CLINICAL AND GENETIC CHARACTERISTICS OF CHILDREN WITH ALAGILLE SYNDROME IN CHILDREN'S HOSPITAL NO. 1

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ABSTRACT

Introduction: *Alagille syndrome is an autosomal dominant disorder that affects many various organs.*

Objectives: *Determining the prevalence of clinical and genetic characteristics of children with Alagille syndrome in Children's Hospital No.1.*

Subjects and methods: *Patients diagnosed with Alagille syndrome and treated at Children's Hospital No. 1 from February 2015 to December 2018. Descriptive case-series design.*

Results: *Thirty two children diagnosed with Alagille syndrome were studied. The proportions of hepatic, facial, spinal, cardiac and ocular abnormality were 96.9%; 87.5%; 78.1%; 75%; 59.4%, respectively. The levels of cholesterol, ALP, Triglyceride, AST, ALT, total bilirubin, GGT, and direct bilirubinwere higher than the normal thresholds. JAG1 gene mutation was detected in 75% of our patients. The rate of nonsense mutations was 33%, missensemutation 29%, frame-shift 21%, splicing 13% andmicrodeletion 4%.*

Conclusion: *We express our sincere need to continue research with a larger sample size to discover the genotype-phenotype correlation as well as specific factors that can relate to the prognosis of liver disease.*

1. INTRODUCTION

Alagille syndrome (ALGS) is an autosomal dominant disorder affecting many organs such as the liver, heart, eyes, spine and face [15]. The disease was first described by Daniel Alagille in 1969 [2], with diagnostic criteria including intrahepatic biliary hypoplasia associated with at least three major clinical symptoms. The disease is very rare, with a prevalance of 1:70,000 neonates, caused by JAG1genetic mutation [10] and a small percentage of NOTCH2genetic mutation (<1%) [13].

Early diagnosis of ALGS is very important because the disease often presents with a variety of symptoms, similar to other diseases, especially biliary atresia, especially in the neonatal period. As a result, they are often misdiagnosed and subjected to unnecessary interventions such as Kasai surgery, severely affecting prognosis of the disease [7].

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At Children's Hospital No. 1, genetic testing is not currently available, diagnosis of ALGS is mainly based on clinical manifestations, often misdiagnosed with biliary atresia, affecting the patient's quality of life. From those challenges as well as from the results and limitations of previous study by Lin C. Henry and Hoang Le Phuc on Vietnamese children with ALGS, the study was conducted with the following two objectives to determine:

1. Prevalence of clinical and subclinical characteristics of children with Alagille syndrome

2. Prevalence of JAG1 and NOTCH2 genetic mutations in children with Alagille syndrome.

2. METHODOLOGY

Research design: Case-series description.

Research subject: All pediatric patients with Alagille syndrome treated at Children's Hospital No.1 from February 2015 to December 2018.

Inclusion criteria: Pediatric patients are under 16 years old; Haveat least three of the five major clinical symptoms including [1] liver abnormality, [2] cardiac abnormality, [3] spinal abnormality, [4] ocular abnormality and [5] facial abnormality; Agree to participate in the study.

Sample size: All samples eligible for inclusion and no exclusion criteria.

Sampling method: Non-probability, in which sequential sampling method is used.

Data processing and analysis: Epidata 3.1 software is used for data entry. Stata 13 is used for data analysis. For descriptive statistics, continuous variables are described by mean, standard deviation if normally distributed and median, interquartile range if not normally distributed. The nominal, binary and ordinal variables are analyzed and presented in terms of frequency and percentage (%). All tests are considered to be statistically significant when $p<0.05$.

