



Smith-Lemli-Opitz syndrome: Clinical and biochemical findings in Brazilian patients

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Abstract

Smith-Lemli-Opitz syndrome (SLOS) or RSH syndrome comprises multiple congenital anomalies and mental retardation. The underlying defect is a deficiency in the activity of Δ^7 -sterol reductase, which decreases cholesterol and increases 7-dehydrocholesterol (7-DHC) levels. Our aim was to identify and evaluate the frequency of SLOS manifestations in a group of Brazilian patients. Based on our own data and those reported previously, we present a simple method that allows the estimation of probabilities favoring the diagnosis of SLOS. We evaluated 30 patients clinically and determined their plasma levels of cholesterol and 7-dehydrocholesterol. In 11 patients, the diagnosis was confirmed by ultraviolet spectrophotometry (UV). Of 19 patients with normal laboratory results, 17 showed a high probability favoring the diagnosis of SLOS. The most significant signs and symptoms observed in over 2/3 of the biochemically confirmed cases were mental retardation (10/11), delayed neuropsychomotor development (10/11), syndactyly of 2nd/3rd toes (10/11), and craniofacial anomalies including microcephaly (11/11), incompletely rotated ears (8/11), palpebral ptosis (10/11), anteverted nostrils (10/11), and micrognathia (9/11). Genital anomalies were found in all male patients (6/6).

Key words: Smith-Lemli-Opitz syndrome, cholesterol metabolism, 7-dehydrocholesterol.

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Introduction

Smith-Lemli-Opitz (SLOS) or RSH syndrome (MIM 270400) comprises multiple congenital anomalies, mental retardation and an autosomal recessive inborn error of cholesterol metabolism. SLOS is caused by mutations in the gene *DHCR7* (Wassif *et al.*, 1998; Fitzky *et al.*, 1998; Moebius *et al.*, 1998; Waterham *et al.*, 1998) which encodes 3 β -hydroxysterol- Δ^7 -reductase (Δ^7 -sterol reductase), an enzyme that catalyzes the conversion of 7-dehydrocholesterol (7-DHC) to cholesterol (Irons *et al.*, 1993; Tint *et al.*, 1994; Shefer *et al.*, 1995). Over 100 different mutations of *DHCR7* have been identified in SLOS, all leading to low tissue cholesterol and accumulation of precursor 7-DHC (Yu *et al.*, 2000; Witsch-Baumgartner *et al.*, 2000; Evans *et al.*, 2001; Correa-Cerro and Porter, 2005; Scalco *et al.*, 2005).

The incidence of SLOS is not known accurately; estimates range from 1:10,000 to 1:60,000 (Lowry and Yong,

1980; Ryan *et al.*, 1998; Kelley, 2000; Kelley and Hennekan, 2000; Nowaczyk *et al.*, 2001). This variation may be due to different criteria used in the evaluation of patients.

The clinical manifestations most frequently reported in SLOS include craniofacial anomalies, mental retardation, syndactyly of the second and third toes, and genital malformations in males. Facial anomalies are typical and comprise microcephaly, narrow frontal area, epicanthic folds, ptosis, short nose, broad nasal bridge, anteverted nostrils, micrognathia, and posteriorly angulated or low-set ears. Strabismus, cataract, high-arched or cleft palate, and short neck are also common. Limb defects include postaxial polydactyly of feet and syndactyly of the second and third toes. Genital anomalies (cryptorchidism, hypospadias) are reported in males. Other findings include: delayed global developmental progress with hypotonia and neuropsychomotor disturbances; pyloric stenosis; infection susceptibility during infancy; and various cardiac, renal, and pulmonary anomalies (Curry *et al.*, 1987; Guzzeta *et al.*, 1996; Cunniff *et al.*, 1997; Ryan *et al.*, 1998; Krajewska-Walasek *et al.*, 1999; Nowaczyk and Wayne, 2001; Haas and Kelley, 2001).

The clinical diagnosis of SLOS remains problematic because signs and symptoms presented by affected individuals range from minor anomalies associated with behavioral and learning disabilities to severe malformations and mental retardation, or even neonatal death (Porter, 2000). Biochemical analyses of cholesterol and 7-dehydrocholesterol levels are fundamental to establish a diagnosis of SLOS.

The purpose of the present work was to identify the most common and important findings in SLOS, to compare our data with those of the literature and thus further to delineate the classical SLOS phenotype. In addition, the frequency of the diverse signs and symptoms was used to determine probability figures favoring the diagnosis of SLOS in patients exhibiting negative biochemical results, in order to help the differential diagnosis of SLOS.

