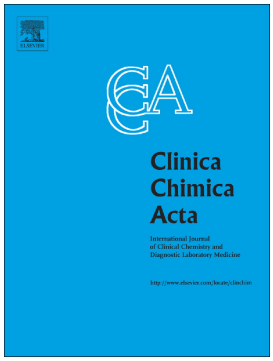

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Early diagnosis of Mucopolysaccharidoses in developing countries: a low cost and easy execution approach.

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The guidelines for an accurate diagnosis of Mucopolysaccharidoses (MPS) recommend first line screening methods on urine sample to determine quantitative and qualitative glycosaminoglycans (GAG) and successively the identification of specific enzyme deficiency and genetic analysis [1]. Actually several methods, requiring sophisticated equipments, are available [2-6]; nevertheless, the great majority of world population cannot benefit of the above approaches due to their high cost.

The aim of the present study was to apply a combined method based on the quantitative evaluation and qualitative characterization of the urinary GAGs for the diagnosis of MPS.

The GAG evaluation [7] has been performed on 1 ml of spot urine samples collected from 625 newborns (356 males and 269 females) at the 2^{nd} and 3^{rd} day of life. As regard as the total amount of urinary GAGs, performed by 1,9-dimethylmethylene blue (DMB) method, the higher values, up to 745 µg/mg Creatinine (Cr), were found in newborns with Cr lower than 40 mg/dl; on the contrary, in newborns with Cr levels higher than 40 mg/dl the range of GAG concentration was between 63.9 and 384.0 µg/mg Cr (Tab.1). No differences between males and females were observed. A significant difference of GAG concentration was found in urine samples with Cr levels < 40 mg/dl compared to urine samples with Cr levels > 40 mg/dl ($p<0.0001$). These results call the attention of the role of Cr levels to obtain reference values of GAGs in normal term newborns.

As regards as the qualitative characterization, the electrophoretic GAG pattern shows in all samples a major fast band of chondroitin A sulfate while a slower faint band, corresponding to heparan-sulfate, was observed in about 60% of urine samples.

Our results demonstrate that the combined method is the first step for the identification of all types of MPS; in fact, performing a qualitative and quantitative analyses simultaneously, a 100% of sensitivity and specificity are obtained [7]. On the contrary, DMB test alone can give false negative results, especially concerning the identification of MPS III and IV patients [6, 8]. Moreover, our combined method can be applied not only in the neonatal period, but also in the first months of life, such as in the occasion of vaccines administration or of a hospitalization for any health problems. It is important to underline that in the above reported situations, it is possible to identify the MPS patients in pre-symptomatic phase, using the reference values of urinary GAGs of the first year of life, recently published by our group [7].

We are aware that in developing countries there are situations of serious deficiencies in welfare state, even for health care system and the treatment of very common diseases; nevertheless, it is our opinion that to have available a simple method for diagnosis of these rare metabolic diseases could be helpful for the importance of genetic counseling and pre-symptomatic early treatment. Moreover, the use of the combined method could be applied in the poor countries where clusters of these diseases or when a high percentage of consanguineous marriages are present. In conclusion, this combined test represents an

important tool for its simplicity, short working time, low cost, and it does not require any sophisticated equipments.

Table 1. Urinary GAG concentration (µg GAG/mg Creatinine) according to Creatinine (CR) values < 40 mg/dl a) and >40 mg/dl b) in healthy newborns.

Creatinine (CR) mg/dl		**DMB** µg GAG/mg CR	
a) range	*n*	**mean ± SD**	**MIN-MAX**
1-10	*12*	492.0 ± 158.7	191.7 - 745.1
11-20	*53*	296.9 ± 77.4	162.7 - 568.6
21-30	*61*	262.2 ± 80.3	163.4 - 511.2
31-40	*63*	246.4 ± 66.8	142.7 - 429.1
	n tot		
1-40	*189*	281.5 ± 100.7	142.7 - 745.1
b) range	*n*	**mean ± SD**	**MIN-MAX**
41-50	*51*	203.0 ± 51.1	117.5 - 361.1
51-60	*44*	220.9 ± 56.4	132.2 - 384.0
61-70	*47*	198.4 ± 60.6	99.4 - 360.7
71-80	*44*	192.6 ± 61.8	84.7 - 364.8
81-90	*28*	187.8 ± 61.8	95.4 - 355.1
91-100	*39*	172.3 ± 45.7	102.1 - 306.5
101-110	*26*	178.0 ± 43.3	94.8 - 262.7
111-120	*39*	178.7 ± 51.6	100.9 - 347.8
121-130	*30*	158.9 ± 32.3	82.7 - 228.8
131-140	*24*	152.7 ± 53.5	63.9 - 291.9
141-150	*16*	182.5 ± 61.7	83.2 - 316.7
151-160	*21*	177.8 ± 68.3	94.6 - 313
161-170	*10*	153.2 ± 62.3	71.3 - 281.7
171-200	*20*	165.5 ± 49.0	91.6 - 304.1
	n tot		
41-200	*436*	185.6 ± 56.9	63.9 - 384

Contributors

OG, AG, LS, CC, and GVC designed the study and prepared the last draft of the manuscript; LZ, LP, TG, RTM, CM, LR and NV performed the experimental procedures and analyses; DC, AF, MM, VPC recruited the newborn urinary samples; OG and GVC performed the statistical analysis and wrote the first draft. All authors read and approved the final manuscript.

Declaration of interest

The authors declare that they have no competing interests.

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