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TRAN THI THAM

SITUATION OF CONGENITAL AND ACQUIRED NEUTROPENIA IN CHILDREN

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ABSTRACT OF MEDICAL PHD THESIS
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Supervisors:

1. Asso. Prof. Le Thi Minh Huong MD, PhD
2. Asso. Prof. Vu Van Quang MD, PhD

Reviewers:

1.
2.
3.

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LIST OF PUBLISHED ARTICLES RELATING TO THE THESIS

1 **Tran Thi Tham**, Vu Van Quang, Le Thi Minh Huong (2019), "Situation of neutropenia at Vietnam National Children's Hospital from January to June 2018", *Vietnam Medical Journal*, Volume 482, p.337 - 342.

2 **Tran Thi Tham**, Vu Van Quang, Le Thi Minh Huong (2019),
"Situation of neutropenia at Haiphong Children's Hospital in 2017", *Vietnam Medical Journal*, Volume 484, p. 683 - 688.

3 **Tran Thi Tham**, Vu Van Quang, Le Thi Minh Huong, Phan Huu Phuc, Nguyen Thanh Binh, Taizo Wada (2017), "Severe congenital neutropenia caused by ELANE 2 mutation at Vietnam National Children's Hospital: a case report", *Journal of Pediatrics*, Volume 10(2), p.64 - 68.

4 **Tham Thi Tran**, Quang Van Vu, Taizo Wada, Akihiro Yachie, Huong Le Thi Minh and Sang Ngoc Nguyen (2018), "Novel *HAX1* gene mutation in a Vietnamese boy with severe congenital neutropenia", *Hindawi, Case reports in Pediatrics*, Volume 2018.

INTRODUCTION

Neutrophils play important roles in the immune system. A child with neutropenia may increase the risk of infections. Neutropenia is characterized by an absolute reduction in the number of neutrophils in peripheral blood. According to age, the criterias of neutropenia are different: Under 1 year old (≤ 1G/l); Upper 1 year old (≤ 1.5G/l). Neutropenia levels can be classified as follow: mild (1 - 1.5G/l); Moderate (0.5 - 1.0G/l), severe (0.2G/l - 0.5G/l) and very severe (≤ 0.2G/l). According to times, neutropenia can be divided into two groups: Acute and chronic neutropenia. In addition, neutropenia can be classified congenital and acquired neutropenia based on pathological mechanisms. Congenital neutropenia is caused by encode gene mutations with prevalence approximately 1/200.000. Some gene mutations are related to congenital neutropenia: *ELANE, HAX1, G6PC3*.... There are few studies with neutropenia in Vietnam. How is the situation of neutropenia in Vietnamese children? How are the causes and classifications of neutropenia in those children? What specially are the clinical and laboratory features of congenital neutropenia group? Therefore, we carried out the thesis with the following objects:

1. To determine proportion and classification of neutropenia in children presenting outside the neonatal period in Haiphong Children's Hospital from 01/01/2017 to 31/12/2017 and Vietnam National Children's Hospital from 01/01/2018 to 30/06/2018.

2. To analyses clinical, laboratory features and gene mutation results of congenital neutropenia.

NEW CONTRIBUTIONS OF THE THESIS

This is the first study to show the proportions and classifications of neutropenia as well as congenital neutropenia in children in our country.

The results of this thesis will contribute into diagnosis, treatment and management of patients who suffer from neutropenia in general and congenital neutropenia in particular. Besides, the study has been found the new *HAX1* gene mutation that causes congenital neutropenia. This is the novel mutation that has never reported in any article in the word.

STRUCTURE OF THE RESEARCH PAPER

The thesis consists of 125 pages: Introduction (2 pages); Chapter 1 - Overview (30 pages); Chapter 2 - Subjects and methods (20 pages); Chapter 3 - Results (38 pages); Chapter 4 - Discussion (32 pages); Conclusion (2 pages); Recommendation (1 page). There are 177 references, including 9 Vietnamese resources and 168 English references. The thesis includes 46 tables, 22 figures and 4 diagrams.