Patients and Methods

Patients

The study group included 30 patients (21M, 9F) with findings consistent with SLOS, who were clinically and biochemically evaluated from 2000 through 2002.

The study protocol was approved by both local and national committees of ethics, and informed parental consent was obtained in each case. To standardize clinical ascertainment and anamnestic procedures, the patients were evaluated according to the same protocol.

Figure 1 shows the facial appearance of all 11 patients whose diagnosis was confirmed biochemically.

Biochemical tests

Cholesterol analysis

Total plasma cholesterol was determined by an enzymatic colorimetric method (Kit Sera-Pak[®] Cholesterol, fast color, Bayer[®]) and auto-analyser RAX Bayer, as described by Allain *et al.* (1974).

7-DHC analysis

7-DHC was determined by UV spectrophotometry. For this purpose, 200 μ L of plasma were transferred to glass tubes and vortexed for 10 s after addition of 200 μ L of ethanol. Subsequently, 1 mL of n-hexane was added and the mixture vortexed for another 20 s. After centrifugation at 400 g for 1 min, the clear n-hexane layer was collected in a 1 mL quartz cuvet and used for UV measurement. The samples were scanned using a n-hexane blank in the reference beam, and absorption maxima at 271, 282 and 234 nm were used for detection of 7-DHC in plasma samples; for quantitative analysis, absorption maxima at 234 and 282 nm were used, as proposed by Honda *et al.* (1997).

Statistical analysis

Table 1 shows the frequencies of SLOS findings in our group of 11 confirmed patients and in cases from the lit-



Figure 1 - Facial appearance of our confirmed cases of Smith-Lemli-Opitz syndrome (SLOS): (A) patient 1, (B) patient 2, (C) patient 3, (D) patient 4, (E) patient 5, (F) patient 6, (G) patient 7, (H) patient 8, (I) patient 9, (J) patient 10, (K) patient 11.

erature. The frequencies of signs/symptoms pertinent to the diagnosis of SLOS were calculated from literature reports, using two different estimation methods (Pardono *et al.*, 2003). Estimate 1 was obtained using the formula $P/(P+A+N)$, where P is the number of cases in which the finding was present, A is the number of cases in which it was absent, and N is the number of cases in which it was not

mentioned. In estimate 1, it is hypothesized that, whenever the anomaly is not mentioned in a reference paper, it is absent. Estimate 2 was obtained using the formula $P/(P+A)$; when the finding is not mentioned, it is assumed that it was not searched for by the authors. The true value of the estimate is obviously an unknown quantity, but its upper and lower limits are given by estimates 1 and 2, respectively. A

Table 1 - Estimated frequencies of the signs/symptoms found in SLOS patients (literature and present study).