3. RESULTS

3.1. Research sample characteristics

Table 1. Characteristics of study participants

Characteristics	Gender			p
	Total (n=32)	Female (n=14)	Male (n=18)	
Age (month)[§]	6.5 (2 - 51.5)	3.5 (2 - 31)	20 (4 - 112)	0.035
Age group (months)				
>4	17 (53.1)	5 (35,7)	12 (66,7)	0. 082[ᶜ]
≤4	15 (46.9)	9 (64,3)	6 (33.3)	
Address				
Ho Chi Minh City	7 (21.9)	4 (28.6)	3 (16,7)	0. 669[ᶜ]
Other provinces/cities	25 (78.1)	10 (71.4)	15 (83.3)	

[§] Report on median and interquartile range, Mann – Whitney test

[ᶜ] Fisher's exact test

3.2. Clinical and subclinical characteristics of children with Alagille syndrome

Table 2. Characteristics of main clinical abnormalities

Characteristics	Frequency (n=32)	Ratio%
Liver abnormality	31	96.9
Facial abnormality	28	87.5
Spinal abnormality	25	78.1
Cardiacabnormality	24	75
Ocular abnormality	19	59.4
Kidney abnormality	2	6 , 3

Table 3. Subclinical characteristics (N=32)

Characteristics	Result of Median (Interquartile range)		The number of times higher than normal
BTP (µmol/L)	153	68.3 - 202.3	7.5
BTT (µmol/L)	83.1	35.1 - 104	24.3
AST (U/L)	218	178 - 347.2	3.6
ALT (U/L)	213.3	99.2 - 305	4.7
ALP (U/L)	482.3	396.3 - 739.9	1.4
GGT (U/L)	470.9	229.7 - 1099	21.4
Cholesterol (mmol/L)	6.5	5.4 - 12.8	1.3
Triglycerides (mmol/L)	2.5	2.1 - 3.9	1.5

3.3. Characteristics of genetic mutations in children with Alagille syndrome

There were32 cases with clinical diagnosis of ALGS analyzed forJAG1 and NOTCH2genetic mutations. As a result, 24 cases (75%) were found to have JAG1genetic mutations. No cases were found to have NOTCH2 genetic mutations.

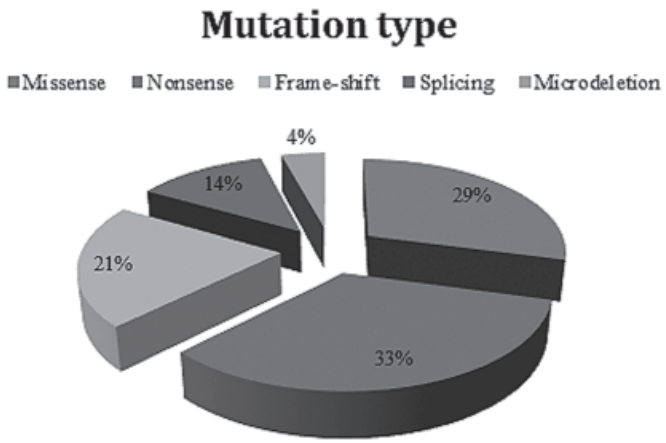

Chart 1. Types of JAG1genetic mutations (N = 24)