Chapter 1

OVERVIEW

1.1. The classification of neutropenia

- According to age: Under 1 year old (Absolute neutrophil count ≤ 1000 cell/mm^3 - 1.0G/l), Upper 1 year old (Absolute neutrophil count ≤ 1500 cell/mm^3 - 1.5G/l).
- According to neutropenia reduction level: mild (1000 - 1500 cell/mm^3), moderate (500 - 1000 cell/mm^3), severe (≤ 500 cell/mm^3), very severe (≤ 200 cell/mm^3)
- According to time: Acute neutropenia and chronic neutropenia (Neutropenia prolonged over 3 months)

- According to cause: Congenital neutropenia or neutropenia because of hereditary causes comprises: Kostmann syndrome, Cyclic neutropenia, Shwanchman-Diamond syndrome...Acquired neutropenia including decrease production: Chemotherapy, infection, nutrition, blood diseases (Acute leukemia, Aplastic anemia, Myelodysplastic syndrome), Metabolic diseases. Increased destruction: Autoimmune neutropenia, Drug - induced neutropenia...Idiopathic neutropenia.

1.2. Congenital neutropenia: This is inherited disorder of hematopoiesis that is characterized by severe neutropenia in peripheral blood prolonged over 3 months. The disorders consist of: Disorders of myelopoiesis (Severe congenital neutropenia, Cyclic neutropenia, Kostmann disease); Disorders of ribosomal and telomere dysfunction (Shwanchman - Diamond); Disorders of metabolism (Barth syndrome, G6PC3 gene mutations); Disorders of vesicular transport (Chediak-Higashi syndrome, Cohen Syndrome); Disorders of immune function (Hyper IgM syndrome, Wiskott-Aldrich syndrome).

1.3. Clinical, laboratory features of congenital neutropenia

Clinical features:

- **Clinical symptoms were caused by neutropenia**: Recurrent infections in organs such as: navel, respiratory, skin, soft issue and skin abscess, mouth ulcer, gingivitis, periodontitis, gastritis, diarrhea, urinary tract infections, sepsis, fungus and other signs depend on gene mutations: *HAX1* gene mutation cause severe congenital neutropenia with neurological symptoms (delayed metal development and epileptic seizures). Neutropenia with abnormal of

the urogenital tract or the heart are symptoms of patients with *G6PC3* gene mutation.

- Clinical symptoms are consequences of hereditary neutropenia: Blood cell lines reduction, myelodysplastic syndrome, acute leukemia, splenectomy, hepatomegaly, growth retardation, osteopenia, osteoporosis, vasculitis....

Laboratory features:

- Cell Blood Count: Severe neutropenia or very severe neutropenia even without infection and prolong over 3 months.

- Genetic analysis: Some gene mutations cause neutropenia: *ELANE, HAX1, G6PC3, SBDS, WAS, GFl1*...with different prevalences. Among them, *ELANE* gene mutations make up approximately 50-60%.

1.4. Situation of neutropenia researches

- In the world: There are a lot of studies about neutropenia with various respects from overview to specific research. From the 1999 to 2004, the study of United States National Health and Nutritional Examination Survey indicated different prevalence of neutropenia between races. In 2010, Ruti Sella et al evaluated 120 children with a clinical suspicion of autoimmune neutropenia. Their results showed that the patients suffer from recurrent infections in various organs. Some authors have in-depth studies about rare cases. However, the researches about congenital neutropenia were limited with single study. The epidemiological investigations about primary immunodeficiency diseases did not mention to congenital neutropenia excluding a study of Iran in 2006.

- In Vietnam: To date, there are few studies about neutropenia in general and congenital neutropenia in particular. In 2003, Bui Van

Vien et al studied neutropenia and infection in children with acute lymphoblastic leukemia in the induction phase of chemotherapy. Tran Viet Ha studied about infection caused by bacteria in patients who suffered from hematopoiesis with neutropenia at National Institute of Hematology - Blood Transfusion. Nghiem Thi Minh Chau and Nguyen Hoang Thanh evaluated clinical and laboratory characteristics of febrile neutropenia. However, the researches were often carried out adult. Recently, Ngo Ngoc Duc studied epidemiological, clinical, laboratory characteristics of 102 cases with neutropenia at Haiphong Children's Hospital. In Vietnam, there is not any author who has studied congenital neutropenia caused by gene mutations so far. Therefore, diagnosis, management as well as genetic advisory for family members of the patients are extreme difficulties.

