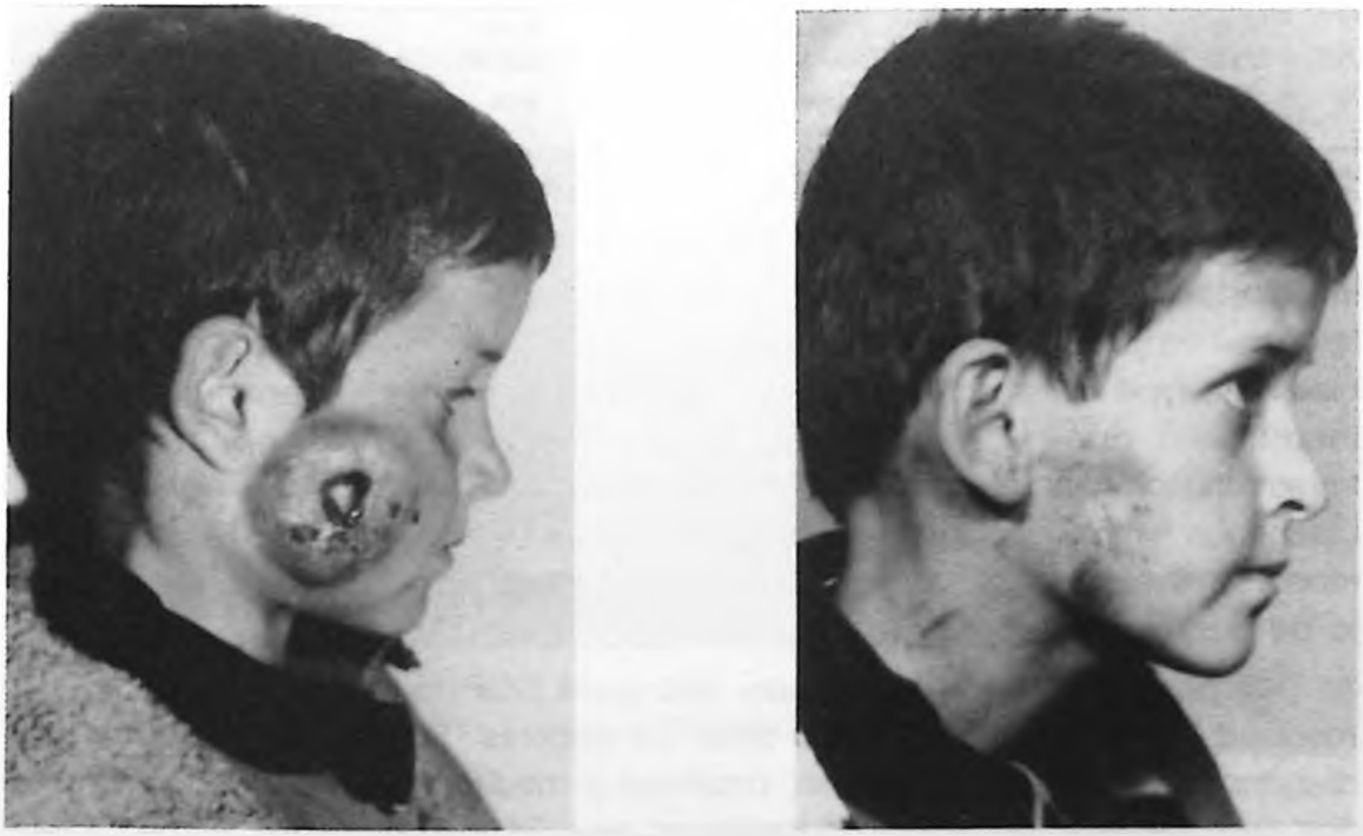


Fig. 1: An irregular lesion on the right cheek (Case 6).



Figs. 2-3: The patient with a large, hard mass on the right cheek at the time of first admission, and appearance after induction therapy (Case 7).

The time interval between the occurrence of the lesion and histopathological diagnosis was one to six months. In patients with primary skin involvement of NHL, systemic involvement did not occur until the last check-up. Clinical and laboratory findings of patients are presented in Table I.

Table I: Selected Characteristics of Patients with Skin Involvement of Non-Hodgkin's Lymphoma

Patient No.	Age* Sex (years)	Duration of Symptoms (months)	Stage	Histopathologic Subgroup	Lesion Region	Other Organ Involvement	Therapy (duration)	Survival and Status
1	11F	2	I E	Lymphoblastic	Right cheek	None	COP 24 months	NED 36 months
2	4 M	1	IV	Large cell/ anaplastic	Right cervical	Cervical Supraclavicular Axillary LAP Liver, spleen	LSA2 L2 26 months	NED 38 months
3	7 F	6	I E	Lymphoblastic	Right cheek	None	LSA2 L2 24 months	NED 36 months
4	4 M	1	III	Small convoluted non-Burkitt	Abdominal wall	Mesenteric mass	LSA2 L2 5 months	Quit in the 5th month
5	15 M	2	III	Large cell immunoblastic	Abdominal wall	Supraclavicular Left axillary LAP	LSA2 L2 17th month replapse than BFM	Death 12 months from relapse
6	10 F	5	I E	Unclassified	Right cheek	None	LSA2 L2	NED 17 months
7	11 M	3	IV	Unclassified	Right cheek	Bone marrow	LSA L2	Under therapy 10th month
8	9 M	1	IV	Unclassified	Abdominal wall	Bone Thyroid	COMP	Under therapy 9th month

Male : male.
LAP : lymphadenopathy.
NED : No evidence of disease.

In histopathologic assessments of cutaneous lymphoma, two lymphoblastic, two large cell immunoblastic/anaplastic and one small cell non-convoluted, non-Burkitt histologic type were identified (Figs. 3-5). In three referral patients, because of insufficient material the subgroup identification could not be made. Immunohistologic assessment was performed in only one case with primary cutaneous lymphoma and one case with stage IV; HLA-DR, CD19 and CD20 monoclonal antibodies, which are primary B lymphocyte markers, were found to be positive in both cases.

All patients received chemotherapy. We gave COP in the first case and the modified LSA2 L2 protocol to the other six patients. The last patient, who was diagnosed with B-cell lymphoma, received a modified COMP protocol. In the first two, the average length of treatment was 24 months, while in the COMP protocol, the treatment length was 12 months. Two of three patients with primary cutaneous lymphoma had been in remission for three years, while the other's

remission duration was 17 months. One of the two patients with cutaneous and extracutaneous NHL has been disease-free for 38 months, while the other patient progressed to systemic disease in the 17th month of therapy died 12 months after the relapse and 27 months following the first diagnosis, although a new therapy protocol had been started (Table I).

Of the patients who were in stage IV at the first diagnosis, one patient developed an early relapse in the second month of therapy and bilateral kidney and skin involvement were observed, but the patient chose not to continue with treatment and follow-up. The other two patients in stage IV were still under therapy and disease free after 9 and 10 months.

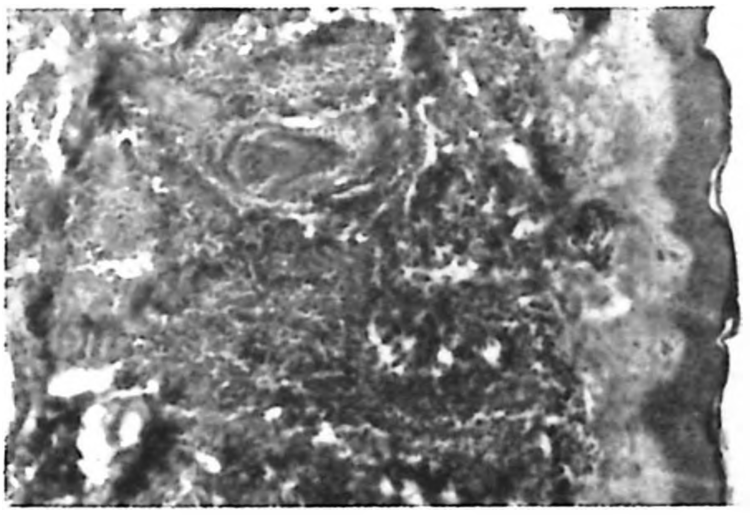


Fig. 4: Diffuse infiltration of lymphoblastic cells in the dermis (H + E, original magnification x 33).

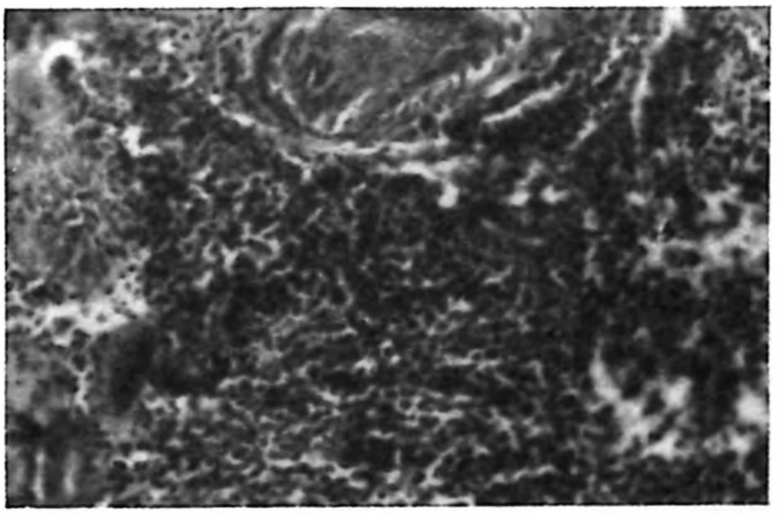


Fig. 5: Lymphoblastic cells surrounding a hair follicle and eccrine glands (H + E, original magnification x 66).

Discussion

It is often difficult to make a correct diagnosis due to the clinical appearance of malignant lymphomas in the skin. This causes some confusion in the differential

diagnosis of some benign lymphoreticular diseases with malignant lymphomas, such as: cutaneous lymphoid hyperplasia, cutaneous lymphoplasia, lymphocytoma cutis, lymphadenosis benigna cutis, reactive lymphoid hyperplasia, Spiegler-Fendt sarcoid, insect bite granuloma, pytriasis lychenoides, varialiformis acuta and pseudolymphomas[7,8].

Primary cutaneous lymphoma is a rare disease, and in some published articles this condition is presented to be a late finding of the disease[9].

The primary disease in the skin may be subclinical for months or even years before histopathological diagnosis. In a report of six cases by Shelly et al.[10], the duration between histopathologic diagnosis and the occurrence of the lesion varied from one to eight years. Skin involvement of lymphoma in reports is generally a solitary, hyperemic nodule, often in the head on neck, similar to our four cases.

Histopathologically and immunohistologically, skin lymphoma is a heterogenous disease. On the other hand, histopathological and immunohistological studies are very rare in this disease. The diagnosis of subgroups of NHL in children varies in different series. Based on the published large series, lymphoblastic lymphoma and large cell lymphoma are the two most commonly encountered types[1-4,11]. In our study, two cases were lymphoblastic, and two cases had large cell NHL similar to those findings. Approximately 50 percent of primary skin NHL is of T-cell origin, and the remaining is of B-cell origin, whereas non-T and non-B lymphomas are very rare[11]. Only two cases in which we could perform immunohistology showed that the origin was B-cell.

There are various treatment modalities. Contrary to the studies that propose only radiation, because of the high relapse rate with this therapy many reports suggest radiation therapy plus chemotherapy or chemotherapy alone[1,2,7]. We administered chemotherapy to all our patients. The prognosis of skin lymphoma depends on the histopathology and stage of disease[1]. When compared to skin and non-skin large cell NHL, primary skin large cell NHL is associated with prolonged survival. While some authors have showed that even if these lymphomas were limited to the skin they could still have a bad prognosis, others have indicated a better overall prognosis[1,2,12-14]. In general, the time interval between occurrence of primary skin lymphoma and systemic illness is six months to five years. In our cases, none of the primary skin lymphomas showed systemic disease.

In conclusion, although the disease is not very commonly seen, we believe that it has a better prognosis than that with other organ involvement. Studies must be done regarding the selection of clinical, histopathologic and immunohistologic assessments as well as treatment protocols for skin lymphomas.

REFERENCES

1. Joly P, Charlotte F, Leibowitch M, et al. Cutaneous lymphomas other than mycosis fungoides: follow-up study of 52 patients. J Clin Oncol 1991; 9: 1994-2001.
2. Grümayer ER, Ladenstein RL, Slavc I, et al. B-cell differentiation pattern of cutaneous lymphomas in infancy and childhood. Cancer 1988; 61: 303-308.
3. Wood GS, Burke JS, Horning S, Doggett RS, Levy R, Warnke RA. The immunologic and clinicopathologic heterogeneity of cutaneous lymphomas other than mycosis fungoides. Blood 1983; 62: 464-472.
4. Grob JJ, Horchowski N, Tubiana N, et al. Non lymphoblastic T cell lymphomas with prevalent skin involvement different from mycosis fungoides or Sezary's syndrome. Dermatologica 1988; 177: 82-97.
5. Murphy S. The management of childhood non-Hodgkin's lymphoma. Cancer Treat Rep 1977; 61: 1161-1165.
6. Magrath IT. Malignant non-Hodgkin's lymphomas In: Pizzo PA, Poplack DG (eds). Principles and Practice of Pediatric Oncology. Philadelphia: J.B. Lippincott Company; 1989: 415-456.
7. Burke JS, Hoppe RT, Cibull ML, Dorfman RF. Cutaneous malignant lymphoma: pathologic study of 50 cases with clinical analysis of 37. Cancer 1981; 47: 300-310.
8. Fisher ER, Park EJ, Wechsler HL. Histologic identification of malignant lymphoma cutis. Am J Clin Pathol 1976; 65: 149-158.
9. Wolk BH. Primary malignant lymphoma cutis. CMA Journal 1977; 117: 750-753.
10. Shelley WB, Wood MG. Observations on occult malignant lymphomas in the skin. Cancer 1976; 38: 1757-1770.
11. Zaatari GS, Chan WC, Kim TH, Williams DL, Kletzel M. Malignant lymphoma of the skin in children. Cancer 1987; 59: 1040-1045.
12. Feller AC, Sterry W. Large cell anaplastic lymphoma of the skin. Brit J Dermatol 1989; 121: 593-602.
13. Long JC, Mihm MC, Qazi R. Malignant lymphoma of the skin; a clinicopathologic study of lymphoma other than mycosis fungoides diagnosed by skin biopsy. Cancer 1976; 38: 1282-1296.
14. Archer CB, Chu AC. Cutaneous B-cell lymphoma. J R Soc Med 1985; 78: 28-29.

The Turkish Journal of Pediatrics 1995; 37: 125-140

THE HISTORY OF X-CHROMOSOME INACTIVATION AND RELATION OF RECENT FINDINGS TO UNDERSTANDING OF HUMAN X-LINKED CONDITIONS*

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SUMMARY: Lyon MF. (Genetics Division, MRC Radiobiology Unit, Chilton, Didcot, Oxon, U.K.). The history of X-chromosome inactivation and relation of recent findings to understanding of human X-linked conditions. Turk J Pediatr 1995; 37: 125-140.

This paper represents the text of two lectures given on the occasion of a Workshop on the X-Chromosome held at Hacettepe University, Ankara, in September 1994. The history of the development of ideas concerning the mechanism of X-chromosome inactivation is traced from the finding of the sex chromatin body in 1949. Important recent findings concern the Xist gene, which is a candidate gene for the X-inactivation center, from which inactivation is initiated. These recent findings shed new light on the abnormalities seen in human patients with X-linked genetic diseases or with aneuploidy of the X-chromosome. *Key words: X-chromosome, X-inactivation, Xist gene.*

Preludes to the Theory:

The first clue that led to the discovery of X-chromosome inactivation came in 1949 when Barr and Bertram found that the cell nuclei of female mammals contained the sex chromatin or Barr body, lying against the nuclear membrane (1-3; throughout this paper wherever possible reference is made to reviews rather than original articles). Human females with Turner's syndrome were found to have no sex chromatin, and this led Paul Polani to the idea that they might have only a single X-chromosome. In the late 1950s good methods for studying human chromosomes became available, and in 1959 Polani with Charles Ford and other colleagues showed that Turner's females were indeed chromosomally XO. In the same year Welshons and Russell found that chromosomally XO mice were not only female, but unlike the human Turner's, morphologically normal. Thus, a female mouse needs only one X-chromosome for normal development. Another important clue in the same year was Ohno's finding that the sex chromation body was formed from a single condensed X-chromosome. Around the same time the first mouse X-linked genes were discovered, and it emerged that female mice heterozygous for X-linked genes affecting coat color or texture showed variegated coats with patches of mutant and normal fur. Putting these various facts together led to the suggestion that one X-chromosome of female mammals becomes

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inactive early in embryonic development[1,2]. Either the maternal or the paternally derived X may be inactivated in different cells. Once inactivation has occurred, the same X remains active throughout all cell divisions in the lifetime of the animal, with the result that in the adult there are large patches of cells with the same X active.

The function of X-chromosome inactivation was suggested to be dosage compensation, in that it resulted in males and females both having effectively a single dose of X-linked gene products. The inactive X-chromosome was not only transcriptionally inactive, but it was also condensed and heterochromatic and replicated its DNA late in the cell cycle.

Thus, the theory was first put forward in 1961, but still today the mechanism of X-inactivation is not understood. The present paper traces the milestones over the years in the development of ideas leading up to the present state of knowledge.

Development of Details of the Theory:

In the first period, in the 1960s, the theory was developed and extended. In the human, many X-chromosome aneuploids were found, such as XXY, XXX, or XXXXY, and in these cases the maximum number of sex chromatin bodies was always one less than the number of X-chromosomes present. Thus, the theory was modified to say not that one X was inactive but rather that a single X remained active. Later, triploids were found first in the human and then in the mouse, and these could have either one or two active X-chromosomes, whereas in tetraploid mouse embryos two X-chromosomes were active. Thus, it was considered that there was a counting mechanism, maintaining one X-chromosome active per two autosome sets[2,3].

Another point concerning X-chromosome aneuploids in the human was the need to explain their malformations, particularly those of XO females. If only one X-chromosome is active, one would expect XOs to be normal, as they are in the mouse, whereas in the human nearly all XOs die prenatally and the few survivors are malformed with Turner's syndrome. The suggestion was made that there might be a pairing segment between the X- and the Y- chromosomes, with homologous genes. For genes in the pairing segment, dosage compensation would not be needed and hence these genes might not be inactivated. The organism would thus need two doses of these genes, and the single dose present in XO individuals might lead to abnormality. It is now known that indeed in the human X, some genes do escape inactivation.

X-Chromosome Activity in Germ Cells:

In the germ cells a different picture was seen. Ohno showed that in female germ cells, at the oogonial or early stage, one X was condensed, just as in somatic

cells, but later, in oocytes, both X-chromosomes looked active. That is, there was reactivation of the X-chromosome. This was confirmed when Gartler showed that in human females heterozygous for the X-linked enzyme glucose-6-phosphate dehydrogenase (G6pd), both alleles were active in the same cell. In the mouse the reactivation was shown to take place just as the cells began to enter meiosis. In male germ cells, by contrast, the single X became condensed and inactive early in spermatogenesis. Thus single-X-chromosome activity is a feature of somatic cells only, and germ cells show a different pattern of activity in the two sexes[3].