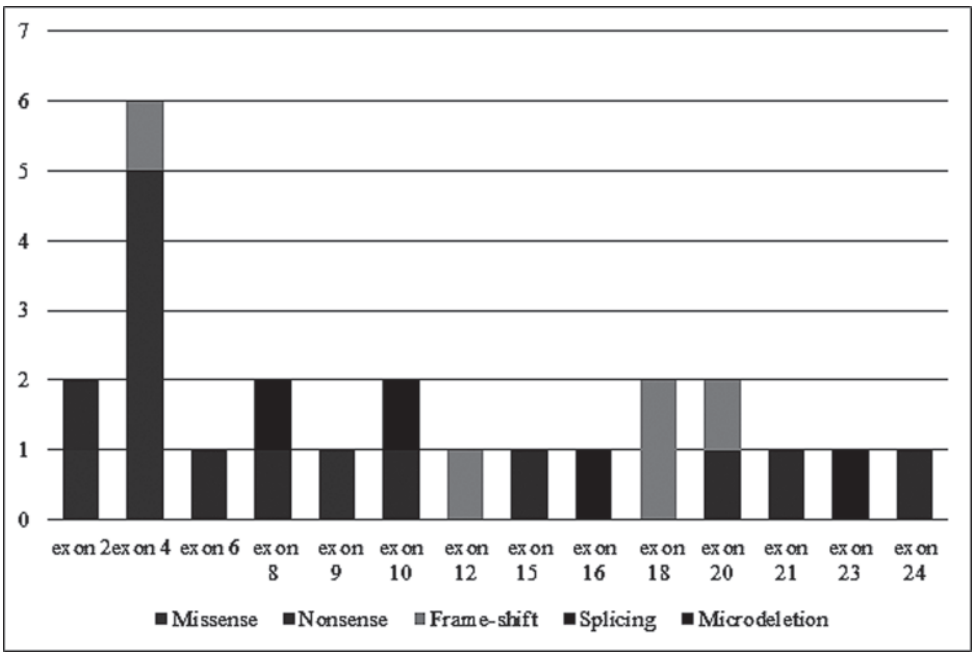

Chart 2 . JAG1 genetic mutation site (N = 24)

4. DISCUSSION

4.1. Research sample characteristics

Median age with detected disease was 6.5 months. The disease detection age of menwas later than that of women (20 months versus 3.5 months). The age group >4 months (17 cases) was more than the group ≤4 months (15 cases),in which the group ≤4 months from Ho Chi Minh City was 3 cases (43%) not different from this same age group from other provinces thatwas 12 cases (48%). That showed that geography was not the cause of late detection of the disease. Compared with the study on 41 children with ALGS by Ahn KJ (2015), the average age with detected disease was very early, 1 month old, of which 6 cases were detected in fetal stage and only 2 cases were detected after 4 months (1 month). Thus, the diagnosis of ALGS at Children's Hospital No. 1 is late even though this is a last-line pediatric medical facility. This may be because the patient's relatives have not been aware of the disease, so the examination is delayed as well as the doctor's limitation in diagnosis such as inexperience or limitedfacilities of diagnosing the disease.

The majority of patients came from other provinces (78.1%) other than Ho Chi Minh City (21.9 %). Due to the fact that Children's Hospital No. 1is the last line in pediatric treatment in the South of Vietnam, the vast majority of patients come from neighboring provinces.

4.2. Clinical characteristics

In the studies on clinical characteristics of children with ALGS, our sample size (n=32) was larger than the researchsample size of Cho JM (n=19) and Lin HC – Hoang Le Phuc (n=21) but smaller than that of Alagille D (n=80), Emerick (n=92) and Subramaniam (n=117) [3,4,6,12,14]. Similar to the above studies, liver abnormalities were very common, ranging from 85% (Emerick KM, 1999) to 100% in the study of Lin HC (2012). This was possibly due to the fact that Lin HC's study only admitted patients in the

Department of Gastroenterology, all had liver abnormalities (100%). Cardiac abnormalities in our study were much lower than those of other authors, accounting for only 75%, while in other studies ≥ 85%. This difference may be due to the way the sampleswere selected. Our study was mainly conducted in the Department of Gastroenterology, so most of the patients had liver abnormalities. In addition, it may be due to differences in sample size or racial differences. Spinal abnormalities ranged from 39% (Subramaniam, 2011) to 94.1% (Lin HC, 2012). Similar to the study of Lin HC and Hoang Le Phuc, spinal abnormalities (78.1%) in our study were higher than those of other authors. This may be a common characteristic of Vietnamese children with ALGS. Meanwhile, our ocular abnormalities (59.4%) are very low compared to those of authors Emerick (78%), Alagille D (88%), Lin HC (100%) but similar to those of authors Cho. JM (53%) and Subramaniam (61%). However, because the study of Lin HC and Hoang Le Phuc only observed 2 cases, the 100% frequency failed to accurately reflect the frequency of ocular abnormalitiesand was not a representative of the study sample. Facial abnormalities in our study were 87.5%. This wasalso a common abnormality in the study of Cho JM (100%), Emerick (96%) and Alagille D (95%).