Chapter 2

SUBJECTS AND METHODS

2.1. Research subjects, location and timing

2.1.1. Research subjects

1846 neutropenia patients who were admitted at Haiphong Children's Hospital (n = 416) and Vietnam National Children's Hospital (n = 1430), were selected for the research.

Selection criteria:

- Children from 1 month to 15 years old
- The selected criteria of neutropenia: According to Nguyen Cong Khanh: Under 1 year old $\leq$ 1.0 G/l, Upper 1 year old $\leq$ 1.5G/l.
- Congenital neutropenia: Confirming gene mutations

Exclusion criteria:

The parents of the children suspected of congenital neutropenia disagree with the children participating in the research.

2.1.2. Research duration

The research had done from 01/01/2017 to 31/12/2017 at Haiphong Children's Hospital and 01/01/2018 to 30/06/2018 at Vietnam National Children's Hospital.

2.2. Methods

2.1.1. Research Design: The study was divided into 2 periods

The first period: Case series study (Object 1): At Haiphong Children's Hospital and Vietnam National Children's Hospital consist of two purposes:

- Determining proportion of neutropenia among admitted patients
- Classifying neutropenia according to: age, time, reduction level, cause (pathological mechanism...)

The second period: Recruitment research (object 2): Congenital neutropenia was recruited from neutropenia patients

+ Selection criteria:

* Severe neutropenia (≤ 0.5 G/l), chronic neutropenia (Neutropenia prolonged over 3 months)

* Severe infection (life-threatening infections such as: sepsis, infection shock) or recurrent infections.

* Excluding other diseases causing severe neutropenia: Acute leukemia, aplastic anemia, myelodysplastic syndrome, system disease, autoimmune neutropenia.

+ Collecting informations about prehistory, medical history, medical record, then discussing with Japanese professors, adding information to the medical record, checking other tests (if necessary),

collecting gDNA. Finally, DNA samples were sent to Laboratory Center of Pediatric Department, Kanazawa University, Japan. They are analyzed to find gene mutations.

2.2.2. Sample size and sampling methods:

- Sample size for object1: Proportion and classification of neutropenia: Sampling technique: Convenient, all patients were confirmed neutropenia taking part in the study.

- Sample size for object 2: Congenital neutropenia. This is a rare disease with prevalence approximately 1/200.000 of alive newborn. Thus, we expected to find 5 - 10 congenital neutropenia patients at Haiphong Children's Hospital and Vietnam National Children's Hospital.

2.3. Variables and research index

Some standards of research variables and index

- Neutropenia according to time:

+ Acute neutropenia: Neutropenia prolongs under 3 months after infection, drug, chemotherapy…

+ Chronic neutropenia: Neutropenia prolongs over 3 months consist of congenital neutropenia, cyclic neutropenia, autoimmune neutropenia (Primary autoimmune neutropenia, Secondary autoimmune neutropenia), chronic idiopathic neutropenia.

- Neutropenia according to the cause:

+ Congenital neutropenia (Primary): Because of gene mutations

+Acquired neutropenia (Secondary): Neutropenia after another disease. Acquired neutropenia are divided 2 groups:

* Decreased production: Causes exert an influence on bone marrow leading to neutropenia: infection (virus, bacteria), blood

disease (acute leukemia, aplastic anemia, myelodysplastic syndrome), chemotherapy treats cancers.

* Increased destruction: Antineutrophil antibodies (Antineutrophil cytoplasmic antibody: Autoimmune neutropenia, Antinuclear antibodies: Systemic lupus erythematosus) and Drug implicated in causing neutropenia: Antiepileptic drugs (Carbamazepine, Valproate), antibiotic drugs (Sulfonamide, penicillin....)