The function of changes in X-chromosome activity in germ cells is probably quite different from that in somatic cells and may be involved in meiotic pairing. Miklos[4] and also recently McKee and Handel[5] suggested that the presence of unpaired pairing sites at meiosis is in some way detrimental to a germ cell. The suggestion is that when chromatin is in an active state, it can take part in meiotic pairing, and if any pairing sites do not have a meiotic partner there is a harmful effect. When chromatin is in an inactive state, its pairing sites are in some way protected. Thus, in a female germ cell the reactivation of the inactive X-chromosome may be necessary for meiotic pairing and recombination, and saturation of the pairing sites. In the male germ cell, inactivation of the single X-chromosome is needed to protect the pairing sites which have no homologue on the Y. Such a system could explain the observed effects of X-autosome translocations on male fertility in the mouse. All reciprocal translocations known so far cause male sterility. This could be because inactivation spreads from the X into the attached autosome and inactivates pairing sites there, leaving unpaired and deleterious pairing sites on the homologous autosome. In Cattanach's translocation, on the other hand, there is an insertion of autosomal material into the X chromosome, and inactivation can pass through the insertion. There are two types of male with the Cattanach translocation: those with a corresponding deletion of the autosome, and those with two intact autosomes. Those with the deletion may be sterile and the others fertile, and this can be understood if the pairing sites of the inserted autosomal segment are inactivated. Animals with the deletion would then have unsaturated sites on the homologous autosome, whereas in those with two intact autosomes these sites would be fully paired[6].

There must in addition be other effects of differential X dosage in germ cells. In both the human and mouse, males with more than one X-chromosome, XXY or XX, are sterile due to death of all germ cells at the spermatogonial stage. In the mouse it is known that in such males any germ cells that by chance lose an X and become XO survive and undergo spermatogenesis[7]. The death of XX germ cells occurs too early to be concerned with pairing, and is in the wrong direction. Thus there must be some other effect of double X-chromosome dosage

which is lethal to male germ cells. Conversely, in XO females the rate of loss of oocytes from the ovary is abnormally high, leading to sterility in humans and curtailed reproductive life in mice. This could either be an effect of unsaturated pairing sites or of X gene dosage itself.

Ohno's Law:

Another important development in the 1960s was Ohno's Law[2]. This law states that a gene X-linked in one mammalian species is X-linked in all. Ohno's ationale in formulating this was that, because of single-X activity, if in evolution a gene was transferred by a translocation from the X-chromosome to an autosome or vice versa, there would be a wrong effective gene dosage and so such a translocation would be hamful and thus would be eliminated. There is now extensive evidence that Ohno's Law is true. Genes have been studied in many mammalian species, and the same genes are X-linked in all. This contrasts with the autosomes in which there is much exchange of genes. The two species in which knowledge of chromosome mapping is most detailed are man and mouse. Maps of where the human homologues of mapped mouse genes lie show that each mouse autosome carries genes with homologues on at least three different human autosomes. Thus, the X-chromosome, as Ohno postulated, indeed differs from the autosomes in having its genes conserved throughout mammalian evolution. However, the X-chromosome has clearly undergone rearrangements during evolution. The shapes of the mouse and human X-chromosomes are very different. On the basis of the specific genes carried, the chromosome can be divided into several segments. Within a segment the order of genes has been conserved in evolution, but the segments have been rearranged with respect to each other[8].

X-Chromosome Imprinting:

Ohno's Law has been very valuable, as it has enabled the study of X-linked genes in many species. In particular, it has enabled the study of X-inactivation in marsupials. A surprise, found in 1971, was that in marsupials, rather than random inactivation of one or other X-chromosome seen in eutherian mammals, the X from the father, the paternally derived X, was inactive in all cells[9]. This is an example of genetic imprinting and was probably the first example of this phenomenon found in mammals. Soon afterwards Takagi and Sasaki[10] showed that preferential inactivation of the paternal X-chromosome occurs also in the trophectoderm and primitive endoderm cell lineages of rat and mouse embryos. It may occur also in extraembryonic lineages of humans, but this is a controversial point. In parthenogenetic mouse embryos, in which all chromosomes are maternally derived, both in the embryo itself and in the extraembryonic membranes X-inactivation can still occur. In addition, in XO mice with only a

paternal X-chromosome, this remains active. Thus, the mechanism which maintains a single X active can override the imprint.

In view of the inactivation of the X in male germ cells, it was suggested that paternal X-inactivation might be the primitive type in evolution, and might have evolved from an earlier inactivation of the X in male germ cells[11, 12].

Ohno[13] pointed out that some fish and amphibia do not have distinguishable sex chromosomes and that the easily distinguishable X and Y of mammals must have arisen during the evolution of vertebrates. The suggestion was that in the early evolution of mammals, as the X and Y began to diverge in size there was a need for inactivation of the X-chromosome in male germ cells to protect the pairing sites which no longer had homologues on the Y. Later in evolution this inactivation of the X-chromosome in sperm was carried over into the new zygote at fertilization and formed a starting point for the evolution of somatic X-inactivation.

X-Chromosome Activity in Early Embryos:

Other major advances in understanding of X-inactivation in the 1970s were brought about by increased knowledge of early mammalian embryos. An early question was whether in the embryo the X-chromosomes were inactive and a single one was activated, or the reverse, with both Xes at first active and one being inactivated. Epstein, studying activity of the X-linked enzyme HPRT in early embryos, showed that the latter was correct[3]. In the early female embryo both X-chromosomes are active, and it is indeed correct to speak of inactivation. Nobuo Takagi and Sohaila Rastan and others showed that an inactive X appeared first in the trophectoderm at the late blastocyst stage at about four days and somewhat later, at about five days, in the primitive ectoderm which gives rise to the embryo proper.

Another development in the 1970s was the study of cultured embryonic cells, first embryonal carcinoma or EC cells, and later embryonic stem cells, ES cells. In some lines of EC cells and in most ES cells, both X chromosomes in female cell lines are active. Martin et al.[14] showed that when such cell lines were allowed to differentiate, X-inactivation took place. This was an important step forward because it meant that ES cell lines constituted a suitable system in which X-inactivation could be experimentally studied.

Initiation of X-Inactivation and the X-Inactivation Center:

Another important step was the concept that X-inactivation could be considered as having two main components: initiation and maintenance[3] initiation is the onset of X-inactivation in the early embryo. Once initiation has occurred, each X-chromosome faithfully replicates its active or inactive state at each cell division throughout the life of that animal, and this is the maintenance or stabilization of inactivation. Initiation of X-inactivation is thought to take place through the

X-inactivation center on the X-chromosome. Evidence for this comes from the study of mouse X-autosome translocations.

When autosomal coat color genes are translocated to the X-chromosome, inactivation spreads into the attached autosomal material and causes variegation for these color genes, but it only spreads into one of the two autosomal segments. In addition, only one of the two segments into which the X is broken shows late replication and dark Kanda staining. An example is T37H in which the large translocation product shows dark Kanda staining but the small one does not. This suggests that only one of the X-chromosomal segments is undergoing inactivation. This was studied in the translocations T37H and T38H, both of which have a breakpoint near the sparse-fur or ornithine transcarbamylase (Otc) locus, sparse-fur being a deficient allele of Otc. Animals heterozygous for the translocation and for sparse-fur were bred, and OTC activity was studied by histochemical staining of the liver. Translocation T38H showed the expected mosaic pattern of dark positive cells and light OTC negative cells. With T37H, however, all cells stained positive for OTC, indicating that the normal allele on the translocated X-chromosome was active in all cells[3]. By in situ hybridization it was shown that in the two translocations, the locus of Otc was on opposite sides of the breakpoint. The interpretation was that in T37H the Otc locus is separated from the inactivation center and so is remaining active in all cells.

As already mentioned, there is thought to be a counting mechanism which maintains one X-chromosome active per two autosome sets, and it is suggested that this acts by blocking one inactivation centre. The chromosome with the blocked center remains active. All other centers in the cell (normally one) then initiate inactivation which spreads in a cis-limited manner along the chromosome in both directions. Segments without a center remain active.

By studying different translocations and deletions, the position of the center has been found to be in chromosome band D in the mouse and band Xq13 in the human. From this region a human gene was cloned, termed XIST, or X-inactive specific transcript, with a unique pattern of expression. It is expressed only from the inactive and not the active X-chromosome[15]. The corresponding gene maps to the region of the inactivation center in the mouse also, and shows a similar expression pattern[16, 17]. From both its position and its unique expression from the inactive X-chromosome only, it is thought that Xist must have some important significance in inactivation.

Sohaila Rastan and colleagues studied the expression of Xist in the mouse embryo[18, 19]. It was not expressed at the 2-cell stage, but was expressed at both the 4-cell and 8-cell stages. At first only the paternal allele was expressed, and expression of the maternal allele was not seen until the late blastocyst stage.

In the extraembryonic tissues, where paternal X-inactivation occurs, only the paternal allele was expressed. Others showed that in male mice Xist was expressed only in the testes, and specifically in the germ cells, where the X is inactive[20-22].

In female germ cells, where reactivation of the X occurs, expression of Xist disappears around the time of reactivation[22]. In XX embryonic stem cells Xist was not expressed while the cells were undifferentiated and both X-chromosomes were active, but Xist expression appeared when the cells were allowed to differentiate and X-inactivation occurred[18]. Thus, in various respects the expression of Xist is appropriate for it to have a causal role in X-inactivation. Expression occurs before inactivation, and is imprinted when it first appears and so on. However, in other respects the expression is an enigma. In the embryo it occurs too early, when both X-chromosomes are active, and so some other factor must be needed to actually bring about inactivation. In addition, Xist is expressed in all cells of the adult female, and at present the significance of this is not known.

The sequence of Xist has been studied in humans and mouse by Hunt Willard[23] and Sohaila Rastan[24], respectively. The structure of the gene is well conserved among mammals. In both human and mouse there is no substantial open reading frame. The transcript remained in the nucleus and appeared associated with the inactive X. Thus it appears that Xist does not code for a protein and may act through RNA.

The Role of DNA Methylation in X-Inactivation:

The question is what the inactivation center is actually doing. It is presumed to be initiating the spread of inactivation along the chromosome, and thus one needs to know the mechanism of spreading. In 1975 Art Riggs, Holliday and Pugh suggested that DNA methylation might be the basis of spreading. A methylase might be an enzyme that could run along the chromosome in an appropriate way. Since then evidence has been obtained that the inactive X-chromosome indeed shows differential methylation[3, 25-27].

The CpG islands in the 5' promoter regions of genes on the inactive X are heavily methylated. Treatment with demethylating agents, such as 5-azacytidine, will cause partial reactivation, and the reactivated genes can be shown to have lost their methylation. Thus, the methylation does have a functional effect. However, this methylation is not found in marsupials, nor is it in mouse germ cells, and probably not in mouse extraembryonic lineages, which show paternal X-inactivation[3].

Thus, X-inactivation can occur without methylation of CpG islands. In addition, methylation appears later in embryonic development than the initiation of X-inactivation. Thus, it is thought that methylation of CpG islands is not the spreading mechanism. The various types of X-inactivation in which methylation is not seen are all less stable than in typical random inactivation in the embryo

proper of eutherians. In marsupials, reversal of X-inactivation can occur either in cultured cells or in some tissues in vivo[9]. In female germ cells of eutherians, reactivation occurs as the cells enter meiosis, and in extraembryonic cells reactivation has been reported. Thus, the suggestion is that methylation is involved in the stabilization and maintenance of inactivation, and exactly what is involved in the spreading of inactivation along the chromosome still remains unknown. Recently a new property of the inactive X-chromosome lack of acetylated histone 4, has been discovered[28]. The set of properties of the inactive X, including condensation, late replication and lack of acetylated histone 4, are those of heterochromatin. Thus, the spreading mechanism is causing normally euchromatic material to behave as heterochromatin, but it remains to be discovered how this is brought about.

Relation of Recent Findings to X-Inactivation in Humans:

The recent advances in knowledge of X-inactivation are not only of interest because of the potential insight they may give into the mechanism of inactivation. In addition they help to give better understanding of the findings in humans with X-chromosome aneuploidies or X-linked genetic diseases.

A first point to consider concerns the shapes, sizes and numbers of patches seen in females heterozygous for X-linked genes. If mouse coat color is taken as an example, the pattern in heterozygotes is complicated with some large patches of solid color and other areas where small patches of one or another color intermingle. A similar pattern is seen in chimaeras produced by experimentally aggregating two embryos of different color. Thus, the actual pattern is not peculiar to X-inactivation but is due to the way cells have grown and mingled in development. Certain features of the pattern are that the patches tend to run transversely and not to cross the midline. This can be understood by remembering that the pigment is formed by melanoblasts that migrate out from the neural crest. Each spot is formed by clonal growth of one or more precursor cells, and the different sizes of spots are due to coalescing of various spots of the same color. Different regions of the body may be populated by the precursors of one or a few pigment-forming cells. One gets the best match to the observed pattern by imagining that each region of the body is populated by two precursor cells. If they are both pigmented or both white, a large patch of solid color results. However, if a region is populated by one white and one pigmented precursor cell, the two types of cell mingle as they spread across the body and give a region with many small spots of color[29]. Pigment cells have been used merely as an example. Using other means of marking cells, such as by histochemical staining, one can look at various tissues, and similarly work out whether particular structures are derived from a single precursor cell or many. Dillwyn Williams[30] has used staining for ornithine transcarbamylase and for glucose 6 phosphate

dehydrogenase to study the patches in various mouse tissues. He has found that intestinal crypts are formed from a single cell, but the villi from many precursor cells. Thyroid acini are of multicellular origin, but thyroid nodules are of single cell origin. X-inactivation has been used to study the single or multicellular origin of tumors, but obviously it is important to know that the tissue of origin of the tumor has a multicellular origin before studying the tumor. Thus, in every human X-linked disease, the mosaic pattern of patches varies with the particular cell type involved. One particular pattern of interest is Blaschko's lines, which are lines on the skin in a pattern somewhat resembling that in mouse coat color, and which are seen in females with the X-linked disease incontinentia pigmenti.

X-Choromosome Aneuploidies and Escape of Genes From Inactivation:

In considering the various chromosome abnormalities affecting the X-chromosome, as mentioned earlier one would expect XO females to be normal, as they are in the mouse, but in humans most die prenatally and the few survivors have Turner's syndrome. It was postulated early on that there might be a pairing segment of the X-chromosome carrying genes with homologues on the Y, and that genes in such a pairing segment might escape inactivation, as they would not need dosage compensation. It is now known that there are various genes on the human X that escape inactivation[3, 31, 32], and thus the abnormalities of human XOs may indeed be due to wrong dosage of some of these genes. Some of the genes concerned, such as MIC2, are in the pairing segment, and others such as ZFX and STS are near it. However, others, such as UBE1 and RPS4X, are some distance away. This is of interest in relation to the mechanism of X-inactivation because these genes have normally inactivated genes on both sides of them. This means that the signal for spreading of inactivation must have run past these genes, and either the genes resisted the inactivation or the stabilizing mechanism must have failed so that reactivation promptly occurred. In any case, study of these genes may provide valuable information on spreading.

In the mouse, from the work of Sohaila Rastan and colleagues, the homologous genes, including Zfx and Rps4, undergo inactivation normally[3]. Up to now only one mouse gene is known which unequivocally escapes inactivation[33]. Thus, this provides a plausible explanation for the fact that XO mice are normal, in contrast to human XOs.

Another type of X-chromosome anomaly for which an explanation has recently been found is the small ring-X chromosome. Several females with one normal X and one small ring-X have been found, and these females have shown severe phenotypes with various congenital malformations and with mental retardation[34]. It was not clear why such a small chromosome should produce a severe effect when females with larger deleted X-chromosomes were less severely affected.

Barbara Migeon showed that in these small ring X-chromosomes, in some cases the Xist gene was deleted, and in other cases although it was present it was not expressed[34]. Thus, the interpretation was that the ring X lacked a functional X-inactivation center and was remaining active. Studies of the expression of some X-linked genes showed this to be so[35]. The abnormal phenotype of these females was thus thought to be due to excess dosage of cretain X-linked genes.

Some Causes of Non-Random Inactivation:

1. Imprinting:

In the mouse, an abnormal phenotype thought to be due to excess X-chromosome dosage is seen in animals with additional X-chromosomes, if these are derived from the mother, In the human, XXY or XXX females in which two X-chromosomes are of maternal origin are normally viable, but in the mouse such females die early in gestation. Nobuo Takagi[36] showed that such embryos tended to have two X-chromosomes active in the extraembryonic cell lineages, where imprinting was occurring, and the development of these extraembryonic tissues was abnormal. Thus, this work suggests that the maternal X in the mouse bears an imprint tending to prevent its inactivation in the relevant tissues. In the human, Barbara Migeon[37] has found that either X-chromosome can be expressed in the extraembryonic tissues, and this, together with the viability of X^mX^mY and $X^mX^mX^p$ humans, is part of the evidence that imprinting may not occur in human X-inactivation.

Sohaila Rastan and her colleagues have carried out two pieces of work on the imprinting of the Xist gene in the mouse which are relevant to the question of imprinting of the X-chromosome in humans.

In the first[38] they studied the methylation of sites in the 5' region of the gene. As already mentioned, the inactive X shows differential methylation; in addition, methylation has been suggested as a basis for imprinting, and thus methylation is a reasonable feature to study. In the male, where the Xist gene is inactive, its 5' region was heavily methylated, whereas in the female the allele on the active X was methylated, and that on the inactive X unmethylated. In the male germ cell, the Xist gene showed demethylation in the course of spermatogenesis. Thus, it is possible that differential methylation of Xist in male and female germ cells may constitute the imprint. The allele in the female germ cell, where both X-chromosomes are active, may well be methylated, but this is not known. Thus, there is no conclusive evidence at present that differential methylation does indeed constitute the imprint, but it is a possibility.

In the second piece of work, Kay et al.[19] studied the expression of Xist in parthenogenotes, gynogenotes and androgenotes. In androgenotes, which are

embryos with all their chromosomes derived from the father, and therefore with demethylated Xist, the gene was expressed at the four-cell stage as in normal embryos, whereas in parthenogenotes and gynogenotes, both of which have only maternally derived chromosomes, Xist expression appeared only later at the morula to blastocyst stage. Thus, this provides evidence that the expression of Xist does indeed show imprinting and that the imprint apparently disappears with time. There were also other findings. When expression did appear in gynogenotes, either allele could be expressed, which is as one might expect since both are maternally either allele could be expressed, which is as one might expect since both are maternally derived. In contrast to gynogenotes, androgenotes can be chromosomally of three types: X^pX^p, X^pY, or YY. The YY embryos would be expected to die early because of their lack of X-linked gene products. The evidence indicated that Xist was expressed in all the surviving embryos, both XX and XY. Thus, the counting mechanism which maintains one X active was apparently not functional at this early stage, but must develop later. Thus, what we see is that an imprint is present early, preventing expression of Xist in maternally derived X-chromosomes of gynogenotes and parthenogenotes, and this disappears as development proceeds. Conversely, the counting mechanism is not present at first and appears as development continues. A further finding in androgenotes was that Xist expression was not maintained as development progressed, as it is in normal embryos, but was down-regulated at the eight-cell stage and disappeared by the morula to blastocyst stage. The authors interpret this to mean that there must be a maternally expressed gene involved in maintaining Xist expression in the early embryo[19].