4.3. Characteristics of biochemical tests

The results of the study showed that all tests BTP, BTT, AST, ALT, ALP, GGT, Cholesterol, Triglycerid had values increase compared to the normal threshold, in which two indices BTT and GGT increased very high compared to normal threshold, up to 24.3 times and 21.4 times, respectively. This was consistent with pathophysiology, reflecting the cholestasis of children with ALGS. The median values of BTP and BTT in our study were 153µmol/L (8.9mg/dL) and 83.1µmol/L (4.8mg/dL), respectively. This result was similar to the study result of 19 children with ALGS by Cho JM (2014), BTP and BTT were 8.5 ± 2.7mg/dL and 4.9 ± 1.6mg/dL (4). In the Subramaniam study (2011), cholesterol >5mmol/L was found in 52/86 (60.4%) patients [14]. We had similar results with a Cholesterol median of 6.55mmol/L.

4.4. Characteristics of genetic mutations in children with Alagille syndrome

The result of genetic analysis of 32 cases of Alagille syndrome showed 24 pathogenic JAG1 genetic mutations (75%) and no NOTCH2 genetic mutations were found. The JAG1 geneticmutation rate in our study was similar to that of Li L (76.9%) [11], Jurkiewicz D (74.3%) [8], Cho JM (74%) [4], higher than that ofCrosnier C (63%) [5], Krantz ID (69%) [9] and lower than than that of Lin HC (86%) [12]. In general, the detection rate of JAG1 genetic mutation by us and the authors was not as high as expected compared with the medical literature (>90%). The limitation in our study was that we had not investigated the large deletionand the loss of the entire JAG1 gene.

Most of the studies showed the most common frameshift mutation, different from the studies as well known by us related to the nonsense mutation. This was important because nonsense mutations often affected the associated protein structure more than frameshift mutations. Besides, we also detected a microdeletion case (4%) and no large deletioncase (we failedto investigate in our study). This frequency was lower than that of Li L (7.1%), Jurkiewicz D (30.7%), Lin HC (16.7%). The explanation for the above difference may be due to technical differences. genetic analysis (no long - term deletion and whole-genome mutations have been investigated), sample size difference or race difference.

Research resultshowedthat exon 4 (6cases) was more often affected than other exons such as exons 2, 6, 8, 9, 10, 12, 15, 16, 18, 20, 21, 23, 24 but in general, all exons could be affected. This result was similar to that of Li L, Jurkiewicz D, Cho JM, Lin HC, Crosnier C, Krantz ID [4,5,8,9,11,12].

5. CONCLUSION

The resultof a study on 32 children with Alagille syndrome at Children's Hospital No. 1, conducted from February 2015 to December 2018 showed:

The mean age upon detecting the disease was 6.5 months. The prevalence of men is 1.3 times more than that ofwomen. The proportion of patients from the southern provinces was 78.1%.

The rates of liver abnormalities, facial abnormalities, spinal abnormalities, cardiac abnormalities and ocular abnormality abnormalities was 96.9%; 87.5%; 78.1%; 75%; 59.4 %, respectively.

The indices of Cholesterol, ALP, Triglycerid, AST, ALT, BTP, GGT, B TT increased by 1.3; 1.4; 1.4; 3.6; 4.7; 7.5, 21.4, respectively; 24.3 times higher than normal threshold.

JAG1 genetic mutation rate was 75%. The rate of non-sense mutations was 33%, missensemutation 29%, frameshift 21%, splicing 13%, and microdeletion 4%. Mutations were distributed across most exons.

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