- My diagnosis based on diagnosis of treatment doctor and clinical symptoms as well as test results.

2.4. Methods and data collection techniques

2.4.1. Conduct research and data collection

+ We designed diagrams for diagnosis, following, management. The diagrams are suitable with arrangement in the ward of the hospital.

+ We announced the diagram for emergency and treatment departments.

Data collection of the first period:

- At Haiphong Children's Hospital

+ We used neutropenia patient list of Hematology Laboratory Department.

+ From this list, you could find the departments and units where neutropenia patients were treating to collect necessary information into the research record.

- At Vietnam National Children's Hospital:

+ Got the list of neutropenia patients from the Information Technology department by screening software according to the research requirement.

+ From neutropenia patient list, basing on medical record codes, logging on electronic medical records of the departments to select necessary informations.

Data collection of the second period: selecting patients with suspected congenital neutropenia for genetic analysis.

- Discharged neutropenia patients were re-examined and following outpatient room of Immunology - Allergy Department at Vietnam National Children's Hospital and Nephrology - Hematology - Endocrinology Department at Haiphong Children's Hospital. The neutrophils of patients were retested every 2 weeks until absolute neutrophil count returned normal.
- Genomic DNA of patients suspected congenital neutropenia was isolated for genetic analysis.

2.5. Manage, process and analyze data

- The data was processed by medical statictis method with SPSS software 22.0.

2.6. Ethical issues

- The study was followed the approval research protocol of Haiphong University of Medicine and Pharmacy, received the consensus of Vietnam National Children's Hospital and Haiphong Children's Hospital.
- For patients are sampled DNA to send genetic analysis, the study received permittion of their parents. All personal informations are kept confidential and only used for study.

Chapter 3

RESULTS

From January 1, 2017 to June 31, 2018, this study collected 1846 neutropenia patients in total with the following characteristics:

3.1. The proportion and classification of neutropenia in children

***The proportion of neutropenia among admitted patients*:** The proportion is 0.9% at Haiphong Children's Hospital and 4.15% at Vietnam National Children's Hospital. The proportion is 2.25% in both hospitals.

Age: The proportion of upper 1 years old patients (61.8%) are higher than under 1 years old patients (38.2%). The difference was significant with $p < 0.05$.

Sex: Male (n = 1117; 60.5%) is higher than female (n = 729; 39.5%). The male/female ratio was 1.5/1. The difference was significant with $p < 0.05$

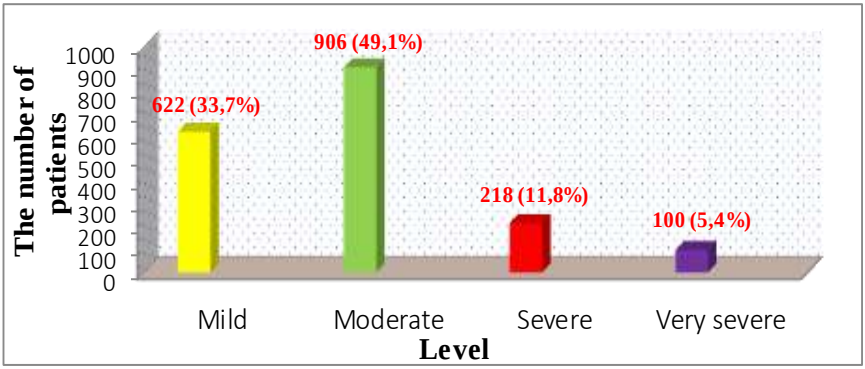

Figure 3.1: The classification of neutropenia according to severe level

Comments: In neutropenia patients, moderate neutropenia is the highest (n = 906, 49.1%), the lowest is very severe neutropenia (n = 100, 5.4%).

The classification of neutropenia according to time: Most of patients are acute neutropenia (92.7%). They are higher than chronic neutropenia (7.3%). The difference is significant with $p < 0.05$.