This work raises the question whether the counting mechanism is a relatively late evolutionary development in X-inactivation. In normal embryos such a mechanism is not needed if there is imprinting. Males are always X^mY and would have an active X-chromosome, and females are X^mX^p and would have one active and one inactive X. In eutherian mammals we know of the counting mechanism through many aneuploids and polyploids. In marsupials, far fewer aneuploids are known, and it remains possible that marsupials do not have a counting mechanism but rely on imprinting. It may be that imprinting is the ancestral form of X-inactivation. In eutherian mammals X-inactivation has been delayed until a stage when the imprint has worn off, and a counting mechanism developed in its place, and this is apparently associated with a more stable type of X-inactivation. It may be that in the human embryo X-inactivation occurs relatively later than in the mouse, so that even in the extraembryonic membranes the imprint has been lost.

2. Chromosome Anomalies:

An important feature seen in humans with X-chromosome anomalies such as translocations and deletions is non-random inactivation. Evidence concerning the explanation for this comes from mice. In mice most translocations show

inactivation of one or another X-chromosome, but one, called T16H or Searle's translocation, shows non-random inactivation with the translocated X active in all cells. The result is that mice heterozygous for this translocation, and for a mutant gene, express the allele of the gene on the translocated X only, and show a phenotype like that of a male. The question is whether the actual initiation of inactivation is abnormal, so that only one inactivation center responds, or whether inactivation first occurs at random and then cell selection eliminates cells with the translocated X inactive. Takagi[39] showed that cell selection was the explanation. He studied embryos at the time of inactivation and found some cells with one segment of the translocated X-inactive but the other segment remaining active, presumably through lack of the inactivation center. Thus, such cells have a double abnormality. They have excess X-chromosome activity, and also a deficiency of autosomal gene activity through the spread of inactivation into the autosomal segment attached to the segment of X-chromosome undergoing inactivation. When Takagi looked at somewhat older embryos, cells with the translocated X inactive had disappeared and only cells with the normal X inactive and the translocated X active remained. Thus, by analogy with the mouse situation, it is thought that the non-random inactivation seen in humans heterozygous for X-autosome translocations is again due to cell selection. Similarly, in humans heterozygous for deleted X-chromosomes, the normal X is active in all cells, and again this is thought due to cell selection. In such females any mutant genes on the active X are expressed fully as in a male. This is particularly important in translocations where the translocation break itself may interrupt genes and so cause a mutation. For example, some females affected with Duchenne muscular dystrophy have been found to carry translocations disrupting the gene.

3. Cell Selection in Certain Tissues:

In some cases non-random inactivation is seen only in certain tissues. This is thought to be due to cell selection against cells with the mutant allele active, in those cell lineages in which the gene acts. This is seen for example in females heterozygous for X-linked immunodeficiencies, such as Wiskott-Aldrich syndrome. Here, in heterozygotes, B and T lymphocytes, granulocytes and platelets all have the normal X active in all cells, but erythrocytes and fibroblasts have a mixture of cells with the normal or the mutant X active[40-42].

In diseases where the gene product is not known, as in Wiskott-Aldrich syndrome until very recently, this non-random inactivation is useful in detecting heterozygotes. One looks in the fibroblasts for X-linked markers for which the female might be heterozygous. If in the lymphocytes there is non-random inactivation, detected either by gene expression or by differential methylation, then the female is a heterozygote for the disease.

Another possible cause of apparent non-random inactivation is passage of the gene product from one cell to another. An example is Duchenne's muscular dystrophy. Each fiber in skeletal muscle is multinucleate and may include cells of both types with the normal or the abnormal X-chromosome active. If any cells with the normal X active are present in a particular fiber, dystrophin will be formed and the fiber will look normal. Thus, the proportion of normal-looking fibers is well above 50 percent.

4. Reactivation of X-linked genes:

In the mouse another type of departure from typical inactivation that has been seen is due to reactivation of specific genes occurring in some cells. Typically X-inactivation is very stable in eutherian mammals and it used to be thought that reactivation did not occur in somatic cells, but it is now known that it can occur at a low level. This was seen using the translocation T16H, which as discussed above causes non-random inactivation with the translocated X active. Females were bred heterozygous for the translocation and for sparse-fur, the deficient allele of ornithine transcarbamylase (OTC), with the deficient allele on the active X. On histochemical staining for OTC in the liver one would expect the mutant deficient allele to be active in all cells, and hence the sections should look uniformly negative for OTC. In fact a few positive cells were seen, and these became more numerous with age. This was attributed to reactivation of the Otc gene in some cells[8]. A similar phenomenon had been seen earlier by Cattanach in animals carrying his translocation opposite T16H. Cattanach's translocation carries the normal allele of albino translocated to the X-chromosome, and the animals were bred so that with the Cattanach translocation inactive in all cells, they should have been albino. In fact they had small patches of pigment, and became darker with age. Thus, this phenomenon is not confined to the Otc locus. However, it does not occur with all genes and has not yet been found in man, although the possibility of it occuring with particular X-linked diseases cannot be excluded.

A question that has arisen lately is whether loss of the Xist gene from a chromosome that had already been inactivated would cause its reactivation. Two pieces of work have been done on this. In one case, Brown and Willard[43] took cultured human cells and induced deletions of the Xist gene in them. In the other case, Tony Monaco and Veronica Buckle and colleagues[44] studied two isodicentric X chromosomes found in patients with leukemia, and found these two chromosomes had lost the Xist gene. In both cases these X-chromosomes without the Xist gene remained inactivated. Thus, there is no evidence that the presence of the Xist gene is needed to maintain inactivation once it has occurred.

5. Non-Random Initiation of X-Inactivation:

Another question is whether there can be true departure from random inactivation at the time of the actual initiation. In the mouse this has been found. Cattanach

found a genetic factor termed Xce. Various strains of mice had different alleles of Xce which affected the probability of the X carrying them being the active one. The allele Xce^c was most likely to remain active, with Xce^b next, and Xce^a weakest. The result was that in females that were genetically heterozygous for Xce^c and Xce^a, instead of a 50 percent ratio, 70 percent of cells had the X-chromosome with Xce^c active, and only 30 percent had Xce^a active. This was thought not to be due to cell selection but to be a true effect on the choice of X for inactivation at the time of initiation. The Xce factor maps to the same point as the inactivation center and is thought probably to be part of the center[3]. There is some evidence that the Xce effect might be produced by different alleles of the Xist gene. Kay et al.[18] found that expression of Xist varied in different Xce alleles, with the strongest Xce allele having the weakest Xist expression. This is interesting but not conclusive evidence that Xce and Xist may be different aspects of the same thing. However, Simmler et al.[45] found apparent crossing-over between Xce and Xist, suggesting that they involve distinct genetic loci. In humans, it is possible that some cases of heterozygotes showing non-random inactivation may be due to similar Xce-like effects, but this is very difficult to investigate in humans.

Conclusions:

Thus, consideration of the history of X-inactivation shows that the present time is an exciting one. The situation is poised for possible further important developments, particularly concerning the role of the Xist gene and its relation to the X-inactivation center. Furthermore, recent developments have provided new insights into the abnormalities seen in human X-linked diseases and X-chromosome aneuploidies. It is likely that further understanding will emerge in the near future.

REFERENCES

1. Lyon MF. Sex chromatin and gene action in the mammalian X-chromosome. Amer J Hum Genet 1962; 14: 135-148.
2. Lyon MF. Some milestones in the history of X-chromosome inactivation. Annu Rev Genet 1992; 26: 16-28.
3. Lyon MF. X-chromosome inactivation. In: Wachtel SS (ed). Molecular Genetics of Sex Determination. San Diego: Academic Press; 1994: 123-142.
4. Miklos GL. Sex-chromosome pairing and male fertility. Cytogenet Cell Genet 1974; 13: 558-577.
5. McKee BD, Handel MA. Sex chromosomes, recombination, and chromatin conformation. Cahromosoma 1993; 102: 71-80.
6. Lyon MF. X-chromosome inactivation as a system of gene dosage compensation to regulate gene expression. Prog Nucleic Acid Res Mol Biol 1989; 36: 119-130.
7. Lyon MF. Sex chromosome activity in germ cells. In: Coutinho E, Fuchs F (eds). Physiology and Genetics of Reproduction, Vol 4 A. New York: Plenum Press; 1974: 63-71.

8. Lyon MF. The William Allan memorial award address: X-chromosome inactivation and the location and expression of X-linked genes. Am J Hum Genet 1988; 42: 8-16.
9. Cooper DW, Johnston PG, Watson JM, Graves JA. X-inactivation in marsupials and monotremes. Sem Dev Biol 1993; 4: 117-128.
10. Takagi N, Sasaki M. Preferential inactivation of the paternally derived X-chromosome in the extraembryonic membranes of the mouse. Nature 1975; 256: 640-642.
11. Cooper DW. A directed genetic change model for X-chromosome inactivation in eutherian mammals. Nature 1971; 230: 292-294.
12. Lifschytz E, Lindsley DL. The role of X-chromosome inactivation during spermatogenesis. Proc Natl Acad Sci USA 1972; 69: 182-186.
13. Ohno S. Sex Chromosomes and Sex-linked Genes. Berlin: Springer-Verlag; 1967.
14. Martin GR, Epstein CJ, Travis B, et al. X-chromosome inactivation during differentiation of female teratocarcinoma stem cells in vitro. Nature 1978; 271: 329-333.
15. Brown CJ, Ballabio A, Rupert JL, et al. A gene from the region of the human X-inactivation centre is expressed exclusively from the inactive X-chromosome. Nature 1991; 349: 38-44.
16. Brockdorff N, Ashworth A, Kay GF, et al. Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome. Nature 1991; 351: 329-331.
17. Borsani G, Tonlorenzi R, Simmler MC, et al. Characterization of a murine gene expressed from the inactive X chromosome. Nature 1991; 351: 325-329.
18. Kay GF, Penny GD, Patel D, Ashworth A, Brockdorff N, Rastan S. Expression of Xist during mouse development suggests a role in the initiation of X-chromosome inactivation. Cell 1993; 72: 171-182.
19. Kay GF, Barton SC, Surani MA, Rastan S. Imprinting and X chromosome counting mechanisms determine Xist expression in early mouse development. Cell 1994; 77: 639-650.
20. Richler C, Soreq H, Wahrman J. Inactivation in mammalian testis is correlated with inactive X-specific transcription. Nature Genetics 1992; 2: 192-195.
21. Salido EC, Yen PH, Mohandas TK, Shapiro LJ. Expression of the X-inactivation-associated gene Xist during spermatogenesis. Nature Genetics 1991; 2: 196-199.
22. McCarrey JR, Dilworth DD. Expression of Xist in mouse germ cells correlates with X-chromosome inactivation. Nature Genetics 1992; 2: 200-203.
23. Brown CJ. Hendrich BD, Rupert JL, et al. The human XIST gene; analysis of a 17kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. Cell 1992; 71: 527-542.
24. Brockdorff N, Ashworth A, Kay GF, et al. The product of the mouse Xist gene is a 15kb inactive X-sepicific transcript containing no conserved ORF and located in the nucleus. Cell 1992; 71: 515-526.
25. Riggs AD. DNa methylation and late replication probably aid cell memory, and type I DNA reeling could aid chromosome folding and enhanced function. Phil Trans Roy Soc B 1990; 326: 285-297.
26. Riggs AD, Pfeifer GP. X-chromosome inactivation and cell memory. Trends in Genetics 1992; 8: 169-174.
27. Bird AP. The essentials of DNA methylation. Cell (Cambridge, Mass.) 1992; 70: 5-8.
28. Jeppesen P, Turner BM. The inactive X-chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. Cell 1993; 74: 281-289.
29. Lyon MF. Genetic activity of sex chromosomes in somatic cells of mammals. Phil Trans Roy Soc B 1970; 259: 41-52.

30. Thomas GE, Williams ED. The demonstration of tissue clonality by X-linked enzyme histochemistry. J Pathol 1988; 155: 101-108.

31. Migeon BR. X-chromosome inactivation: molecular mechanisms and genetic consequences. Trends in Genetics 1994; 10: 230-235.

32. Ellison J, Passage M, Yu L-C, Yen P, Mohandas TK, Shapiro L. Directed isolation of human genes that escape X-inactivation. Somat Cell Mol Genet 1992; 18: 259-268.

33. Agulnik AI, Mitchell MJ, Mattei M-G, et al. A novel X gene with a widely transcribed Y-linked homologue escapes X-inactivation in mouse and human. Hum Molec Genet 1994; 879-984.

34. Migeon BR, Luo S, Stasiowski BA, et al. Deficient transcription of XIST in females with tiny ring X chromosomes and severe phenotypes. Proc Natl Acad Sci 1993; 90: 12025-12029.

35. Migeon BR, Luo S, Jani M, et al. The severe phenotype of females with tiny ring X chromosomes is associated with inability of these chromosomes to undergo X inactivation. 1994; 55: 497-504.

36. Shaof C, Takagi N, An extra maternally derived X chromosome is deleterious to early mouse development. Development 1990; 110: 969-975.

37. Migeon BR, Wolf SF, Axelman J, Kaslow DC, Schmidt M. Incomplete X chromosome dosage compensation in chorionic villi of human placenta. Proc Natl Acad Sci USA 1985; 82: 3390-3394.

38. Norris DP, Patel D, Kay GF, et al. Evidence that random and imprinted Xist expression is controlled by preemptive methylation. Cell 1994; 77: 41-51.

39. Takagi N. Primary and secondary nonrandom X chromosome inactivation in early female mouse embryos carrying Searle's translocation T (X;16)16H. Chromosoma 1980; 81: 439-459.

40. Gealy WJ, Dwyer JM, Harley JB. Allelic exclusion of glucose-6-phosphate dehydrogenase in platelets and T lymphocytes from a Wiskott-Aldrich syndrome carrier. Lancet 1980; 1: 63-65.

41. Greer WL, Kwong PC, Peacocke M, Ip P, Rubin LA, Siminovitch KA. X-chromosome inactivation in the Wiskott-Aldrich syndrome: a marker for detection of the carrier state and identification of cell lineages expressing the gene defect. Genomics 1989; 4: 60-67.

42. Prchal JT, Caroll AJ, Prchal JF, et al. Wiskott-Aldrich syndrome: cellular impairments and their implication for carrier detection. Blood 1980; 56: 1048-1054.

43. Brown CJ, Willard HF. The human X-inactivation center is not required for maintenance of X-chromosome inactivation. Nature 1994; 368: 154-156.

44. Rack KA, Chelly J, Gibbons RJ, et al. Absence of the XIST gene from late-replicating isodicentric X chromosomes in leukaemia. Hum Molec Genet 1994; 3: 1053-1059.

45. Simmler M-C, Cattanach BM, Rasberry C, Rougeulle C, Avner P. Mapping the murine Xce locus with $(CA)_n$ repeats. Mamm Genome 1993; 4: 523-530.

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BARE LYMPHOCYTE SYNDROME WITH LACK OF HLA CLASS I AND II ANTIGENS

Presentation of Two Cases*

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SUMMARY: Ersoy F, Sanal Ö, Tezcan İ, Yel L, Metin A. (Immunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Bare lymphocyte syndrome with lack of HLA class I and II antigens. Presentation of two cases. Turk J Pediatr 1995; 37: 141-146.

Bare lymphocyte syndrome (BLS) is a rare disorder characterized by deficient expression of human leukocyte antigens (HLA antigens) and combined immunodeficiency to various degrees. Recurrent severe infections especially due to opportunistic organisms are common. Here, we present two patients with BLS who lack both class I and II antigens (Type III). They had the typical clinical and immunologic findings of BLS. The first patient showed marked improvement in pulmonary symptoms resulting from cytomegalovirus infection by means of gancyclovir treatment. However, intramuscular injections of interferon-α (IFN-α) had no beneficial effect in either the expression of HLA antigens or the clinical status. The second patient died of septicemia while being prepared for bone marrow transplantation. *Key words: bare lymphocyte syndrome, HLA antigens, combined immunodeficiency, interferon-α.*

"Bare lymphocyte syndrome (BLS)" or "major histocompatibility complex (MHC) class I or II deficiency syndrome" is a rare disorder in which patients have a deficient expression of human leukocyte antigens (HLA antigens). The usual clinical manifestations consist of recurrent severe and opportunistic infections, persistent gastroenteritis and overwhelming septicemia[1-3]. The first patient with BLS in the literature was found to have a defect in the expression of HLA class I antigens (Type I)[4]. However, BLS may also involve only class II antigen expression (Type II), or both class I and class II antigen expression (Type III)[1,2]. In Type I, beta-2 microglobulin, a structural component of class I antigens, is not found on the surface of the cells either[1,3,5].

We present here two patients with defective expression of both class I and II antigens.

Case Reports

Case 1

ÖS, the fourth child of consanguineous parents (first cousins), was admitted to our hospital with the complaints of fever, tachypnea and diarrhea at the age of 5.5 months.

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The past history revealed that she had been born with a body weight of 3800 g (90-95th percentile), and her clinical condition at birth and development during the first 3.5 months had been seemingly normal. Then, however, she had recurrent otitis media, urinary tract and pulmonary infections despite appropriate antibiotic therapy. BCG and two doses of DTP-OPV vaccinations had been administered without any complication. Her two female siblings were healthy, but a male sibling who had been diagnosed with combined immunodeficiency (CID) had died of intractable pneumonia at the age of eight months. Postmortem tissue specimens had revealed lymphoid depletion and absence of germinal center formation in the spleen, consistent with severe combined immunodeficiency (SCID), and pneumocystis carinii pneumonitis.

On admission, her weight was 6500 g (25th percentile), she had respiratory failure, oral candidiasis and hepatomegaly. The laboratory data are shown in Table I.

Table I: Laboratory Data of The Patients

	Case 1	Case2
Hemoglobin (g/dl)	11.8	9.8
White cell count (/mm^3)	5800	7200
Total lymph.count (/mm^3)	1450	1944
Serum immunoglobulins		
IgG (mg/dl)	< 52.4	59.2
IgM (mg/dl)	< 13.1	< 13.1
IgA (mg/dl)	< 21.0	< 21.0
DTH: PHA	(–)	(+)
Candida	(–)	(–)
Tetanus	(–)	(–)
PPD	(–)	(–)
Lymphocyte markers*		
CD3	10% (326)	32% (622)
CD4	11% (358)	8% (156)
CD8	5% (163)	28% (544)
CD20	4% (130)	38% (738)
CD56	6% (196)	ND
Lymphocyte stimulations		
PHA (cpm)	48499	63867
Con-A (cpm)	39787	49059
Media (cpm)	1673	3300
MLR	uninformative	ND
HLA Class I	(–)	(–)
Class II	(–)	(–)

DTH : delayed type hypersensitivity reaction.
MLR : mixed lymphocyte reaction.
ND : not determined.
* : absolute counts per mm^3 are shown in parenthesis.