Signal/symptom	SLOS patients								
	Literature (n = 34)*			Present sample			Combined sample		
	Est.1	Est.2	Comb.	Est.1	Est.2	Comb.	Est.1	Est.2	Comb. Est
Microcephaly	34/34	34/34	1.00	11/11	11/11	1.00	45/45	45/45	1.00
Prominent occipitus	8/34	8/14	0.31	-	-	-	-	-	-
High forehead	22/34	22/24	0.83	4/11	4/11	0.36	26/45	26/35	0.66
Large ears	13/34	13/13	-	6/11	6/11	0.55	19/45	19/24	0.59
Low-set ears	16/34	16/31	0.49	-	-	-	-	-	-
Rotated ears	11/34	11/16	0.44	8/11	8/11	0.73	19/45	19/27	0.54
Hypertelorism	6/34	6/17	0.22	-	-	-	-	-	-
Epicanthic folds	23/34	23/26	0.81	6/11	6/11	0.55	29/45	29/37	0.72
Ptosis	23/34	23/27	0.78	10/11	10/11	0.91	33/45	33/38	0.81
Strabismus	9/34	9/16	0.35	5/11	5/11	0.45	14/45	14/27	0.38
Cataract	2/34	2/11	0.07	2/11	2/11	0.18	4/45	4/22	0.11
Medial hypoplastic eyebrows	-	-	-	8/11	8/11	0.73	-	-	-
Anteverted nostrils	28/34	28/34	0.82	10/11	10/11	0.91	38/45	38/45	0.84
Narrow nasal bridge	12/34	12/13	0.67	-	-	-	-	-	-
Flattened nose	-	-	-	9/11	9/11	0.82	-	-	-
Long filtrum	-	-	-	5/11	5/11	0.45	-	-	-
Micrognathia	20/34	20/22	0.80	9/11	9/11	0.82	29/45	29/33	0.79
Bifid uvula	6/34	6/13	0.23	-	-	-	-	-	-
Cleft palate	9/34	9/34	0.26	4/11	4/11	0.36	13/45	13/45	0.29
High palate	-	-	-	6/11	6/11	0.55	-	-	-
Tented upper lip	-	-	-	10/11	10/11	0.91	-	-	-
Short neck	18/34	18/21	0.71	7/11	7/11	0.64	25/45	25/32	0.67
Polydactyly	7/34	7/34	0.21	-	-	-	-	-	-
Simian crease	20/34	20/34	0.59	3/11	3/11	0.27	23/45	23/45	0.51
Toe syndactyly (2/3)	32/34	32/34	0.94	11/11	11/11	1.00	43/45	43/45	0.96
Toe polydactyly	2/34	2/21	0.07	-	-	-	-	-	-
Club foot	9/34	9/13	0.38	-	-	-	-	-	-
Cryptorchidism	13/22	13/22	0.59	2/6	2/6	0.33	15/28	15/28	0.54
Hypospadias	10/22	10/22	0.45	3/6	3/5	0.50	13/28	13/28	0.4
Ambiguous genitalia	-	-	-	3/6	3/6	0.50	-	-	-
Muscular hypertrophy	8/34	8/34	0.24	7/11	7/11	0.64	15/45	15/45	0.33
Cardiac anomalies	4/34	4/08	0.15	-	-	-	-	-	-
Piloric stenosis	6/34	6/33	0.18	-	-	-	-	-	-
Feeding difficulties	16/34	16/21	0.60	-	-	-	-	-	-
Neuropsychomotor development delay	20/34	20/21	0.87	10/11	10/11	0.91	30/45	30/32	0.86
Growth retardation	7/34	7/34	0.27	-	-	-	-	-	-
Mental retardation	34/34	34/34	1.00	10/11	10/11	0.91	44/45	44/45	0.98

*Smith, Lemli and Opitz, 1964; Wallace *et al.*, 1994; Comier-Daire *et al.*, 1996; Guzzeta *et al.*, 1996; Krajewska-Walasek *et al.*, 1999. Combined sample = literature + present study.

combined estimate may be obtained by weighing estimates 1 and 2 (Est1 and Est2) by the reciprocals of their respective binomial variances (Var1 and Var2): $\text{comb. est.} = (\text{Est1}/\text{Var1} + \text{Est2}/\text{Var2}) / (1/\text{Var1} + 1/\text{Var2})$. Identical methodology was applied to three different conditions that should be considered in the differential diagnosis of SLOS: alpha-thalassemia/mental retardation syndrome (MIM 141750), Opitz (G/BBB) syndrome (MIM300000), and Aarskog syndrome (MIM 305400) (Grier *et al.*, 1983; Cappa *et al.*, 1987; Fryns *et al.*, 1992; Guion-De-Almeida and Richieri Costa, 1992; Mc Donald *et al.*, 1993; Teebi *et al.*, 1993; Fernandez *et al.*, 1994; Gibbons *et al.*, 1995; Reardon *et al.*, 1995). The estimates thus obtained are shown in Table 2. These values allow the calculation of the probabilities favoring the diagnosis of SLOS in relation to each one of the other three conditions. If no information about a given trait is available, the probability rate (likelihood) takes the indifferent value 1. To exemplify the use of the table data, let us suppose that in a given patient suspected of having SLOS or Opitz syndrome, only the first four features shown in the table were searched and that the first three features were present and the last one was absent. In this case, the likelihood favoring the diagnosis of SLOS

is the product: $95/5 \times 66/30 \times 54/80 \times 78/5 = 440.754$, that is, given this set of anomalies, the probabilities favoring both diagnoses are in the ratio of 440.754: 1; therefore, the probability of SLOS diagnosis is obtained by normalizing 440.754 and 1: $P1 = 440.754 / (440.754 + 1) = 440.754 / 441.754 = 0.9977$.

Results

The clinical diagnosis of SLOS was confirmed biochemically by abnormally high levels of 7-dehydrocholesterol and low plasma cholesterol levels in 11 of the 30 patients studied (Table 3). In these 11 patients, cholesterol and 7-dehydrocholesterol levels ranged from 50 to 143 mg/dL and 1.56 to 28.81 mg/dL, respectively. The remaining 19 patients had 7-dehydrocholesterol levels that could not be quantified and normal cholesterol levels (94-221 mg/dL).