The classification of neutropenia according to the causes: Acquired neutropenia are the highest (92.4%). There are 5 patients with congenital neutropenia and 135 patients with idiopathic neutropenia.

Table 3.7: Some causes of acquired neutropenia

Acquired neutropenia	**Number of patient (n)**	**Frequency (%)**
Infectious disease	1460	85.6
Blood disease	151	8.9
Autoimmune	6	0.4
Chemotherapy	59	3.5
Drug	10	0.6
Other	20	1.2
Total	**1706**	**100**

Comments: In acquired neutropenia group, rate of neutropenia after infectious disease is the highest with 85.6% (n = 1460). The lowest is autoimmune neutropenia with 0.4% (n = 6).

Isolation of bacteria in neutropenia patients: Distribution analysis of microbiological isolates revealed 22.2% (n = 14) Gram-positive baccili, 60.3% (n = 38) Gram-negative baccili. In addition, there is 17.4% atypical bacteria.

Isolation of virus in neutropenia patients: Some common viruses in neutropenia patients comprise: Influenza (n = 85; 30%), Dengue fever (n = 31; 11%), *CMV* (n = 31; 11%). Moreover, there are another viruses such as: *EBV*, varicella, *RSV*, hepatitis virus, *HIV*, mealse..

3.2. Clincal, laboratory features and genetic analysis results of 05 congenital neutropenia patients

3.2.1. Clinical features

Table 3.24. Prehistory of infection before diagnosis

Case	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at the first infection	8 months	2 months	24 days	1 days	7 months
Organ at the first infection	Pneumonia	Soft Issue ulcer after ear	Navel inflammation	Inflammation at sacrum area	Otitis
Time of the first infection period	2 weeks	2 weeks	3 weeks	4 weeks	2 weeks
Warning signs of primary immunodeficiency disease	Yes	Yes	Yes	Yes	Yes

Comments: All congenital neutropenia patients often were infected early. Duration of treatment is from 2 to 4 weeks. The patients have at least one of the warning signs of primary immunodeficiency disease.

Table 3.25: Infectious organs from newborn to before diagnosis

Case	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Navel			x		
Lung	x		x	x	x
Ear	x	x	x	x	x
Skin, soft tissue	x	x	x	x	x
Mouth	x		x	x	
Brain				x	

*** *Comments:*** Congenital neutropenia patients usually suffer from infection at respiratory tract (pneumonia, otitis), skin and soft tissue (pustulosis on skin, cutaneous abscess), gingivitis, encephalitis...

Table 3.27: Clinical symptoms of hospitalized episodes

Case	Episode I	Episode II	Episode III	Episode IV	Episode V	Episode VI	Episode VII
Patient 1	Fever, cough	Fever	Fever				
Patient 2	Fever	Fever	Fever	Fever	Fever	Fever	Fever
Patient 3	Fever, cough	Cough	Fever	Fever	Abdominal pain		
Patient 4	Fever, Pustulosis on skin	Fever, Pustulosis on skin	Fever	Fever, lymphadenitis neck	Fever		
Patient 5	Scalp ulcer	Fever, left cheek swelling	Fever	Fever			

**** Comments:*** Most of patients were hospitalized because of fever. In addition, some patients have other symptoms such as: cough, scalp ulcer, pustulosis on skin.

3.2.2. Laboratory features

Table 3.29: The number of leucocyte cells according to age before diagnosis of patient 1

Age (month)	WBC (G/l)	NEUT (G/l)	LYM (G/l)	MONO (G/l)
10	20.1	0.6	12.4	6.6
17	10.2	0.58	4.8	5.08
18	9.2	**0.01**	5.9	2.67
23	13.2	3.43	6.6	3.17
41[a]	6.48	1.6	2.6	1.9
41[b]	7.09	**0.21**	2.26	3.55
42	7.98	0.95	3.19	3.43
43	7.7	0.6	4.46	2.77
45	6.79	0.33	3.87	2.77
48[a]	9.54	1.33	7.5	0.57
48[b]	8.8	5.34	3.69	0.9
49	9.34	**0.09**	7.65	1.12
57	6.64	**0.03**	2.98	3.09
58	5.18	**0.28**	3.26	1.21
70	6.76	**0.02**	2.88	2.94