There was radiologic evidence of interstitial pneumonia. HLA class I and II antigens were negative by microcytotoxicity assay. However, HLA A2, A3, B51, B50, BW4 and BW6 antigens could be detected on peripheral blood mononuclear cells after in vitro treatment with IFN-α. Oligotyping for DR loci was kindly performed by M. Oudshoorn (Blood Bank, Leiden, Netherlands) and found to be identical to one of her sisters (Table II).

Table II: HLA Antigens of The Cases and The Family Members

Case 1	: Negative
	: A2, A3, B50, B51, BW4, BW6 (After in vitro IFN-α treatment)
Father	: A2, A3, B51, CW-, DR4, DR11, DQ7, DQ8, BW4, DR52, DR53
Mother	: A1, A3, B7, B50, CW6, CW7, DR15, DR17, DQ2, DQ6, BW6, DR51, DR52
Sibling 1:	A1, A2, B7, B51, CW7, DR4, DR15, DQ6, DQ8, BW4, BW6, DR51, DR53
Sibling 2:	A2, A3, B50, B51, CW6, DR4, DR17, DQ2, DQ8, BW4, BW6, DR52, DR53
Oligotyping for the DR-loci:	
Case1	: DRB1*0402, DRB1*0301, DRB3*0202
Sibling 2:	DRB1*0402, DRB1*0301, DRB3*0202
Case 2	: Negative
Father	: A3, B44, BW4, DR4, DR53, DQ7
Mother	: A11, A23, B27, B49, BW4, DR1, DR11, DR52, DR53, DQ5, DQ7
Sibling 1:	A3, A23, B44, B49, BW4, DR4, DR11, DR52, DR53, DQ7
Sibling 1:	A3, A11, B44, B27, BW4, DR4, DR11, DR52, DR53, DQ7

Intravenous immunoglobulin (IVIG), antibiotic and antifungal therapy were initiated. As cytomegalovirus (CMV) inclusion bodies were identified in the urine, gancyclovir treatment was given for three weeks and resulted in marked improvement in pulmonary findings and a gradual decrease in the elevated transaminases and the hepatomegaly. She was discharged with intravenous immunoglobulin, prophylactic trimethoprim-sulfamethoxasole and ketoconazole. During follow-up at the outpatient clinic, she had chronic diarrhea which led to failure to thrive and finally to marasmus. Intramuscular injections of human interferon-α administered for seven weeks in the hope of ameliorating the symptoms was of no benefit to either the disease course or the expression of HLA antigens. At 22 months, she weighed 5100 g (< 5th percentile) and BCG lymphadenitis appeared in her left axillary region. She was referred to Prof. Dr. J.M.J.J. Vossen (Department of Pediatrics, University Hospital of Leiden) for bone marrow transplantation (BMT) from her HLA identical sibling.

Case 2

The second case, ÖF, was also a female infant referred to our hospital because of pneumonia at the age of four months. The part history revealed that she had

been born with a body weight of 2200 g (< 5th percentile) and had begun to suffer from persistent cough, recurrent fever and diarrhea during the neonatal period. BCG had been administered at birth with no complication. She was the fourth child of consanguineous parents (first cousins). A male sibling had died of persistent pneumonia and gastroenteritis at home at the age of six months. Two female siblings were healthy.

On referral, she weighed 5000 g (25th percentile) and the physical examination revealed palpable small cervical lymph nodes, oral candidiasis, intercostal retractions and bilateral rales. Atelectasis, hyperinflation and bronchial thickening were seen on the chest roentgenogram. The laboratory data are shown in Table I. HLA class I and II antigens were negative by microcytotoxicity method. In vitro treatment of lymphocytes by IFN-α had no effect on the expression of HLA antigens (Table II).

The patient was given IVIG, antibacterial and antifungal therapy. During follow-up, intractable diarrhea requiring hospital care persisted on two occasions. She succumbed to death at home, presumably because of septicemia three months after the diagnosis.

Discussion

BLS was first described by Touraine et al.[4] in 1978. Several additional cases have subsequently been reported[2, 3, 5-7]. Among these, two siblings born to a family of Turkish origin have been reported by Schuurman et al.[5] Having an autosomal recessive form of inheritance, relatively frequent occurrence of BLS can be expected in our country because of the high incidence of marriages among relatives.

The HLA antigens function as important recognition signals on the surface of lymphocytes and play a crucial role in modulating lymphocyte functions such as antigen presentation to immunoregulatory T cells (especially CD4 helper cells) and T cell development in the thymus. In BLS, as a result, both T and B cell functions are impaired to various degrees[1, 2, 5]. Apart from a few patients reported lacking HLA class I antigens without suffering from apparent immunodeficiencies, all others described in the literature have a combined type of immunodeficiency with slight variations in the degree of the defects and the severity of the disease[2, 3, 5-8]. Both of our cases presented with recurrent lower respiratory tract infections, gastroenteritis and oral candidiasis in early infancy, as did the vast majority of the patients in the review by Haas and Steihm[2]. Despite all the supportive therapy, IVIG, prophylactic antifungal and antibacterial agents, the inevitable, gradually developing marasmic state of our first patient was noteworthy.

Although both patients had hypogammaglobulinemia; while T and B cells were diminished in the first patient, diminished T cells and slightly increased B cells were discovered in the second patient. This finding supports the view that

defective T-cell function leads to deficient B-cell response to antigenic stimulation. Negative delayed hypersensitivity skin test (DTH) reactions of Case 1 were consistent with most of the cases in the literature[2]. However, in the second case, DTH response to one of the mitogens (PHA) was slightly positive, as in Tourain's case[4]. Normal or near-normal proliferative response to mitogens, a characteristic finding of BLS, was observed in both of our patients. Although absent proliferative response to specific antigens is another consistent finding in BLS, we could not demonstrate it as our tests failed.

It has been reported that both α and β human interferons could enhance the expression of HLA class I antigens in vitro[9]. We observed the same effect in Case 1. On the other hand, intramuscular injections of IFN-α were beneficial neither in alleviating clinical symptoms nor in expression of HLA antigens in the same patient.

BMT is now accepted as the treatment of choice when an HLA-matched sibling is available[10]. It is necessary to perform genotyping with DNA probes to determine the HLA antigens of the patient in order to choose the donor[11]. Successful BMT from a haploidentical parent or from an unrelated donor has also been reported[7, 12]. Furthermore, successful in utero transplantation of fetal liver stem cells in two patients with BLS diagnosed prenatally has already been mentioned as an alternative to therapeutic abortion[13]. However, the overall prognosis of BLS is still poor. Elucidation of the precise molecular pathogenesis of the illness and cloning of the regulatory genes for HLA antigen expression may contribute to the development of more promising therapeutic approaches, such as gene therapy.

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REFERENCES

1. Ammann AJ, Hong R. Disorders of the T-cell system. In: Stiehm ER (ed). Immunologic Disorders in Infants and Children. Philadelphia: W.B. Saunders Company; 1989: 257-315.
2. Haas A, Stiehm ER. Failure to thrive, thrush, and hypogammaglobulinemia in a 6-month-old infant. Ann Allergy 1987; 59: 103, 141-144.
3. Touraine JL. The bare-lymphocyte syndrome: report on the registry. Lancet 1981; 1: 319-320.
4. Touraine JL, Betuel H, Souillet G, Jeune M. Combined immunodeficiency disease associated with absence of cell-surface HLA-A and -B antigens. J Pediatr 1978; 93: 47-51.
5. Schuurman RKB, van Rood JJ, Vossen JM, et al. Failure of lymphocyte-membrane HLA-A and -B expression in two siblings with combined immunodeficiency. Clin Immunol Immunopathol 1979; 14: 418-434.

6. Sugiyama Y, Maeda H, Okumura K, Takaku F. Progressive sinobronchiectasis associated with the "bare lymphocyte syndrome" in an adult. Chest 1986; 3: 398-401.
7. Will N, Seger RA, Betzler C, et al. Bare lymphocyte syndrome-combined immunodeficiency and neutrophil dysfunction. Eur J Pediatr 1990; 149: 700-704.
8. Payne R, Brodsky FM, Peterlin BM, Young LM. "Bare lymphocytes" without immunodeficiency. Hum Immunol 1983; 6: 219-227.
9. Durandy A, Virelizier JL, Griscelli C. Enhancement by interferon of membrane HLA antigens in patients with combined immunodeficiency with defective HLA expression. Clin Exp Immunol 1983; 52: 173-178.
10. Parkman R. The biology of bone marrow transplantation for severe combined immune deficiency. Adv Immunol 1991; 49: 381-410.
11. Marcadet A, Cohen D, Dausset J, Fischer A, Duraney A, Griscelli C. Genotyping with DNA probes in combined immunodeficiency syndrome with defective expression of HLA. N Engl J Med 1985; 312: 1287-1292.
12. Casper JT, Ash RA, Kirchner P, Hunter JB, Havens PL, Chusid MJ. Successful treatment with an unrelated-donor bone marrow transplant in an HLA-deficient patient with severe combined immune deficiency ("bare lymphocyte syndrome"). J Pediatr 1990; 116: 262-265.
13. Touraine JL. In utero transplantation of fetal liver stem cells in humans. Blood Cells 1991; 17: 379-387.

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PARTIAL C4 DEFICIENCY IN TWO CHILDREN WITH SYSTEMIC LUPUS ERYTHEMATOSUS*

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SUMMARY: Bakkaloğlu A, Pascual M, Schifferli JA, Özen S, Beşbaş N, Saatçi Ü. (Nephrology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey, and Clinique Medicale, Hopital Cantonal Universitaire, Basel, Switzerland). Partial C4 deficiency in two children with systemic lupus erythematosus. Turk J Pediatr 1995; 37: 147-151.

Systemic lupus erythematosus (SLE) is a rare disease in childhood. Here two cases with SLE are presented, both with C4 null alleles yielding a functional C4 deficiency. The first case, a 14-year-old girl with a C4A null allele only, had a mild disease course, whereas the second child, a seven-year-old boy with both C4A0 and C4B0, had a more relentless course leading to death in five years. We conclude that complement activation by the classical pathway might be an essential mechanism that protects against the emergence of autoimmune or immune-complex disases, and that the deficient state in our patients predisposed them to the early development of SLE. *Key words: systemic lupus erythematosus, C4 null allele, childhood.*

Systemic lupus erythematosus (SLE) has been reported in children of all ages, although it is very rare before the age of ten. The prevalence appears to be high in Turkey, since 56 children with SLE have been seen during the last ten years at our hospital. Various genetic and environmental factors may contribute to the initiation of the disease process. Among these factors are deficiencies of complement proteins of the classical pathway (C1q, C1r, C1s, C4, and C2)[1]. Such deficiencies also favor bacterial infections which may contribute to the initiation of the autoimmune process. A partial deficiency of C4 expression has also been reported in SLE[2]. Two forms of C4 are coded on chromosome 6-C4A and C4B-so that C4 synthesis is due to the expression of four genes (two for each). The two forms differ in their capacity to bind covalently to cell surfaces and proteins. Whereas C4B binds essentially to hydroxyl residues, C4A binds

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more efficiently to amino residues[3,4]. For example C4B is capable of inducing the lysis of erythrocytes five-to-ten-fold more efficiently than C4A, whereas C4A binds preferentially to immune complexes[5,6]. It was therefore of particular interest not only that SLE was associated with partial C4 deficiency, but also that C4A was the culprit for this association[2]. The presence of a C4AQ0 allele (lack of expression of one of the C4A gene products) is found in approximately 15 percent of normal individuals and in 30 percent of SLE patients. Even more striking is the observation that a double C4AQ0 (homozygous C4AQ0, i.e. no circulating C4A in plasma) is found in 11 percent of SLE patients and in only one percent of normal individuals[7-11].

We have recently begun to determine the C4 alleles in children with SLE and their families, and found a partial C4 deficiency in two patients studied because of their persistently low C4 levels.

Case Reports

Case 1

This 14-year-old girl presented with polyarthralgia, arthritis in the interphalangeal joints and a butterfly rash following a hepatitis A infection. She was the third child of the family, and her parents were distantly related.

Laboratory examinations were as follows: Hb 12.80 g/dl, white blood cell (WBC) 6400/mm^3, erythrocyte sedimentation rate 128 mm/hr, C3: 30 mg/dl, C4 < 5 mg/dl, C1q: 36 mg/dl (normal), C5: 11 mg/dl (normal), ANA:+, Anti DNA: 17 unit/ml (normal: 0-5). Urine examination was normal.

The child was diagnosed with SLE, and corticosteroid treatment was initiated at a dose of 2 mg/kg/day. At the end of six weeks, the patient was in clinical remission. The complement levels at the time were C3: 74 mg/dl, C4 < 5 mg/dl. The corticosteroid dose was lowered and hydroxychloroquine was instituted. Her C4 levels remained below 5 mg/dl although there was no activation of the disease for a year.

The C4 assays and HLA typing of the patient and the family were thus studied. The C4 functional assay (hemolytic) revealed a 28 percent function for C4. The patient as well as the father and one sister had C4AQ0 (Fig. 1). These three family members were the only ones with HLA-B8 antigen on a supratype of HLA-A1, B8, BfS, C4AQ0, B1,-DR4.

Case 2

This seven-year-old boy was diagnosed with SLE in 1987, when he presented with aphthous stomatitis, cutaneous lesions, Raynaud phenomenon and Coombs+ hemolytic anemia. The parents were first cousins.

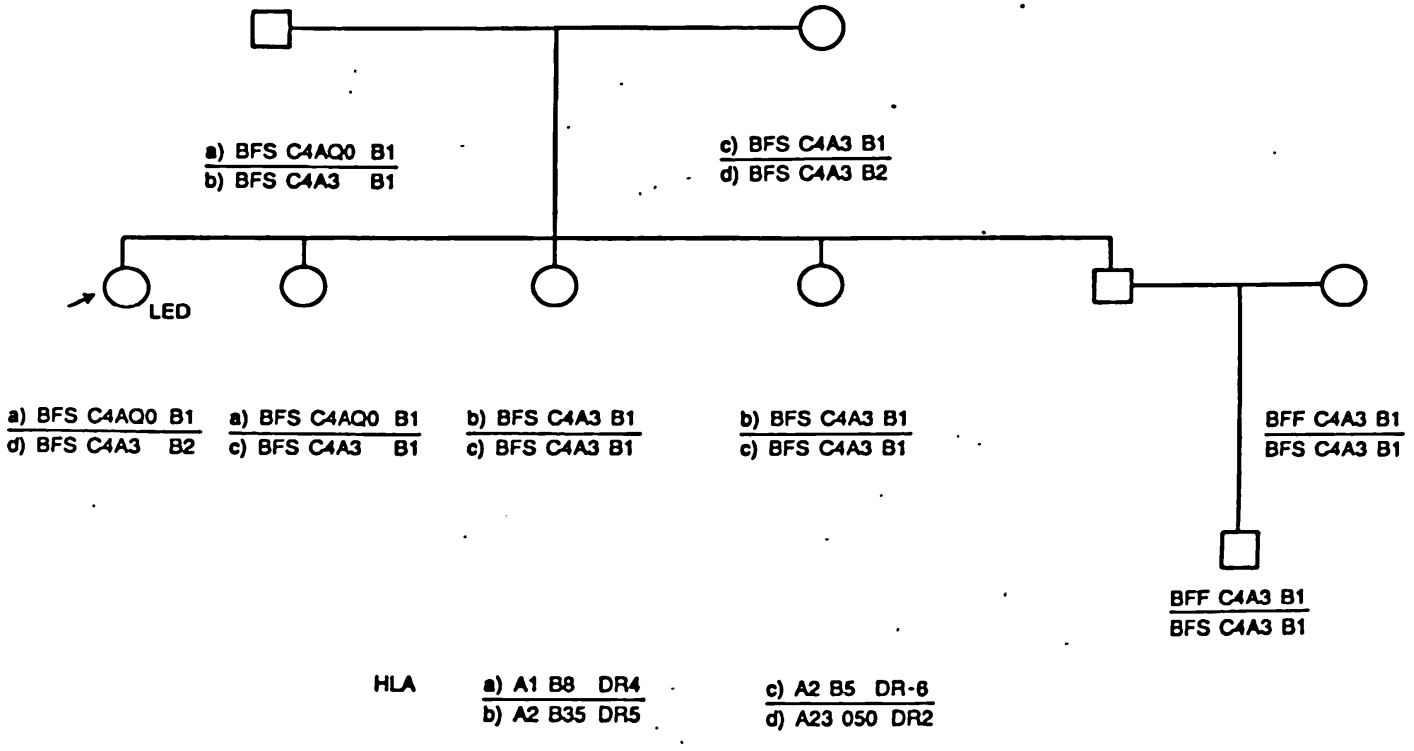

Fig. 1: The patient as well as the father and one sister had C4AQ0.

Laboratory studies revealed Hb 8.20 g/dl, WBC 3400/mm^3, sedimentation rate 22 mm/hr, C3: 48 mg/dl, C4: 8 mg/dl, ANA +, and Anti DNA: 48 unit/ml.

During the follow-up period, the patient developed cerebral involvement and showed a resistant disease course. He received oral and intravenous corticosteroids, and the disease activity was partially controlled after the addition of cyclophosphamide. During his five-year follow-up period, his C4 levels were consistently low although C3 reached normal levels a few times. Five years after the diagnosis he was admitted to the hospital with a secondary viral infection superimposed on his neutropenia and pulmonary edema. This time he was reevaluated because of his atypical facies, and his chromosomal analysis revealed him to be a mosaic Down syndrome. In spite of symptomatic treatment for his pulmonary edema and infection, he died a day after his hospitalization.

The C4 assays were performed and revealed a functional C4 level of 20 percent of normal. Null alleles were present at the C4A and C4B loci. HLA typing was as follows: A3, A23, B51 (5, w4), DRw15-(2, Qw1), DQw6 (Qw1).

Discussion

These two patients had partial C4 deficiencies and SLE. Christiansen et al.[12] have suggested a gene dose effect, starting with total C4 deficiency (C4AQ0, BQ0), which is associated in more than 90 percent of cases with an SLE-like

disease; i.e. total C4 deficiency appears to be associated almost invariably with the expression of autoimmunity and/or an immune complex disease. Double C4A null allele increases the likelihood of developing SLE approximately ten-fold, whereas the presence of only a single C4A null only doubles this likelihood[7-11]. In the young boy described here in whom two C4 gene products were lacking, SLE started before the age of ten, which is very rare.

The results of several series suggest that the association between C4 deficiencies and SLE is due to the lack of C4A or total C4 function[1,2]. However, a recent report provides evidence for the pre-dominant role of class I and II genes over C4, since the C4AQ0 allele was included in a haplotype which was itself a risk factor for SLE (HLA B9-C4AQ0-C4B1-DR3)[13]. This haplotype was also present in our first patient. Despite these latter epidemiological data, it is worth emphasizing that C4 function might be essential in many immune reactions including B-cell memory and the physiological clearance of bacteria, viruses, immune complexes and other substances such as DNA[1,14-17]. Thus complement activation by the classical pathway might be an essential mechanism which protects against the emergence of autoimmune or immune-complex diseases.