The clinical traits observed in our sample of 30 patients are summarized in Table 4. Among the eleven patients with positive biochemical results, only one patient presented apparently normal development (case 11). Of the remaining 10, seven could not walk or talk (cases 1, 2, 4, 5,

Table 2 - Reported frequencies of signs and symptoms in SLOS, Opitz syndrome, Aarskog syndrome and alpha-thalassemia/mental retardation syndrome. Whenever the frequency of a given feature (with the exception of polydactyly) was not increased in a given syndrome, a frequency of 5% was assigned to it.

Signal/symptom	SLO		Opitz		Aarskog		alpha-thal.	
	PRES:	ABS	PRES:	ABS	PRES:	ABS	PRES:	ABS
Microcephaly	95:	5	5:	95	5:	95	5:	95
High palate	66:	34	30:	70	34:	66	5:	95
Rotated ears	54:	46	80:	20	5:	95	65:	35
Hypertelorism	22:	78	95:	5	95:	5	5:	95
Ptosis	81:	19	10:	90	44:	56	5:	95
Strabismus	38:	62	15:	85	5:	95	5:	95
Anteverted nostrils	84:	16	45:	55	75:	25	88:	12
Micrognathia	79:	21	30:	70	67:	33	5:	95
Short neck	67:	33	5:	95	5:	95	5:	95
Syndactyly	96:	4	5:	95	21:	79	5:	95
Polydactyly	7:	93	1:	99	1:	99	1:	99
Cryptorchidism	54:	46	35:	65	5:	95	61:	39
Hypospadias	46:	54	45:	55	5:	95	12:	88
Club feet	38:	62	5:	95	5:	95	59:	41
Hypotrophic musculature	33:	67	5:	95	5:	95	5:	95
Mental retardation	98:	2	5:	95	25:	75	92:	8
Neuropsychomotor development delay	86:	14	5:	95	5:	95	5:	95
Cardiac anomalies	5:	95	65:	35	5:	95	12:	88
Central nervous system defects	5:	95	80:	20	5:	95	5:	95
Clinodactyly	5:	95	5:	95	50:	50	35:	65
Kidney defects	5:	95	5:	95	5:	95	18:	82
“Shawl”scrotum	5:	95	5:	95	87:	13	5:	95

canthic folds, anteverted nostrils, micrognathia, short neck, cutaneous foot syndactyly, and delayed neuropsychomotor development or mental retardation. Other important findings in more than 2/3 of our patients (Table 1) were: tented upper lip (10/11), medial hypoplastic eyebrows (8/11), hypotrophic musculature (8/11), and incompletely rotated ears (8/11). All the boys exhibited some kind of genital alteration (6/6).

Discriminant analysis showed that only 3/30 of our patients presented a low probability favoring the diagnosis of SLOS in relation to the other three conditions, Opitz (G/BBB) syndrome, Aarskog syndrome and alpha-thalassemia/mental retardation (Table 5).

Table 5 - Probability figures favoring the hypothesis of SLOS in each of our 30 patients.

Patients	Probability figures favoring the diagnosis of SLOS relative to		
	Opitz syndrome P1	Aarskog syndrome P2	Alpha-thalassemia/ mental retardation P3
1*	1.0000	1.0000	1.0000
2*	1.0000	1.0000	1.0000
3*	1.0000	1.0000	1.0000
4*	1.0000	1.0000	1.0000
5*	1.0000	1.0000	1.0000
6*	1.0000	1.0000	1.0000
7*	1.0000	1.0000	1.0000
8*	1.0000	1.0000	1.0000
9*	1.0000	1.0000	1.0000
10*	1.0000	1.0000	1.0000
11*	0.9996	1.0000	0.9999
12	0.9999	0.9985	0.8941
13	0.3385	0.0554	0.0766
14	0.9991	0.9252	0.9541
15	1.0000	1.0000	1.0000
16	0.0084	0.2072	0.0042
17	1.0000	1.0000	1.0000
18	0.0001	0.0000	0.0000
19	1.0000	1.0000	0.9959
20	1.0000	1.0000	0.9999
21	0.9767	0.9953	0.7442
22	1.0000	1.0000	1.0000
23	1.0000	1.0000	1.0000
24	1.0000	0.9990	1.0000
25	0.9961	0.9903	0.9492
26	0.0000	0.0000	0.0001
27	0.9992	0.9744	0.0512
28	0.7788	1.0000	0.3485
29	0.998	1.0000	0.3485
30	0.8216	0.9997	0.9427

*Patients confirmed biochemically.