Table 3.30: The number of leucocyte cells according to age before diagnosis of patient 2

Age (Month)		WBC (G/l)	NEUT (G/l)	LYM (G/l)	MONO (G/l)
Newborn		12.73	**0.26**	11.58	0.89
2 months		14.32	1.72	10.31	2.29
3 months		10.49	0.32	8.6	1.57
7 months	Time 1	8.74	**0.087**	6.99	1.66
	Time 2	4.05	**0.02**	2.77	1.26
	Time 3	7.0	0.62	3.55	2.83
	Time 4	10.36	0.72	6.33	3.31
	Time 5	12.41	1.18	6.97	4.26
	Time 6*	12.5	3	5.95	3.55
	Time 7	11.8	3.4	5.24	3.16
	Time 8	15.4	5.4	7.55	2.45

Table 3.31: The number of leucocyte cells before diagnosis of patient 3

Date	WBC (G/l)	NEUT (G/l)	LYM (G/l)	MONO (G/l)
09/06/2016	13.05	0.7	8.7	3.6
13/06/2016	11.3	1.6	7.3	2.4
16/06/2016	10.33	**0.04**	7.55	1.82
28/10/2016	5.0	1.5	2.1	1.4
05/12/2016	14.6	0.15	4.18	9.5
30/12/2016	12.9	0.04	8.69	3.52
03/01/2017	13.7	3.2	6.71	2.19
18/01/2017	19.2	3.2	11.71	3.65
22/01/2017	8.6	0.8	4.82	1.89

Table 3.32: The number of leucocyte cells before diagnosis of patient 5

	WBC (G/l)	**NEUT (G/l)**	**LYM (G/l)**	**MONO (G/l)**
15/08/2016	2.39	**0.002**	1.04	1.33
17/08/2016	5.3	**0.5**	2.23	1.06
20/08/2016	3.2	**0.4**	1.88	0.42
25/08/2016	2.13	**0.34**	0.53	0.34
29/08/2016	1.8	**0.38**	0.29	0.52

**** Comments***: Congenital neutropenia patients were caused gene mutation with severe and very severe neutropenia. Other leucocyte cells are normal.

Table 3.43: Gene analysis results

Ordinal number	**Patient**	**Age**	**Gender**	**Gene**	**Exon**	**Mutation**	**Consequence**
1	Number 1	120	Male	*ELANE*	Exon 3 (R81P)	- Homozygous - G > C	Arginine > Proline
2	Number 2	26	Male	*ELANE*	Exon 3 (301)	- Heterozygous - G > A	Valine > Methionine
3	Number 3	26	Male	*ELANE*	Exon 4 (401)	- Heterozygous - A > C	Glutamine > Proline
4	Number 4	19	Female	*ELANE*	Exon 3 (308)	- Heterozygous - G > T	Arginine > Leucine
5	Number 5	108	Male	*HAX1*	Exon 3	Frameshift (c.423_424ins G, p.Gly143fs)	Change genetic code

**** Comments***: There are 4 males and 1 female of 05 congenital neutropenia patients. There are 4 patients who have gene mutation

points (replace 1 acid amin by another acid amin) on different exons of *ELANE* gene. For patient 5, genetic analysis has found a novel homozygous frameshift mutation (c.423_424insG, p.Gly143fs). The gene mutation makes to change genetic code causing severe consequences. This is a new mutation. The *HAX1* gene mutation is also the first reported in the word.

Chapter 4
DISCUSSION

4.1. The proportion and classification of neutropenia in children except newborn in Haiphong Children's Hospital from 01/01/2017 to 31/12/2017 and Vietnam National Children's Hospital

The proportion of neutropenia in pediatrics diseases: There were 416 neutropenia patients making up 0.9% of total admitted patients at Haiphong Children's Hospital in 2017. The proportion of Vietnam National Children's Hospital is higher than Haiphong Children's Hospital. In the first six months of 2018, there were 1430 neutropenia patients making up 4.15% at Vietnam National Children's Hospital. The proportion of neutropenia is 2.25% in general. This result is similar to the study of Karavanaki et al with 2.0% of admitted patients.