From the foregoing we might suggest that it is not the partial C4 deficiency which causes SLE, but an optimal C4 function which is a protective factor. This hypothesis is supported by the fact that 1 in 100 normal individuals are homozygous for C4AQ0. Without other factors predisposing to SLE, this partial C4 deficiency has no pathological consequences. There is a corollary to this hypothesis, namely that patients who are predisposed to develop SLE and who have a partial C4 deficiency will develop the disease earlier than those in whom C4 function is normal. The two children we studied had partial C4 deficiency in an environment (infections) which might be particularly efficient at triggering immune and possibly autoimmune responses. We plan now to compare in a prospective study the frequency of partial C4 deficiencies in childhood versus adult-onset SLE.

REFERENCES

1. Morgan BP, Walport WJ. Complement deficiency and disease. Immunol Today 1991; 12: 301-306.
2. Hauptmann G, Tappeinerr G, Schifferli JA. Inherited deficiency of the fourth component of human complement. Immunodef Rev 1988; 1: 3-22.
3. Law SK, Dodds AW, Porter RR. A comparison of the properties of two classes, C4A and C4B, of the human complement component C4. EMBO 1984; 3: 1819-1823.
4. Isenman DE, Young JR. Covalent binding properties of the C4A and C4B isotypes of the fourth component of human complement on several C1-bearing cell surfaces. J Immunol 1986; 136: 2542-2550.

5. Schifferli JA, Steiger G, Paccaud JP, Sjoholm AG, Hauptmann G. Difference in the biological properties of the two forms of the fourth component of human complement (C4). Clin Exp Immunol 1986; 63: 473-477.

6. Schifferli JA, Hauptmann G, Paccaud JP. Complement-mediated adherence of immune complexes to human erythrocytes. Difference in the requirements for C4A and C4B. FEBS Lett 1987; 213: 415-418.

7. Dawkins RL, Christiansen FT, Kay PH, et al. Disease associations with complotypes, supratypes and haplotypes. Immunol Rev 1983; 70: 1-22.

8. Fielder AH, Walport MJ, Batchelor JR, et al. Family study of the major histocompatibility complex in patients with SLE: importance of null alleles of C4A and C4B in determining disease susceptibility. Br Med J 1983; 286: 425-428.

9. Howard PF, Hochberg MC, Bias WB, Arnett FC Jr, McLean RH. Relationship between C4 null genes, HLA-D region antigens, and genetic susceptibility to SLE in Caucasian and black Americans. Am J Med 1986; 81: 187-193.

10. Kemp ME, Atkinson JP, Skanes VM, Levine RP, Chaplin DD. Deletion of C4A genes in patients with SLE. Arthritis Rheum 1987; 30: 1015-1022.

11. Reveille JD, Arnett FC, Wilsonn RW, Bias WB, McLean RH. Null alleles of the fourth component of complement and HLA haplotypes in familial SLE. Immunogenetics 1985; 21: 299-311.

12. Christiansen FT, Zhankg WJ, Griffiths M, Mallal SA, Dawkins RL. Major histocompatibility complex (MHC) complement deficiency, ancestral haplotypes and SLE: C4 deficiency explains some but not all of the influence of the MHC. J Rheumatol 1991; 18: 1350-1358.

13. Hartung K, Baur MP, Coldeway R, et al. Major histocompatibility complex haplotypes and Complement C4 alleles in SLE. Results of a multicenter study. J Clin Invest 1992; 90: 1346-1351.

14. Bottger EC, Bitter-Suermann D. Complement and the regulation of humoral immune responses. Immunol Today 1987; 8: 261-264.

15. Mascart-Lemone F, Hauptmann G, Goetz J, et al. Genetic deficiency of C4 presenting with recurrent infections and a SLE-like disease. Genetic and immunologic studies. Am J Med 1993; 75: 295-304.

16. Schifferli JA, Ng YC, Peters DK. The role of complement and its receptor in the elimination of immune complexes. N Engl J Med 1986; 315: 488-495.

17. Robey FA, Jones KD, Steinberg AD. C-reactive protein mediates the solubilization of nuclear DNA by complement in vitro. J Exp Med 1985; 161: 1344-1356.

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NEONATAL LUPUS SYNDROME:
Report of a Case*

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SUMMARY: Gürakan B, Yalçın S, Tekinalp G, Çeliker A. (Neonatology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Neonatal lupus syndrome: report of a case. Turk J Pediatr 1995; 37: 153-156.

Neonatal lupus syndrome (NLS) is characterized by the presence of cutaneous lupus, congenital heart block or both associated with maternal autoantibodies anti-Ro (SS-A) and anti-La (SS-B). NLS is responsible for more than 80 percent of cases with isolated congenital complete heart block. This report describes a neonate with third-degree atrioventricular block who was born to an anti-Ro (SS-A) positive mother. The dermatological and hematological manifestations of NLS were absent. The infant's clinical condition was good during the two months of follow-up with expectant management. *Key words: neonatal lupus, congenital heart block.*

Neonatal lupus syndrome (NLS) is characterized by the presence of cutaneous lupus or congenital heart block in an infant whose mother has connective tissue disease or the autoantibodies anti-Ro (SS-A), anti-La (SS-B), or anti-U1RNP[1]. The annular and erythematous skin lesions in NLS generally appear within the first two months of life and resemble the adult skin lesions of subacute cutaneous lupus erythematosus[2]. Associated hematologic and hepatic abnormalities have also been reported, but are not considered an essential component of the syndrome[3,4]. Except for congenital heart block, the disease resolves with the clearance of the maternal antibodies by the sixth to eighth month of postnatal life.

NLS is responsible for more than 80 percent of cases with isolated congenital complete heart block (CCHB)[5]. The heart block appears during the second half of pregnancy. Although CCHB is compatible with normal life, fetal hydrops, in utero or neonatal deaths may occur. In this report, we describe a neonate with CCHB associated with anti-Ro (SS-A) antibodies, and we discuss current concepts in the prenatal and postnatal management of this rare entity.

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Case Report

This was the first pregnancy of a 26-year-old healthy woman. She was referred to our hospital because of fetal bradycardia in her 33rd week of gestation. Apart from this findings her pregnancy was noted to be unremarkable.

At the time of her presentation, sonography revealed normal cardiac anatomic findings. However, fetal bradycardia of 60-70 beats/min was noted, suggesting isolated CCHB.

The clinical findings and routine blood tests of the mother were normal. Other laboratory tests for connective tissue disease included negative results for ANA, anti-DNA and anti-La (SS-B), but positive results for rheumatoid factor and anti-Ro (SS-A) antibodies (106 IU/ml by ELISA). The IgM antibodies for cytomegalovirus, rubella and toxoplasma were all negative. Fetal echocardiography revealed normal cardiac contractility and size. There was no pericardial effusion. In serial ultrasound scans during follow-up, breathing movements and fetal tone were normal, and the signs for hydrops were absent. In the 37th week of gestation, a cesarean section was performed because of oligohydramnios.

This male baby was 45 cm in length and 2550 g in weight. The Apgar scores were 8 and 9 at one and five minutes, respectively. The heart rate was 64 beats/min, and a pansystolic murmur of 2/6 was heard at the left sternal border. Neither skin lesions nor purpura was observed. The other organ system findings were normal.

Laboratory studies revealed hemoglobin 19 g/dl, white blood cells 15.100/mm^3 with 48% neutrophils, 50% lymphocytes and 2% bands. Platelets were normal in the peripheral smear. Blood sugar, electrolytes, SGOT and SGPT were within normal limits. Anti-Ro (SS-A) antibodies were positive (101 IU/ml), but anti-La antibodies were negative. EKG indicated third-degree atrioventricular (AV) block with a heart rate of 68 beats/min. The chest X-ray showed no cardiomegaly. During six days of follow-up at the hospital, the infant's condition did not change. The daytime heart rates were greater than 50 beats/min. During his first month of life, the holter monitoring revealed heart rates ranging from 42 to 80 beats/min, with a mean of 62 beats/min (Fig. 1). During the two months of follow-up, the infant's clinical condition did not change.

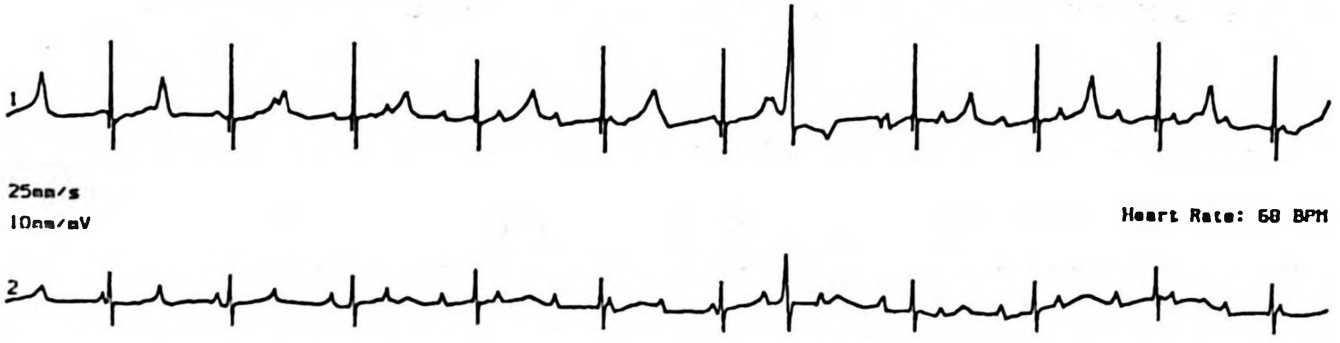

Fig. 1: Third-degree atrioventricular block.

Discussion

The pathogenesis of NLS is presumed to be related to the transplacental passage of maternal autoantibodies anti-Ro (SS-A) and anti-La (SS-B), which are found almost exclusively in connective tissue diseases. Depending on the method, the frequencies of these antibodies vary in controls and in patients with connective tissue diseases. Anti-Ro (SS-A) antibodies are found in more than 40 percent of Sjögren's syndrome patients, 25 to 30 percent of systemic lupus erythematosus (SLE) patients and 5 percent of rheumatoid arthritis patients[5]. Anti-La (SS-B) is not as common as anti-Ro (SS-A). These antibodies, however, are found in only 0.44 to 2.0 percent of the normal population[1].

Anti-Ro (SS-A) and anti-La (SS-B) antibodies are also found in more than 80 percent of mothers of infants with CCHB. The antibodies in human fetal heart tissue and immunoglobulin deposition in a neonatal lupus heart have been demonstrated[6, 7]. In a recent study, inhibition of cardiac repolarization by anti-Ro (SS-A) antibodies was observed[8]. Despite this evidence, the low incidence of NLS in infants born to anti-Ro (SS-A) positive mothers (10%)[9], and the discordance of disease expression in twins and in subsequent pregnancies, raise the possibility of other factors including HLA alloantigen classes, the host response, and environmental etiologies. These antibodies, then, may be necessary, but not sufficient to cause NLS.

Although these antibodies are found mostly in women with connective tissue diseases, mothers of infants with NLS are usually asymptomatic. Connective tissue disease may develop later in these initially asymptomatic mothers[4]. Our patient's mother was also asymptomatic and her laboratory tests were negative other than rheumatoid factor. However, she was advised to be checked regularly.

For infants with skin manifestations alone, the prognosis is excellent and no treatment is necessary. However, for infants with CCHB, the mortality is significant. Therefore, the prenatal and postnatal management is important.

The enviroment in utero is preferred as long as possible because of low circulatory resistance. Treatment is not necessary in cases without fetal cardiac failure.

However, the presence of ascites, pericardial effusion or hydrops requires more active management. Intrauterine therapeutic regimens, including dexamethasone, which is not metabolized by the placenta and is available to the fetus in an active form, and plasmapheresis have been tried[10, 11]. Unfortunately, heart block could not be reversed by these therapies. In our case, the initial echocardiography and ultrasound scan did not indicate cardiac failure. Expectant management was judged to be safe. In fact, our patient was normal at birth, other than bradycardia.

Although CCHB is usually well tolerated, it is sometimes associated with syncope or sudden death. Prophylactic pacemaker insertion is indicated in selected patients with CCHB. Ventricular rate has been emphasized as the most sensitive means of selecting patients for pacing. Rates of less than 50 beats/min for neonates and 40 beats/min for older infants have been advocated as cutoffs.. Recent studies indicate that ventricular rate alone is not the best index. The presence of symptoms, cardiomegaly, a wide QRS or a prolonged QT interval, evidence of junctional instability and decreased ventricular function by echocardiography are the additional advocated guidelines[12, 13]. Our case is two months old now, and none of the above criteria is present. We plan to monitor him at six-month intervals.

REFERENCES

1. Petri M, Watson R, Hochberg MC. Anti-Ro antibodies and neonatal lupus. Rheum Dis Clin North Am 1989; 15: 335-360.
2. Maynard B, Leiferman KM, Peters MS. Neonatal lupus erythematosus syndrome. J Cutan Pathol 1991; 18: 333-338.
3. Watson R, Kang JE, May M, Hudak M, Kickler T, Provost TT. Thrombocytopenia in the neonatal lupus syndrome. Arch Dermatol 1988; 124: 560-563.
4. McCune AB, Weston WL, Lee LA. Maternal and fetal outcome in neonatal lupus erythematosus. Ann Intern Med 1987; 106: 518-523.
5. Scott JS, Maddison PJ, Taylor PV, Esscher E, Scott O, Skinner RP. Connective-tissue disease, antibodies to ribonucleoprotein, and congenital heart block. N Engl J Med 1983; 309: 209-212.
6. Deng JS, Bair LW Jr, Shen-Schwarz S, Ramsey-Goldman R, Medsger T Jr. Localization of Ro (SS-A) antigen in the cardiac conduction system. Arthritis Rheum 1987; 30: 1232-1238.
7. Lee LA, Harmon CE, Huff JC, Norris DA, Weston WL. The demonstration of SS-A/Ro antigen in human fetal tissues and in neonatal and adult skin. J Invest Dermatol 1985; 85: 143-146.
8. Alexander E, Buyon JP, Provost TT, Guarnieri T. Anti-Ro/SS-A antibodies in the pathophysiology of congenital heart block in neonatal lupus syndrome, an experimental model. Arthritis Rheum 1992; 35: 176-189.
9. Watson RM, Lane AT, Barnett NK, Bias WB, Arnett FC, Provost TT. Neonatal lupus erythematosus: a clinical, serological and immunogenetic study with review of the literature. Medicine 1984; 63: 362-378.
10. Buyon JP, Swersky SH, Fox HE, Bierman FZ, Winchester RJ. Intrauterine therapy for presumptive fetal myocarditis with acquired heart block due to systemic lupus erythematosus. Arthritis Rheum 1987; 30: 44-49.
11. Barclay CS, French MAH, Ross LD, Sokol RJ. Successful pregnancy following steroid therapy and plasma exchange in a woman with anti-Ro (SS-A) antibodies. Case report. Br J Obstet Gynaecol 1987; 94: 369-371.
12. Dewey RC, Capeless MA, Levy AM. Use of ambulatory electrocardiographic monitoring to identify high-risk patients with congenital complete heart block. N Engl J Med 1987; 316: 835-839.
13. Sholler GF, Walsh EP. Congenital complete heart block in patients without anatomic cardiac defects. Am Heart J 1989; 118: 1193-1198.

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SANFILIPPO DISEASE TYPE B

A Case Report and Review of the Literature on Recent Advances in Bone Marrow Transplantation*

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SUMMARY: Güngör N, Tunçbilek E. (Genetics Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Sanfilippo disease type B. Turk J Pediatr 1995; 37: 157-163.

A patient with enzymatically proven Sanfilippo disease type B is presented. This type of mucopolysaccharidosis results from deficient α-N-acetylglucosaminidase activity leading to defective degradation of heparan sulphate. As this disease is associated with high morbidity and mortality, different therapeutic approaches are under investigation. Bone marrow transplantation is among these new choices of management. The value of bone marrow transplantation in the mucopolysaccharidoses, especially in MPS IIIB, is discussed with a wide review of the literature. *Key words: Sanfilippo disease, mucopolysaccharidosis, bone marrow transplantation.*

Case Report

A four-and-one-half-year-old girl who had been diagnosed with mucopolysaccharidosis (MPS) in another hospital was admitted to Hacettepe University Children's Hospital, Department of Clinical Genetics. The family asked to be informed about the possibility of bone marrow transplantation (BMT) for this child.

The patient was the product of the eighth pregnancy of a couple with first degree consanguinity. Following three spontaneous abortions, the fourth child (Fig. 1, III-4) had been followed with the clinical diagnosis of mucopolysaccharidosis type III (MPS III; Sanfilippo syndrome) in another hospital. No enzymatic analysis for type determination had been performed. He apparently died at nine years of age as a result of progressive mental and motor deterioration. There is only one healthy child in the family (Fig. 1, III-5). The last two pregnancies were terminated on demand (Fig. 1).

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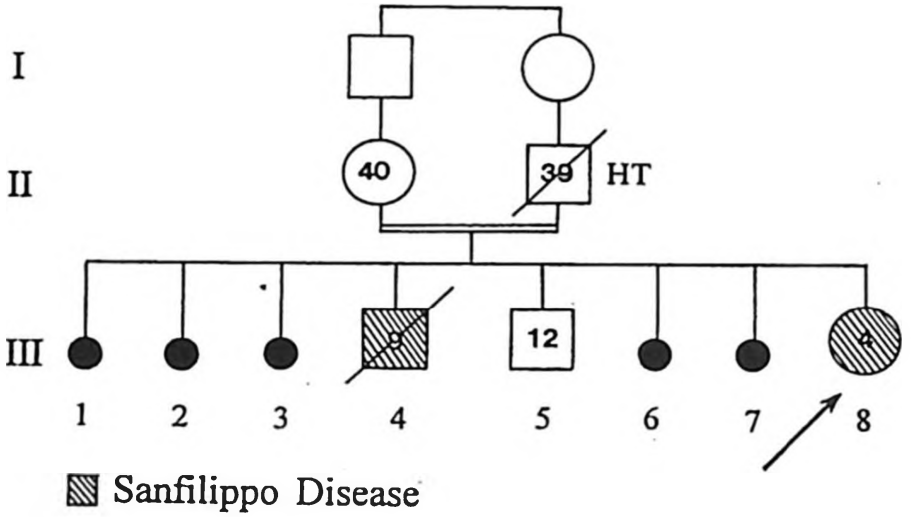

Fig. 1: The Pedigree

The prenatal and birth history of the patient were unremarkable. A sitting position was achieved at eight months of age, and she was reportedly able to walk by one year of age. She could also distinguish her mother. At one-and-a-half years of age, she was taken to another health center because of restlessness and sleep disturbances and a mildly protuberant abdomen. She was diagnosed with MPS III on the grounds of the family history, physical and x-ray findings coupled with a positive test for mucopolysaccharides in a spot urine sample. Genetic counselling was provided.