Discussion

Eleven of the 30 patients suspected of having SLOS exhibited increased levels of 7-dehydrocholesterol. In the literature, studies including controls and SLOS carriers have shown that 7-DHC and cholesterol levels were normal in all controls, whereas in patients with SLOS the 7-DHC levels were always increased; cholesterol levels were normal only in 10% of patients (Axelson, 1991; Nowaczyk *et al.*, 1999). These and other similar studies in the literature, however, do not clearly state the clinical inclusion criteria used in the diagnosis of SLOS in cases which are not completely typical.

The plasma 7-DHC levels found in our 11 patients by UV spectrophotometry were higher than 1mg/dL of plasma, a result consistent with that found by Honda *et al.* (1997).

The clinical diagnosis of SLOS is hindered by the fact that none of its signs or symptoms is pathognomonic. Despite this difficulty, the association of mental retardation with genital anomalies in males and a typical facial appearance seems to be the most important clinical indicator of SLOS (Guzzeta *et al.*, 1996; Cuniff *et al.*, 1997; Ryan *et al.*, 1998; Krajewska-Wallasek *et al.*, 1999). That is exactly what we observed among our biochemically confirmed patients.

Thirty-four cases of the literature were reviewed and their clinical findings were compared with those of our biochemically confirmed SLOS patients. The most frequent findings in our sample were: mental retardation (10/11), microcephaly (11/11), delayed neuropsychomotor development (10/11), and syndactyly of the 2nd and 3rd toes (11/11). The six male patients had genital anomalies. A typical face (with incompletely rotated ears, palpebral ptosis, anteverted nostrils, flattened nasal base, and micrognathia) is easily recognized in all 11 patients. According to the review by Kelley and Hennekan (2000) including 164 individuals with SLOS, the anomalies present in over 50% of the biochemically confirmed cases are: syndactyly of the 2nd/3rd toes (97%), mental retardation (95%), microcephaly (84%), anteverted nostrils (78%), and palpebral ptosis (70%). The corresponding frequencies observed in the present study, therefore, do not differ from those observed in a larger number of affected individuals.

Another facial anomaly commonly found among our SLOS patients, but not frequently reported in the literature (though usually observed in photos), was a tented upper lip (10/10), a characteristic described by Neri and Opitz (1999) as "elongated upper lip" and by Kelley (2000) as "large mouth, which, combined with frequent micrognathia, gives a distinctive appearance". Another facial trait that seems not to have been described in the literature so far, but was frequently observed in our patients (8/11), is the presence of medial hypoplastic eyebrows.

The comparison between the frequencies of the most significant SLOS signs and symptoms observed in our pa-

tients and those observed in three other entities, by using a simplified discriminant analysis technique, led us to conclude that all 11 patients with positive biochemical results and 16 of the 19 with negative results had a high probability of having SLOS. This fact suggests that the latter group of patients should be investigated molecularly.

Recent studies have indicated that the malformations associated with SLOS can be traced to the role cholesterol plays in normal embryonic development. Most malformations seen in SLOS are likely to be related with a role of cholesterol in the hedgehog signal transduction pathway (Roux *et al.*, 2000; Waterham and Wanders, 2000). Hedgehog (Hh) proteins form a family of embryonic signaling molecules, which are important for patterning in embryos. In humans, these proteins are called Sonic hedgehog (SHH), Desert hedgehog (DHH), and Indian hedgehog (IHH). The covalent addition of cholesterol to SHH is essential for its autoprocessing. SHH participates in the development patterning of the ventral forebrain, neural tube and limbs, among other structures. A similar process may be responsible for abnormal responses to DHH and IHH in the development of the genital tract and skeleton, respectively (Porter *et al.*, 1996; Hammerschmidt *et al.*, 1997; Tabin and McMahon, 1997).

Based on the discovery that the impaired function of development proteins (Hh) may explain a number of malformations present in SLOS, our hypothesis, to be tested in future studies, is that some of the patients who show typical malformations of SLOS but negative biochemical results have a defect, not in the DHCR7 gene (that codifies Δ^7 -sterol reductase), but in a developmental gene that codifies for Hh proteins.

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Internet Resources

The URL for data presented here is as follows: Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/OMIM>.

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