Age: 1141 (61.8%) of total 1846 admitted neutropenia patients is upper 1 year old. The proportion of under 1 year - old patients is 38.2% (n = 705). The difference between two age groups was significant with $p < 0.05$. This result is similar to the study of Angelio et al with upper 1 year - old 52%.

Sex: Among research subjects, the proportion of female is 39.5% (n = 729) and the proportion of male is 60.5% (n = 1117). The

male/female ratio is 1.5/1. This result is similar to some studies of other authors in Vietnam as well as in the world. However, the result is different with the study of Andersen et al (the male/female ratio is 0,7) and Anirban (the male/female ratio is 3/1).

Neutropenia classifications by severity: In all subjects, the highest number of patients is moderate neutropenia (n = 906; 49.1%). The lowest number of patients is very severe neutropenia (n = 100; 5.4%). The proportion of other groups in order: 33.7% (n = 622; mild neutropenia), 11.8% (n = 218; severe neutropenia). This research result is similar to Ngo Ngoc Duc as well as other authors in the world.

The classification of neutropenia according to time: Most of patients were acute neutropenia (n = 1712; 92.7%). The prevalence of chronic neutropenia patients was 7.3% (n = 134). This result is similar to the study of Andersen et al.

The classification of neutropenia according to cause: According to this classification, the highest proportion is acquired neutropenia (n = 1706) 92.4%. There are five congenital neutropenia patients (0.3%). Idiopathic neutropenia is 135 patients (7.3%). The result can be explained as follow: There are many causes leading to acquired neutropenia such as: infection, drugs, chemotherapy... On the other hand, congenital neutropenia is caused by gene mutation or severe combine immunodeficiency disease. They are rare diseases in Vietnam. Moreover, there are many difficult about genetic analysis in Vietnam. Consequently, some patients with congenital neutropenia were misdiagnosed with acquired neutropenia due to infectious diseases.

There are many causes of acquired neutropenia in Table 3.7. In which, the number of neutropenia patients relating to infectious diseases is the highest 85.6% (n = 1460). In addition, there are other causes such as: Blood diseases, drug induced - neutropenia, autoimmune neutropenia. Our result is similar to the studies of other authors in the world. In those patients, we found some bacteria types with three groups: Gram- Positive Bacilli (n = 14; 22.2%), Gram - Negative Bacilli (n = 38; 60.3%) and Atypical bacteria (n = 11; 17.5%). The research results of other authors showed that: The prevalence of Gram - Negative Bacilli was higher than Gram-Positive Bacilli in neutropenia patients. In addition to isolating bacteria, our study found some common viruses in neutropenia patients including: influenza (n = 85; 30%), Dengue (n = 31; 11%), *CMV* (n = 31; 11%). The our result is similar to other authors.

4.2. Clinical, laboratory features and genetic analysis results of congenital neutropenia patients

History of infections: The results in Table 3.24 showed that: Five congenital neutropenia patients had infections early. The patient who was infected earliest, was 1 day of age with pervasive inflammation of sacrum area (Patient 4). The patient 3 was navel inflammation when he was 24 days of age. The soft tissue ulcer behind ear appeared at 2 months of Patient 2. The first infectious episodes of Patient 1 and Patient 5 were the latest at 7 - 8 months old with infectious respiratory tract such as: pneumonia, otitis, mastoiditis. Timly treatment of five congenital neutropenia patients was over 2 weeks even 4 weeks. They also accompanied one of the warning signs of primary immunodeficiency disease. The early and persistent infection in our neutropenia patients is similar to the results of

research from several other authors in the world. Popular infectious organs of congenital neutropenia are navel, lung, ear, skin and soft tissue, mouth, and brain (Table 3.25). The patients were infected about average 3 - 4 episodes per year. This result is similar to the study by Tahmineh Salehi et al with average 3.1 hospitalized episodes per year.