At the time of referral, her speech development was slow and poor. She was able to spell about 10-20 words. The parents complained of her restlessness and hyperkinesis. She was easily frightened and became aggressive in stressful situations. The family was now willing to learn whether the patient would benefit from BMT.

The patient weighed 20 kg (75^{th} - 90^{th} percentile). Her height was 108 cm (75^{th} - 90^{th} percentile) and head head circumference was 50.6 cm (within normal limits for age and sex; 49.9 ± 1.3 cm).

The physical examination revealed a mentally retarded patient with difficulty in cooperation. Her general condition was good. She had hirsutism and sparse hair growth. Prominent auriculae, coarse facial features with synophrys, anteverted nostrils, and an open mouth with a large tongue were observed. There was no clouding in her corneas and no joint limitation (Fig. 2). The liver was palpated two centimeters below the right costal arch.

The urinary excretion of mucopolysaccharides was 6.8 mg/dl in the spot urine sample. Her skeletal x-rays were consistent with mild dysostosis multiplex. A dense calvarium with increased diploe space, mildly oar-shaped ribs, and mild tapering at the proximal ends of the metacarpals were observed.

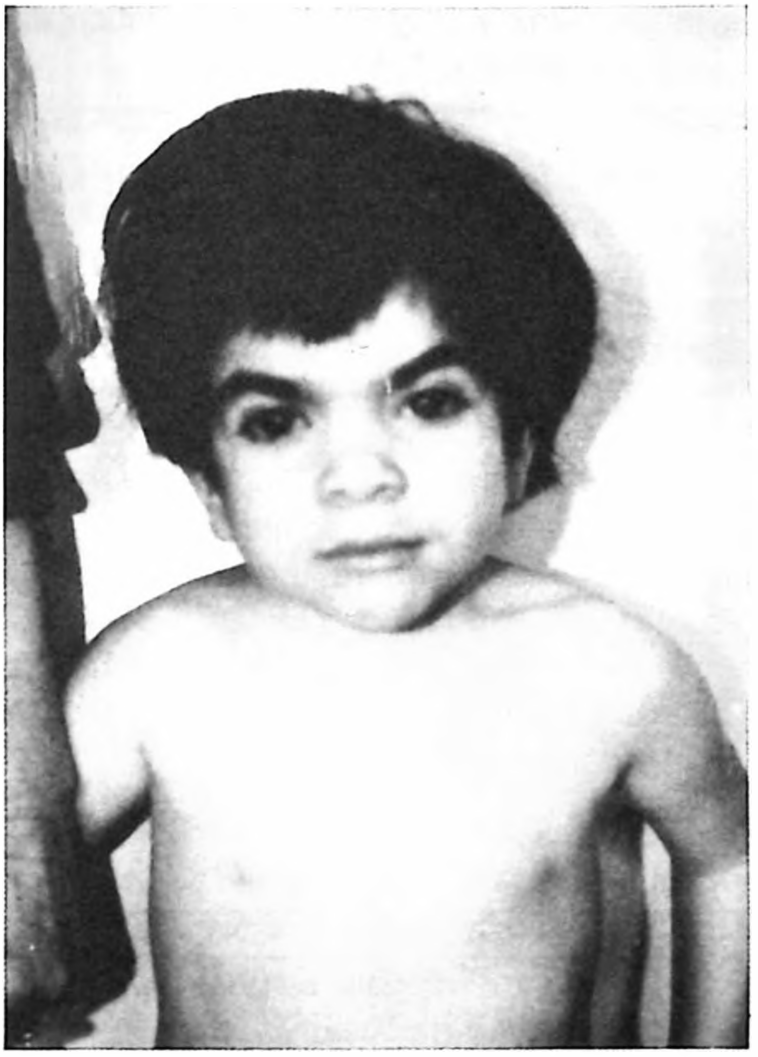

Fig. 2: The patient. Note the coarse facial features including the synophrys and the anteverted nostrils.

Fresh urine glycosaminoglycan analysis revealed large amounts of heparin and heparan sulphate, with heparan sulphate as the predominant fraction. This is typical for MPS III. Further studies on enzyme in leukocytes and plasma showed deficient o-N-acetylglucosaminidase activity. These results indicated a diagnosis of MPS IIIB.

Discussion

The mucopolysaccharidoses are a group of inherited disorders caused by a deficiency of specific lysosomal enzymes. The enzyme deficiency cause interference with cellular function because of excessive accumulation within the cells of partially degraded glycosaminoglycans, which are also excreted excessively in the urine. The type of glycosaminoglycan stored depends on the specific enzyme deficiency, and the classification of the group is now based on these deficiencies, rather than on clinical features[1]. The clinical presentations of patients with different types of MPS are summarized in Table I[1].

Table I: The Clinical Presentations of Patients with Different Types of MPS

Mode of Presentation	Type of MPS
Prominent dysmorphic features	MPS I, MPS II, MPS IIII
Behavior problems and dementia	MPS III
Bone dysplasia	MPS IV, MPS VI

In our patient the behavior problems related to mental retardation were more striking features when compared to the dysmorphic features. This was the initial point leading us to think in favor of MPS III. Sanfilippo syndrome is the most frequent of the mucopolysaccharidoses. The frequency is approximately 1:25,000[2]. The mode of inheritance is autosomal recessive. Excessive excretion of heparan sulphate in the urine is characteristic of this disorder[3,4]. Genetic heterogenetiy is present in Sanfilippo syndrome with the presence of four different enzyme deficiencies, in leukocytes, plasma, fibroblasts and other tissues, each of which forms a different subtype[2]. The enzymatic deficiencies in different subtypes of MPS III are demonstrated in Table II[4].

Table II: The Enzymatic Deficiencies in the Subtypes of MPS III (Sanfilippo Disease)

Subtype	Deficient Enzyme
Sanfilippo A	Heparan sulphate sulphatase
Sanfilippo B	N-acetyl-o-glucosaminidase
Sanfilippo C	Acetyl CoA: o-glucosaminide N-acetyltransferase
Sanfilippo D	N-acetyl-o-glucosamine-6-sulphate sulphatase

Recent studies point to great intertype and intratype variability in Sanfilippo syndrome[2]. This variability includes not only the phenotype of the patients but also the course of the disease. A study of 73 patients with Sanfilippo syndrome (types A, B and C) revealed that MPS IIIA, the most common subtype, was associated with an earlier onset, with more severe clinical manifestations and an earlier age at death. MPS IIIC was slightly less severe than MPS IIIA. The natural course of MPS IIIB seemed to be milder than that associated with types A and C.

On the other hand, an intratype variability was also observed in Sanfilippo syndrome type B, suggesting the existence of two allelic mutations[2]. Intrafamilial variability in Sanfilippo syndrome is another point of interest currently being investigated[2,5].

The mucopolysaccharidoses require a multidisciplinary approach to management involving not only pediatric subspecialties, such as cardiology and ophthalmology, but also other specialists including psychologists[1]. Today no definitively curative

treatment is available. Attempts are being made at enzyme replacement therapy[3, 4, 6, 7]. When the literature is reviewed on this issue, it is seen that in principle, BMT can be used to ameliorate any enzyme deficiency state, provided there is some reason to believe that enzyme-competent cells derived from the marrow or even enzyme released from them could be therapeutic[8]. Recently BMT has been used in proven states of enzyme deficiency (the lysosomal storage disorders). Severe combined immune deficiency, thalassemia and osteopetrosis are a few examples of genetic diseases that are correctable by BMT.

The mucopolysaccharidoses are also among the diseases that may be correctable by BMT[6]. But the use of BMT to treat inborn errors of metabolism involving the central nervous system (CNS) is still a subject of much controversy[3, 4, 6, 8]. Approximately 40 patients with Hurler's/Hunter's syndromes have been transplanted by the Westminster Hospital group using donors with a variety of histocompatibility relationships to the recipients. When using histocompatible donors, prompt donor lymphoid and hematopoietic engraftment have been observed with normalization of enzyme levels in the circulating leukocytes. However, marked improvement in the patient's non-CNS symptomatology would be of little clinical benefit unless it were paralleled by clinical improvement in the patient's CNS function. The long-term neurological outcome of these patients is still a subject of debate. Here the age at transplantation seems to be an important factor. Some patients who received transplantation at an early age (less than 18 months of age) are doing better than would have been predicted by their underlying disease, with some patients now in school with normal intellectual function; patients undergoing transplants at an older age appear to have had minimal benefit[6].

To the best of our knowledge, there are only three children with MPS IIIB who have received BMT and are reported in the medical literature. One of them is a girl whose age at transplant was 5.3 years and whose IQ was only 40. She continued to regress after BMT[3]. The other two patients were twins diagnosed with MPS IIIB at 18 months of age. They were clinically normal at that age and the diagnosis was made during the investigation of an elder sibling previously diagnosed with MPS IIIB. At the same time, another brother of theirs was similarly diagnosed. Nine-year follow-up post-transplant showed that neither twin was as handicapped as her untreated brothers were at the same age. Despite this, one of the twins was hyperactive and had behavioral problems characteristic of the disorder. On the other hand, both twins who showed functioning in the (low) average range according to Griffiths Scales prior to transplant, had a steady decline in cognitive development and had significant developmental delay. The authors find these results difficult to interpret, especially when taking into account the intrafamilial heterogeneic variation observed in MPS IIIB[3].

In conclusion, BMT does have a role in the management of the mucopolysaccharidoses. But in the forms with serious involvement of the brain, either there has not been any important difference in the final outcome of the neurologic deterioration or cases have not been followed long enough to permit an unequivocal conclusion to be drawn. As normal brain tissue is segregated from the circulation by the blood-brain barrier, it is doubtful that marrow-derived elements cross the barrier except under circumstances of injury or inflammation. Thus the direct transfer of enzyme to neurons has little chance of occurring. Considering the fact that BMT itself also entails considerable risks of morbidity and mortality, as well as a large commitment of medical, financial, psychosocial and other resources, it is still a controversial management for mucopolysaccharidoses with CNS involvement. Still, since the number of cases is not many, the disease is associated with an unacceptable morbidity and there is no effective alternative therapy, BMT may be applied to MPS IIIB patients who are as yet clinically unaffected. Providing a compatible related donor and informing the parents of the risks of both the procedure and the prognosis of the untreated disease, are still the most important issues while making the decision. For all these reasons, our patient was not thought to be a suitable candidate for this procedure. We believe that the answers may be found after long-term follow-up of a sufficient number of cases, and investigations at the molecular level will add a lot to our understanding of not only the heterogeneity in the natural course, but also of patterns of improvement after BMT. Therefore we emphasize the importance of proper genetic counselling and attempts at prenatal diagnosis for selected families whose future siblings are at risk of carrying this disorder, which is still practically untreatable.

Acknowledgements

We wish to thank G.T.N. Besley, Ph.D. (Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, University of Manchester) for performing the enzyme assays in our patient.

REFERENCES

1. Wraith JE, Rogers JG, Danks DM. The mucopolysaccharidoses. Aust Paediatr J 1987; 23: 329-334.
2. Van De Kamp JJP, Niermeijer MF, Von Figura K, Giesberts MAH. Genetic heterogeneity and clinical variability in the Sanfilippo syndrome (types A,B, and C). Clin Genet 1981; 20: 152-160.
3. Vellodi A, Young E, New M, et al. Bone marrow transplantation for Sanfilippo disease type B. J Inherit Metab Dis 1992; 15: 911-918.
4. Stevenson RE. Mucopolysaccharidosis III. In Buyse ML (ed). Birth Defects Encyclopedia. Center for Birth Defects Information Services Inc.: Blackwell Scientific Publications, 1990: 1163-1164.
5. Andria G, Di Natale P, Del Giudice E, et al. Sanfilippo B syndrome (MPS IIIB): mild and severe forms within the same sibship. Clin Genet 1979; 15: 500-504.

6. Parkman R. Organ transplantation as therapy for genetic diseases. In Friedmann T (ed). Therapy for Genetic Disease. New York: Oxford University Press, 1991: 95-106.

7. Krivit W, Pierpont ME, Ayaz K, et al. Bone-marrow transplantation in the Maroteaux-Lamy syndrome (mucopolysaccharidosis type VI). Biochemical and clinical status 24 months after transplantation. N Engl J Med 1984; 311: 1606-1611.

8. Barranger JA. Marrow transplantation in genetic disease. N Engl J Med 1984; 311: 1629-1631.

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MUCORMYCOSIS IN A DIABETIC CHILD AND ITS TREATMENT WITH FLUCONAZOLE: A CASE REPORT*

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SUMMARY: Selcen D, Seçmeer G, Aysun S, Kanra G, Önerci M, Gököz A, Ecevit Z, Ceyhan M, Anlar Y. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Mucormycosis in a diabetic child and its treatment with fluconazole: a case report. Turk J Pediatr 1995; 37: 165-168.

We present here a case of a diabetic patient having complaints of painful swelling of the left eye, blurring of the vision and tonic-clonic convulsion. Surgical exploration of the sinuses was performed, and the histopathological examination revealed mucormycosis. Because of the side effects of Amphotericin B, we tried Fluconazole and the patient recovered completely. *Key words: diabetes mellitus, mucormycosis, fluconazole.*

Mucormycosis is a fulminant fungal infection caused by phycomycetes. Specific organisms affecting humans are species of the general Mucor, Rhizophus and Absidia, which are members of the order Mucorales. They may be recovered from nose and stool cultures in healthy individuals, and man usually has a strong natural resistance to infection[1]. In most cases there are predisposing factors, such as diabetes mellitus, leukemia, multiple myeloma, carcinoma, anemia, burns, septicemia, hepatitis, use of antibiotics, steroids and ionizing radiation, among which the most common is diabetes mellitus[2,3].

We report a case of rhinocerebral mucormycosis in a diabetic patient and his treatment with fluconazole.

Case Report

A 15-year-old boy was admitted to the hospital with the complaints of painful swelling of the left eye, blurring of the vision and a tonic-clonic type of convulsion

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of the right side of the body. He had been suffering from insulin-dependent diabetes, which was poorly controlled, for five years. Two months earlier, a painful swelling began in the left eye, and an inflammatory exudate was drained from the cheek. He used three different antibiotics of 10-day duration for a nonspecific infection, but the swelling persisted. On admission, he was cachectic. Ptosis, exophthalmus and chemosis of the left eye, as well as periorbital swelling and redness of the left cheek were noted. Total ophthalmoplegia was present and there was no direct or indirect pupillary light reflex of the left eye and no indirect light reflex of the right eye. His oral hygiene was poor. On the left side of the body, there was a motor deficit of 40 percent. The coronal and orbital computerized tomography showed complete opacification of the left maxillary, ethmoid, frontal and sphenoid sinuses as well as cerebral cortical atrophy. For treatment, sulbactam-ampicillin, chloramphenicol, ornidazole and diphenylhydantoin were initiated. An emergent surgical exploration was performed, and necrotic material was curetted from the left maxillary, ethmoid and sphenoid sinuses.

Histopathological examination of the curetted tissue revealed nonseptate, irregularly branching hyphae, which was diagnosed as mucormycosis. There was no growth from pus of the maxillary sinus, but Staphylococcus aureus was identified from the ethmoidal sinus. The culture on Sabaroud's agar from the maxillary sinus also revealed candida spores. For the treatment of mucormycosis, Amphotericin B was administered. After one week of treatment, the medication was changed to fluconazole because of the side effects of Amphotericin B (hepatotoxicity, chills, fever). For the first week, 100 mg of fluconazole (3 mg/kg/day) by 30 minute intravenous infusion was given; then the oral preparation was continued (100 mg/day for six weeks). With this treatment, the patient recovered completely.

Discussion

Mucormycosis is an opportunistic infection, and there is usually a predisposing condition. In patients with diabetes, such as in our case, most often rhinocerebral mucormycosis develops[3,4].

Rhinocerebral mucormycosis usually begins in the nasal sinus or palate. The mucor fungus has a specific predilection for invading arteries and veins; thus, cavernous sinus thrombosis is frequent[5]. The most common clinical manifestations are headache, dark blood-tinged nasal discharge, periorbital and/or perinasal swelling, ptosis of the eyelid, fixed dilated pupil, loss of extraocular movements, progressive lethargy, black necrotic palate and alveolar ridge of turbinates, decreased visual acuity progressing to blindness and loss of corneal reflex[5-7]. Early amaurosis secondary to occlusion of the central retinal artery helps to distinguish this entity from septic cavernous sinus thrombosis[6]. The diagnosis

depends on histological examination of a biopsy specimen showing characteristic large, irregular, nonseptate, clear branching filaments shown by hematoxylin-eosin and periodic acid schiff (PAS) stains. As the culture may be negative even when fungi are present in large numbers, any suspicious area in the nose or palate should be biopsied[8].

Successful treatment of orbital mucormycosis depends on early diagnosis, control of any underlying disease, systemic antifungal therapy, and aggressive surgical debridement[1, 3, 6, 7, 9, 10]. Until recent studies, Amphotericin B had been the only drug known to be effective in the treatment of mucormycosis[9]. Recently a case of mucormycosis resistant to Amphotericin B was reported[10]; rhinocerebral mucormycosis was cured with ketaconazole. In another case report, fluconazole for the treatment of pulmonary mucormycosis complicating acute leukemia was used[11].

Because of side effects of Amphotericin B, we used a triazole derivative fluconazole. It has an extremely long half-life (22 hours in serum) and a very low protein-binding capacity (11%)[12]. Because of 95 percent absorption from the gastrointestinal tract, oral use can be effective. In our patient, the cure was obtained by treatment with fluconazole. In our opinion, this new agent can be used as an alternate to Amphotericin B.

REFERENCES

1. Anaissie EJ, Shikhani AH. Rhinocerebral mucormycosis with internal carotid occlusion: report of two cases and review of the literature. Laryngoscope 1985; 95: 1107-1113.
2. O'Keefe M, Baining WM, Young JD, Guthrie W. Orbital mucormycosis with survival. Br J Ophthalmol 1986; 70: 634-636.
3. Zak SM, Katz B. Successfully treated spheno-orbital mucormycosis in an otherwise healthy adult. Ann Ophthalmol 1985; 17: 344-348.
4. Sugar AM. Agents of mucormycosis and related species. In: Mandell GL, Douglas RG, Bennett JE (eds). Principles and Practice of Infectious Disease (3rd ed). New York: Churchill Livingstone; 1990; 1962-1970.
5. Rangel-Guerra R, Martinez HR, Sáenz C. Mucormycosis. Report of 11 cases. Arch Neurol 1985; 42: 578-581.
6. Gans ROB, Strack van Schijndel RJM, Laarman DA, et al. Fatal rhinocerebral mucormycosis and diabetic ketoacidosis. Netherlands J Med 1989; 34: 29-34.
7. Van der Westhuijzen AJ, Grotepass FW, Wyma G, Padayachee A.A rapidly fatal palatal ulcer: rhinocerebral mucormycosis. Oral Surg Oral Med Oral Pathol 1989; 68: 32-36.
8. Miller RD, Steinkuller PG, Naegele D. Nonfatal maxillocerebral mucormycosis with orbital involvement in a dehydrated infant. Ann Ophthalmol 1980; 12: 1065-1068.
9. Rakover Y, Vered I, Garzuzi H, Rosen G. Rhinocerebral phycomycosis; combined approach therapy: case report. J Laryngol Otol 1985; 99: 1279-1280.
10. Barnert J, Behr W, Reich H. An amphotericin B-resistant case of rhinocerebral mucormycosis. Infection 1985; 13: 134-136.

11. Funada H, Miyake T, Kanamori K, Okafuji K, Machi T, Matsuda T. Fluconazole therapy for pulmonary mucormycosis complicating acute leukemia. Jpn J Med 1989; 28: 228-231.

12. Jawetz E. Antifungal agents. In: Katzung B.C. (Ed) Basic and Clinical Pharmacology, a Lange Medical Book (3rd ed). 1987: 554-558.

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TYPHOID FEVER WITH VERY HIGH TRANSAMINASE LEVELS*

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SUMMARY: Özen H, Seçmeer G, Kanra G, Ecevit Z, Ceyhan M, Dursun A, Anlar Y. (Infectious Diseases Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Typhoid fever with very high transaminase levels. Turk J Pediatr 1995; 37: 169-171.

Typhoid fever is endemic in developing countries and may cause very different clinical findings. Although hepatic involvement and abnormal liver function tests may be seen in 50% of the patients, intravascular hemolysis and renal involvement are rare. In this report, a 10-year-old patient with enteric fever presenting with hepatitis, severe intravascular hemolysis and glomerulonephritis is presented. To see all of these findings together in a patient with typhoid fever is very rare and may cause diagnostic difficulties. *Key words: typhoid fever, transaminase level.*

Typhoid fever causes endemics as well as occasional epidemics in developing countries. Enteric fever may sometimes present with completely unexpected clinical findings in adition to its classical signs and symptoms[1]. In this report, a patient with enteric fever presenting with hepatitis, severe intravascular hemolysis and glomerulonephritis is discussed.

Case Report

A 10-year-old male patient was hospitalized with the complaints of apathy and jaundice. The history of his present illness revealed that he had been experiencing anorexia, fever and weight loss during the previous 12 days. He became pale and icteric and had dark urine four days earlier, and he became apathetic two days previously. He had received no drugs or toxic substances, and there was no history of hemolytic anemia or hepatitis in his family or close contacts.

The physical examination revealed a confused and spastic child. His fever was 37.2 °C (axillary), pulse rate 116/min, respiratory rate 38/min, and blood pressure 110/70 mmHg. His skin was very pale, his sclera were icteric, and there was pitting edema in his lower extremities. There was no hepatosplenomegaly.

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The laboratory findings were as follows: hemoglobin 0.82 mmol/L, hematocrit 15% and reticulocyte 16%. Blood smear examination revealed anisocytosis, macrocytosis, and rare spherocytosis with 38% normoblastemia. His white blood cell count was 3.2×10^9/L with 81% polymorphonuclear leukocytes (vacuolization in 5% and severe toxic granulation), 1% monocytes, 5% metamyelocytes and 13% lymphocytes. Mean corpuscular volume was 125 fl and many thrombocytes were noted.

The urine was dark-colored and had a pH of 7 and a specific gravity of 1006. Protein and urobilinogen were (++). Glucose and bilirubin were negative, and 10-15 leukocytes plus 10-15 fine granular casts per field were present on microscopic examination.

The blood chemistry showed the following results: sodium 135 mmol/L, potassium 4.2 mmol/L, blood urea nitrogen 20 mmol/L, creatinine 194.5 µmol/L, alkaline phosphatase 154 IU/L, serum aspartate aminotransferase 2682 IU/L (490 ten days later), serum alanine aminotransferase 7655 IU/L (99 ten days later), total bilirubin 51 µmol/L, conjugated bilirubin 13.8 µmol/L, blood glucose 6.3 mmol/L, total protein 59 g/L, and albumin 34 g/L.

The direct, indirect and complementary Coombs tests were negative. Plasma hemoglobin was 14 µmol/L, and haptoglobulin was 380 mg/L (normal: 700-3790 mg/L). In the hemoglobin electrophoresis, hemoglobin A_2 was found to be 1.3% and hemoglobin F 0.9%. The sickling test and G_6PD were within normal limits. Erythroid hyperplasia and megaloblastic changes were detected on bone marrow aspiration. The G_6PD level and blood smears of both parents were within normal limits. Hepatitis markers including HBsAg, HBeAg, anti-HBs, anti-HBe, anti-HBc IgM and IgG, hepatitis C virus antibody, and hepatitis A virus antibody M and G were negative. Paul-Bunnel test and anti-cytomegalovirus IgM were also negative. Serum copper and ceruloplasmin levels were within normal limits. Cold agglutinin and erythrocyte E antigen were negative. The chest x-ray and the cranial computerized tomography were normal, and there was an increase in the echogenicity of both kidneys on ultrasonographic examination. The level of fibrin degradation products was normal. Salmonella typhi was cultured in blood.

Discussion

There are rare reports in the literature of atypical presentations of typhoid fever[1,2]. Hepatic involvement and especially jaundice in typhoid fever is also rare in childhood. The pathogenesis of typhoid hepatitis is not yet known. It is suggested that a high concentration of endotoxin and bacterial multiplication in the liver may cause a granulomatous and mononuclear inflammatory response, necrotic areas and vacuolar degeneration[3,4]. IgG antibodies against Salmonella typhi O antigen have been demonstrated by Calva and his colleagues[5] in liver biopsy specimens.

The liver function test values may be abnormal in 50 percent of patients with or without hepatomegaly[1,3,4]. The level of transaminases is usually less than 200 IU/L and rarely above 1000 IU/L[2,4]. Our literature search failed to disclose any case with severe jaundice and such high transaminase levels. The high level of SGOT may be partly due to hemolysis, but the rise in SGPT cannot be explained by hemolysis. This condition should be differentiated from other infectious hepatitis. In our case, Salmonella typhi was confirmed by serological tests and the blood culture. After treatment, all the liver function tests returned to normal in a short time.

Intravascular hemolysis is also rare in typhoid fever. It is seen particularly in patients with G_6PD deficiency[6]. It may be due to both antimicrobial therapy and a direct effect of the infection[6,7]. G_6PD levels were normal in our patient and his parents, and there was no history of drug intake or exposure to other hemolytic agents. Therefore, hemolysis was considered to be a direct effect of typhoid fever.

During the course of typhoid fever, glomerulonephritis, IgA nephropathy and pyelonephritis can be seen as a direct effect of Salmonella typhi with or without clinical findings and abnormal kidney functions[8,9]. Hemolytic uremic syndrome has also been reported[10]. The disturbance in renal function tests was explained by glomerulonephritis. Deposition of salmonella Vi antigen C_3 and immunoglobulin have been shown in the glomerular capillary wall[9]. The clinical and laboratory findings of our case were not compatible with disseminated intravascular coagulation or hemolytic uremic syndrome.

These findings are very rare in typhoid fever and can cause diagnostic difficulties.

REFERENCES

1. Klotz SA, Jorgensen JH, Buckwold FJ, Craven PC. Typhoid fever: an epidemic with remarkably few clinical signs and symptoms. Arch Intern Med 1984; 144: 533-537.
2. Faierman D, Ross FA, Seckler SG. Typhoid fever complicated by hepatitis, nephritis, and thorombocytopenia. JAMA 1972; 221: 60-61.
3. Ramachandran S, Godfrey JJ, Perera MV. Typhoid hepatitis. JAMA 1974; 230: 236-240.
4. Ayhan A, Gököz A, Karacadağ Ş, Telatar H. The liver in typhoid fever. Am J Gastroenterol 1973; 59: 141-146.
5. Calva JJ, Ruiz-Palacios GM. Salmonella hepatitis: detection of salmonella antigens in the liver of patients with typhoid fever. J Infect Dis 1986; 154: 373-374.
6. Hersko C, Vardy PA. Haemolysis in typhoid fever in children with G-6-PD deficiency. Brit Med J 1967; 1: 214-215.
7. Meyer HA. Acute haemolysis associated with typhoid fever and G-6-PD deficiency. Lancet 1973; i: 729-730.
8. Sitprija V, Pipantanagul V, Boonpucknavig V, Boonpucknaving S. Glomerulitis in typhoid fever. Ann Int Med 1974; 81: 210-213.
9. Indraprasit S, Boonpucknaving V, Boonpucknaving S. IgA nephropathy associated with enteric fever. Nephron 1985; 40: 219-222.
10. Baker NM, Mills AE, Rachman I, Thomas JE. Haemolytic-uraemic syndrome in typhoid fever. Brit Med J 1974; 2: 84-87.

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CONGENITAL DOUBLE-ORIFICE MITRAL VALVE*
A Case Report

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SUMMARY: Yurdakul Y, Arsan S, Karapınar K, Tamim M, Bilgiç A. (Department of Thoracic and Cardiovascular Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey). Congenital double-orifice mitral valve. Turk J Pediatr 1995; 37: 173-176.

A three-year-old girl was admitted to Hacettepe Children's Hospital due to sweating and poor feeding. Echocardiography demonstrated ostium primum atrial septal defect with a cleft mitral valve. After two months of medical follow-up with digitalis, she was subjected to open-heart surgery, and a primum-type atrial septal defect with double mitral orifice was found. To avoid creating acute mitral stenosis, the cleft was only partially sutured and the septal defect was closed with a patch. Her postoperative recovery was uneventful. It is emphasized that surgical intervention should be individualized in each case. *Key words: congenital heart defect, congenital double-orifice mitral valve, atrial septal defect.*

Duplication of the mitral valve is an extremely rare congenital cardiac malformation. In half of the reported cases, the duplication of the mitral valve occurs as an isolated lesion[1]. Atrioventricular septal defect appears to be the most common among the associated cardiovascular anomalies.

This malformation of the valve sometimes presents with no hemodynamic disturbances, but usually produces mitral stenosis and, very rarely, mitral insufficiency[1,2].

A case of duplication of the mitral valve for which the preoperative diagnosis was primum-type interatrial septal defect with a cleft mitral valve is the subject of this paper.

Case Report

A three-year-old girl was admitted to Hacettepe Children's Hospital in December 1992 due to sweating and poor feeding. Physical examination revealed a heart

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rate of 112/min in sinus rhythm and blood pressure of 110/60 mmHg. Precordial thrill, pansystolic murmur, early diastolic murmur, and an ejection systolic murmur in the pulmonary area were noted. Further examination was unremarkable. The electrocardiogram showed left-axis deviation and left ventricular hypertrophy.. Chest x-ray revealed global cardiac enlargement with pulmonary plethora (Fig. 1). Echocardiography demonstrated ostium primum atrial septal defect with a cleft mitral valve. The left ventricular angiogram revealed the characteristic "goose neck" deformity of the left ventricular outflow tract and mitral insufficiency. In cardiac catheterization, the pulmonary-to-systemic flow ratio was 3.9 at the atrial level, the pulmonary artery pressure was 32/6 and the mean left atrial pressure was 6 mmHg.

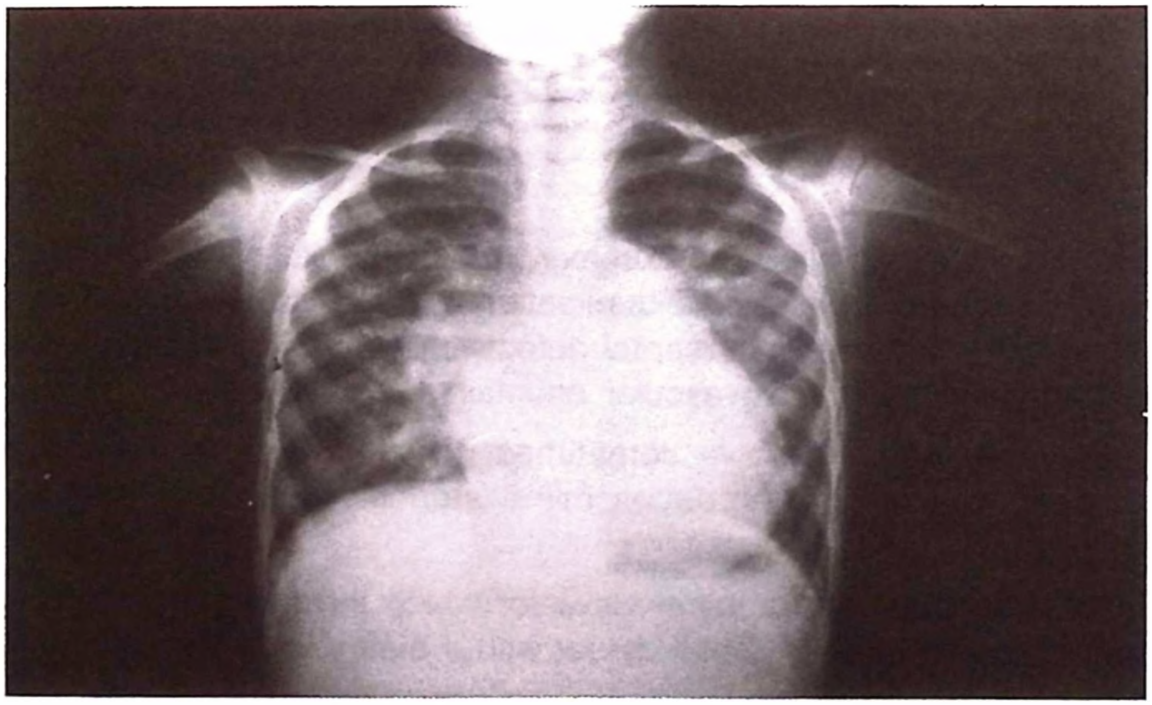

Fig. 1: Chest roentgenogram demostrating cardiomegaly and increased pulmonary vascularity.

After two months of medical follow-up with digitalis, the patient underwent open-heart surgery using the Bentley bubble-oxygenator and DeBakey roller pumps under hypothermia of 25 °C. Before cardiopulmonary by-pass was started, the digital examination through the right atrial appendix verified the presence of mitral insufficiency. Intraoperatively, an ostium primum-type atrial septal defect 1.5 cm in diameter was seen. The mitral valve had a double orifice-one side was 1 cm in diameter, located posteromedially, and the other 2.5 cm in diameter, located anterolaterally with cleft towards its anterior aspect. The double orifice was separated by a fibrotic band, and there were two underlying papillary muscles supplying chordae to both cusps of the appropriate orifice; however, for the area adjacent to the cleft, chordal support arose directly from the ventricular wall. To

avoid creating acute mitral stenosis, the cleft was only partially sutured, and the posteromedially located orifice was left untouched. To close the septal defect, a dacron patch was affixed with interrupted sutures to the ventricular septum, the tricuspid valve and the right atrial wall anterior and inferior to the coronary sinus orifice, and continuous sutures were used along the remainder of the defect.

The patient's postoperative recovery was uneventful, and she was kept on a daily dose of digitalis but rarely on diuretics. Postoperative echocardiography demonstrated minimal mitral insufficiency and double orifice of the mitral valve (Fig. 2).

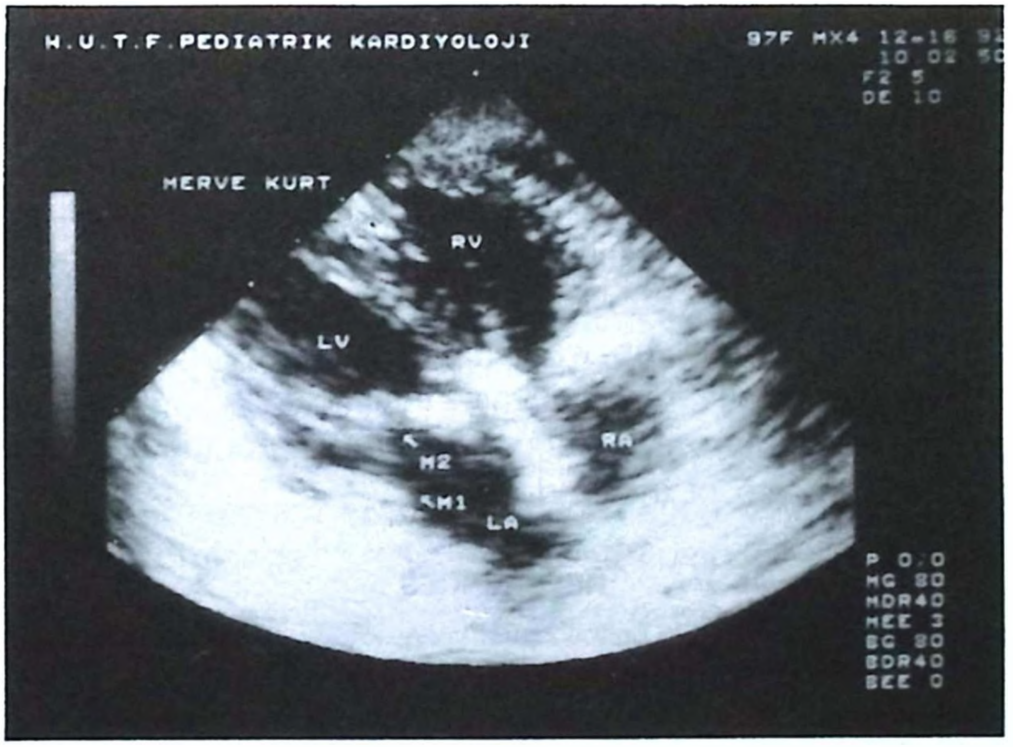

Fig. 2: Postoperative echocardiography showing double orifice of the mitral valve.

Discussion

In 25 percent of the reported cases, partial atrioventricular septal defect is associated with this rare malformation, suggesting that the developmental defect involves endocardial cushion tissue. Since more than two left ventricular papillary muscles are associated with malformation, it is now accepted that the true developmental basis of duplication of the mitral valve is unknown[1]. In this malformation, the two mitral orifices are usually of unequal size, and additional papillary muscles and chordae tendineae may be present[1]. There had been no surgical approach reported until Reed et al's[4] presentation. Since then surgical management has improved tremendously, parallelling the better understanding of the pathology. A careful appraisal of the structure of the valve (site of the accessory orifice, cleft leaflet or a parachute valve) and associated malformations

may guide the surgeons to decide whether any surgical intervention on the valve is warranted[2]. Duplication of the mitral valve may function normally, requiring no repair. In selected cases conservative management may be preferable[2,3].

There are three types of surgical approach: major valve reconstruction, cleft suture, and valve replacement[2,4-8]. Major valve reconstruction by the Amano-Suzuki technique, i.e. division of the fibrous bar, valve reconstruction by cleft suture and leaflet extension may be considered by experienced surgeons when there is a failure of leaflet coaptation due to annular dilatation or leaflet retraction[5]. In some cases the fibrous bar may contain leaflet tissue responsible for keeping the valve competent. If the valve is incompetent due to a cleft leaflet, the cleft may be sutured. Partially cleft suture may be preferred over complete cleft suture because of a lower risk of acute mitral stenosis. However, the possibility of the development of late mitral stenosis still exists. If one orifice is incompetent, its closure may be considered[2]. If the valve is severely damaged causing significant mitral insufficiency or stenosis, valve replacement should be advocated[7,9]. We previously published a case of an 18-month-old child with a double orifice mitral valve who was successfully treated with mitral valve replacement because of severe mitral valve insufficiency[7,9]. Care must be taken to avoid creating iatrogenic valve stenosis by suturing of the cleft or acute mitral incompetence by division of the bridge[2].

REFERENCES

1. Rosenberg J, Roberts WC. Double orifice mitral valve. Study of the anomaly in two calves and summary of the literature in humans. Arch Path 1968; 86: 77-80.
2. Aljafri AM. Double orifice mitral valve. A case report and review of management. J Cardiovasc Surg 1991; 32: 837-839.
3. Saylam A, Oram A, Nazlı N, Yener A, Aytaç A. Double orifice mitral valve associated with ostium primum atrial septal defect. Turk J Pediatr 1976; 18: 58-62.
4. Reed GE, Cortes LE, Clauss RH, et al. The surgical repair of duplication of the mitral orifice. Ann Thorac Surg 1970; 9: 81-85.
5. Amano J, Suzuki A. Surgical treatment of duplication of the mitral valve. J Cardiovasc Surg (Torino) 1986; 27: 323-327.
6. Lee CN, Danielson GK, Shaff HV, Puga FJ, Mair DD. Surgical treatment of double-orifice mitral valve in atrioventricular canal defects. Experience in 25 patients. J Thorac Cardiovasc Surg 1985; 90: 700-705.
7. Yurdakul Y, Bilgiç A, Saylam A, Sarıoğlu T, Köşker S, Aytaç A. Congenital double-orifice mitral valve. Report of a case with valve replacement. Jpn Heart J 1980; 21: 545-550.
8. Yurdakul Y, Sarıoğlu T, Bilgiç A, et al. Onbir yılını dolduran yapay mitral kapaklı bebek hastamız oniki yaşında. Hacettepe Tıp Dergisi 1989; 22: 187-192.
9. Kron J, Standerfer RJ, Starr A. Severe mitral regurgitation in a woman with a double orifice mitral valve. Br Heart tJ 1986; 55: 109-111.

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DIRECT COMMUNICATION BETWEEN PULMONARY ARTERY AND LEFT ATRIUM*

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SUMMARY: Ökten T, Okumuş Y, Kurtoğlu S. (Departments of Pathology and Pediatrics, Erciyes University Faculty of Medicine, Kayseri, Turkey). Direct communication between pulmonary artery and left atrium. Turk J Pediatr 1995; 37: 177-181.

In this article an autopsy finding of a rare congenital anomoly is presented. We found only 28 cases in the literature. Four anatomic variations have been defined, and our case was of type II. The morphogenetic mechanism is a matter for discussion. The previous reports include inadequate pathological examinations. Our histologic findings support the arterial nature of the aneurysmal sac. *Key words: pulmonary artery-left atrium communication, congenital heart disease.*

Direct communication between the pulmonary artery or its branches and the left atrium is a rare anomaly, and only 25 cases had been published as of 1986[1]. Since then three cases (two cases in 1986 and one case in 1989) have been reported[1-3]. The age range of the patients is between one day and 45 years, and only six cases under the age of one year are reported. This anomaly has been classified into four types on the basis of two main features: the presence or absence of an aneurysm in the communication, and the anatomy of the pulmonary venous drainage[4,5]. Our case was compatible with type II.

Because it is a rare congenital anomaly, we present this case with a review of the literature.

Case Report

A three-day-old male baby was admitted to the Neonatal Unit of Erciyes University Faculty of Medicine with complaints of purplish skin color, failure to suck and uneasiness. His past history was noncontributory. There was no consanguinity between his parents. the had three brothers who were alive and healthy.

He was 49 cm in length and his weight was 2800 g. His body temperature was 36 °C. Blood pressure was 60 mmHg by flush method, and pulse was 120 beats per minute. Tachypnea with perioral and peripheral cyanosis was evident. Significant II/IV systolic murmur was heard in the mesocardiac area during auscultation.

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Hemoglobin concentration was 17.7 g/dl. Urinalysis results were within normal limits. Blood gas analysis revealed pH 7.34, pCO_2 45 mmHg, pO_2 25.2 mmHg, HCO_3^- 24.6 mEq/L, O_2 saturation 42.3%, and BE -80.9. The chest roentgenogram showed a double contour on the right border of the cardiac silhouette. The pulmonary vascular markings were decreased. Electrocardiogram revealed spiked p waves at D II.

In the 38th hour of hospitalization, the patient died. Autopsy was then performed:

Macroscopic findings of cardiopulmonary system: Valves were normal. The foramen ovale was open (ostium secundum type). Patent ductus arteriosus was present. The aorta and its branches were normal. The left atrium was dilated and communicated with an aneurysmal sac toward the lower lobe of the right lung. The pulmonary artery was dilated up to 1.5 cm beginning from its exit from the right ventricle and opening to the left atrium which communicated with the aneurysmal sac (Fig. 1). There were three pulmonary veins draining directly into the left atrium below the aneurysmal sac. The left lung consisted of two and the right of three lobes, and there was no pulmonary lobe agenesis. However, there was an aneurysmal sac of 2.5 x 2 x 1 cm dimensions present in the lower lobe of the left lung (Fig. 2).

Fig. 1: Macroscopic appearance of the heart. There is a marked dilatation at the beginning of the pulmonary artery. After the juncture with the left and right branches, the pulmonary artery communicates with the left atrium. The aorta and its branches are normal. Patent ductus arteriosus is also present.

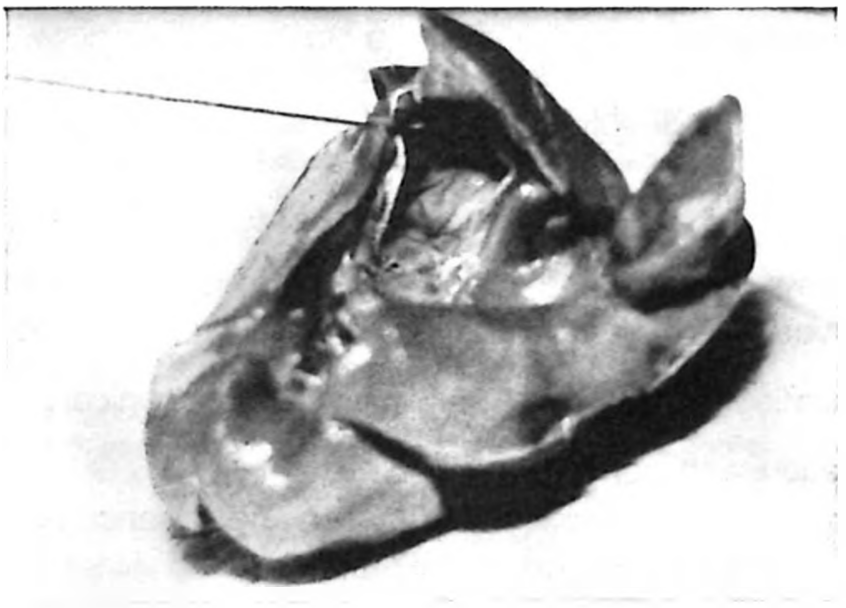

Fig. 2: Macroscopic appearance of the left lung. Note the aneurysmal sac located in the lower lobe.

No pathology was observed macroscopically in the other systems.

Microscopic findings: Congestion due to hypoxia was the only pathology observed in other organs. However, we noted the presence of surrenal ectopia in the testis. Sections prepared from the wall of the aneurysmal sac located in the parenchyma of the lower lobe of the right lung were stained with Voerhoff's elastic stain, Masson's Trichcrom and Hematoxylin-Eosin (Fig. 3). There were no heart muscle fibres in the wall of the sac. The structure was composed of a mixture of connective tissue and smooth muscle fibers. In some sections we clearly observed layers seen in the arterial walls. Furthermore, there were internal and external elastic membranes which led us to suspect that the sac may have originated from an artery.

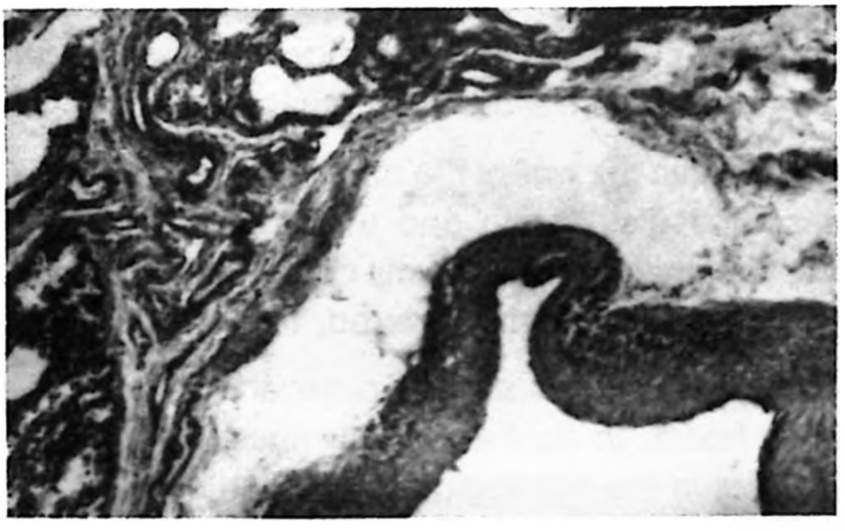

Fig. 3: Microscopic appearance of the aneurysmal sac in the parenchyma of the lower lobe. (H.E. x 40).

Discussion

Direct communication between the pulmonary artery or its branches and the left atrium is a rare anomaly, and only 25 cases had been reported in the literature by 1986[1].

Since then, three cases (two cases in 1986 and one case in 1989) have been reported[1-3]. The age range of the patients reported is one day to 45 years, and only six cases younger than one year have been documented.

Nelson et al.[4] classified the anatomic variations of the communication into three types:

I) Communication with normal veins.
II) Aneurysm with the abnormalities of lobulation and absence of pulmonary veins.
III) All veins draining into the communication.

Ohara et al.[5] added a fourth type to this classification:

IV) Aneurysm with the right veins connected to it.

Our case was compatible with type II. The first case of this type in the literature was presented as a variant of pulmonary arteriovenous fistula in 1961 by Lucas et al[6].

In most of the reported cases in the literature, a surgical approach was chosen rather than an adequate pathological investigation. For this reason the morphogenetic mechanism of the anomaly continues to be a matter of argument. Some have speculated about the persistence of the communication between the pulmonary artery and the pulmonary vein in the first month, when the common pulmonary vein joins the left atrium[1]. Others emphasize the similarity to arteriovenous fistulas originating from agenesis of a pulmonary lobe, the dilated capillary bed giving rise to the communication[1,6]. The presence of abnormal lung lobulation, especially in type II cases, constitutes the starting point of the latter theory. But, as in our case, the existence of normal pulmonary lobulation together with the aneurysm invalidates this theory.

Ohara et al.[5] reported that the wall of the aneurysmal sac can be divided roughly into two layers by the distribution and the population of the elastic fibers. According to this, the inner third of the wall consisted of relatively dense, fibrous connective tissue and regularly multilayered, fine elastic fibers.

The outer two-thirds consisted of a loose collagenous tissue layer with irregular and sparse elastic fibers. In contrast to our case, there were no smooth and skeletal muscle fibers in the histologic examination of the aneurysmal sac. Our findings were mostly different, and led us to consider an arterial nature. Lekuona et al.[1] also stressed that the aneurysmal sac was of arterial origin.

REFERENCES

1. Lekuona I, Cabrera A, Inguanzo R, Cid C, Agosti J. Direct communication between the right pulmonary artery and the left atrium. Thorax 1986; 41: 78-79.
2. Subramanian N, Vasudevan S, Hussain AT. Direct communication of a pulmonary artery with left atrium-report of a case with balloon occlusion studies and successful correction: a case report. Angiology 1986; 37: 136-141.
3. Karnık AM, Nilsson U, Vijayaraghavan G, Hashmi J, Shuhaiber H. Direct communication between the left pulmonary artery and the left atrium. Chest 1989; 96: 937-939.
4. de Souza e Silva NA, Giuliani ER, Ritter DG, Davis GD, Pluth JR. Communication between right pulmonary artery and left atrium. Am J Cardiol 1974; 34: 857-863.
5. Ohara H, Ito K, Kohguchi N, et al. Direct communication between the right pulmonary artery and the left atrium. A case report and review of the literature. J Thorac Cardiovasc Surg 1979; 77: 742-747.
6. Lucas RVJr, Lund GW, Edwards JE. Direct communication of a pulmonary artery with the left atrium. An unusual variant of pulmonary arteriovenous fistula. Circulation 1961; 24: 1409-1414.

The Turkish Journal of Pediatrics 1995; 37: 183-185

LETTER TO EDITOR

INTERMEDIATE TYPE PULMONARY ATRESIA WITH INTACT VENTRICULAR SEPTUM*

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*Alpay Çeliker MD******, *Metin Demircin MD***

Pulmonary atresia (PA) with intact ventricular septum (IVS) is a rare and life-treathening congenital anomaly which causes mortality and morbidity in infancy[1]. We report herein an infant who had PA with IVS.

A five-month-old boy was hospitalized with the signs of cyanosis and severe heart failure in October 1994. His initial diagnosis was atrial septal defect (ASD) and pulmonary stenosis (PS). Because of severe bradycardia and cardiac instability during cardiac catheterization, the procedure was not able to be performed. He was then taken to surgery urgently with ASD plus PS plus functional tricuspid insufficiency, which was diagnosed by echocardiography.

We performed the operation by the standard means of cardiopulmonary bypass using moderate hypothermia. Initially we opened the main pulmonary artery, but we could not find any orifice. Instead of the pulmonary valve, there was fibromuscular tissue (Fig. 1). We extended our incision down to the right ventricular outflow tract, resected the fibromuscular tissue and inserted a cross-annular patch. Then we closed the ASD with a dacron patch. No extra surgical procedure was considered for tricuspid insufficiency because it was not important hemodynamically. At last, the patient was taken to the intensive care unit with no inotropic support.

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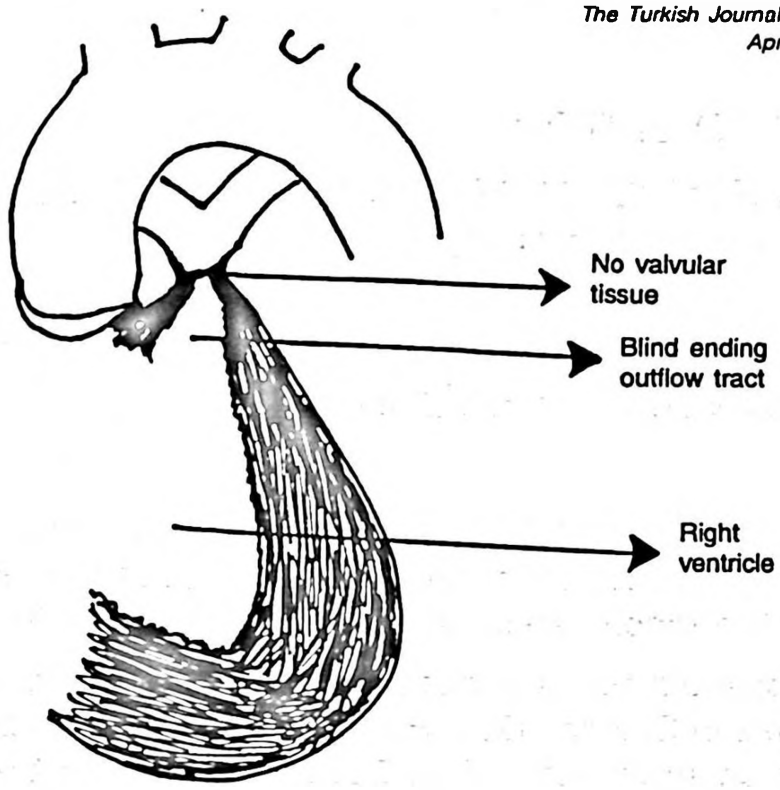

Fig. 1: Schematic drawing of the patient's right ventricular outflow tract. The base of the pulmonary trunk itself is connected to the ventricular mass, but the cavity of the trunk is separated from the ventricle by fibromuscular tissue.

Unfortunately, in the early postoperative period the patient suffered from oliguria which progressed to anuria. Peritoneal dialysis was established, but the patient died in the early postoperative period because of a cardiac arrest.

Pulmonary atresia is a congenital aberration in which there is complete blockage of the pathway from the pulmonary trunk to the ventricular mass[2]. The atresia can take one of three forms –valvular or membranous atresia, muscular atresia, and solitary arterial trunk– the differences being of considerable surgical significance[3, 4]. Also, intermediate types may be found in some cases. In such patients, the decision as to the most suitable procedure may be difficult to make[5].

Our case was an intermediate form of PA with IVS between Types I and II. We decided to continue corrective surgery because the patient had already been taken for open-heart surgery when the exact diagnosis was established.

The preoperative, definitive diagnosis gains importance especially in the intermediate and complex forms of PA with IVS[6, 7]. In order to determine the most suitable treatment, all laboratory studies, invasive (when needed) and noninvasive diagnostic tools must be performed[8].

An angiocardiography needed to have been performed in this case in order to make a definitive diagnosis and change the type of intervention. In this case, if the preoperative diagnosis had been PA with IVS, a palliative procedure could have been our choice of procedure, where a staged repair could have decreased the mortality and morbidity of this child.

REFERENCES

1. Rook GD, Gootman N. Pulmonary atresia with intact ventricular septum. Operative treatment with survival. Am Heart J 1971; 81: 476-481.
2. Zuberbuhler JR, Anderson RH. Morphological variations in pulmonary atresia with intact ventricular septum. Br Heart J 1979; 41: 281-288.
3. deLeval M, Bull C, Stark J, Anderson RH, Taylor JF, Macartney FJ. Pulmonary atresia and intact ventricular septum: surgical management based on a revised classification. Circulation 1982; 66: 272-280.
4. Aziz KU, Olley PM, Rowe RD, Trusler GA, Mustard WT. Survival after systemic to pulmonary arterial shunts in infants less than 30 days old with obstructive lesions of the right heart chambers. Am J Cardiol 1975; 36: 476-483.
5. Stellin G, Santini F, Thiene G, et al. Pulmonary atresia, intact ventricular septum, and Ebstein anomaly of the tricuspid valve. Anatomic and surgical considerations. J Thorac Cardiovasc Surg 1993; 106: 255-261.
6. Dhanavaravibul S, Nora JJ, McNamara DG. Pulmonary valvular atresia with intact ventricular septum: problems in diagnosis and results of treatment. J Pediatr 1970; 77: 1010-1016.
7. Freeman JE, Deleon nSY, Lai S, Fisher EA, OW EP, Pifarrè R. Right ventricle to aorta conduit in pulmonary atresia with intact ventricular septum and coronary sinusoids. Ann Thorac Surg 1993; 56: 1393-1395.
8. Akagi T, Benson LN, Williams WG, Truslerr GA, Freedom RM. Ventriculo-coronary arterial connections in pulmonary atresia with intact ventricular septum, and their influences on ventricular performance and clinical course. Am J Cardiol 1993; 72: 586-590.

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