Routine laboratory findings:

Absolute Neutrophil Count: Before meeting us, the patient 4 was examined in many times because of infections, but her parents did not receive any information about her situation of neutropenia. So, we had not data relating to her absolute neutrophil counts in the past. Before being diagnosed, the patient has had neutropenia in many times but was ignored. The reason may be explained that infectiou diseases are popular in Vietnam, as a result she is misdiagnosed or diagnosed lately. Total of four patients were severe neutropenia to prolong over 3 months. The result is similar to other authors.

Other leucocyte cell lines: This study's result showed that another leucocyte cell lines change according to age but in normal limitations.

Genetic analysis results: There are four patients with *ELANE* gene mutation (from patient 1 to patient 4) and one *HAX*1 gene mutation (Patient 5). Moreover, the total of *ELANE* gene mutation patients are point mutations with replacing this nucleotide by another nucleotide (Table 3.43). In a word, most of gene mutations causing neutropenia are *ELANE*. The previous studies showed that: Mutations in the *ELANE* gene are found in approximately 50 - 60% of congenital neutropenia. The mutations of some other genes also cause

congenital neutropenia with lower prevalence such as: *HAX1, G6PC3, WAS*…

The difference about gene, location, type of mutation leads to different clinical symptoms. This is clearly expressed at organs and frequency of infection as well as prognosis. From Patient 1 to Patient 4, although they present *ELANE* gen mutation, they are different about location of mutation. Hence, they were suffered from different infectious organs such as: pneumonia, gingivitis, foot fungus...

Patient 5: In this patient, mental retardation and delayed development were important signs helping us to decide on analyzing the *HAX1* gene after finding no mutation in the *ELANE* gene. In exon 3 of *HAX1* gene, we found a novel homozygous frameshift mutation (c.423_424insG, p.Gly143fs). This is the very serious mutation because it completely changes the structure of the protein molecule. Structurally, the *HAX1* gene has two types of isomers: one isomer involving neutropenia and another isomer related to the nervous system. Therefore, severe neutropenia patients caused by *HAX1* gene mutation always have neurological abnormalities such as mental retardation, motor or seizures. The *HAX1* gene mutation of our patient was found in exon 3 affecting both transcript variants. As a result, the patient presents severe neutropenia and developmental delay: Two years old could not speak, could not walk, seven years old only spoke short sentences and only knew pointing things he liked. The mutation that was found by our study, is the novel mutation. This mutation is the first published in Vietnam as well as in the word.

CONCLUTIONS

1. *The proportion and classification of neutropenia in children except newborn in Haiphong Children's Hospital from 01/01/2017 to 31/12/2017 and Vietnam National Children's Hospital*

Through the study of 1846 pediatric patients with neutropenia in 2 major pediatric hospitals showed that: The proportion of neutropenia was 2.25%. However, the proportion of Vietnam National Children's Hospital (4.15%) was higher than Haiphong Children's Hospital (0.9%). Most of patients were upper 1 year - old (61.8%). The number of males was higher than that of females. The male/female ratio was 1.5/1.

Classified by the level of neutropenia, this study showed that: Most of patients were moderate neutropenia (49.1%) and mild neutropenia (33.7%). The proportion of severe and very severe neutropenia was 11.8% and 5.4%, respectively. According to time, 92.7% of neutropenia patients were acute. The proportion of acquired neutropenia was 92.4%, specially after infectious diseases (85.6%). Gram-negative bacilli and influenza virus were the most common infectious agents in neutropenia patients.

The study found 5 congenital neutropenia patients confirmed by gene mutations.

2. Clinical, laboratory features and gene mutation results of congenital neutropenia

Prominent clinical feature in pediatric patients with congenital neutropenia was the occurrence of an early infection from the newborn period, which was often severe and recurred many times. Mainly clinical symptom of admitted patient was fever (100%). Common infections of congenital neutropenia patients were

pneumonia, otitis, mouth ulcers, gingivitis, pustulosis on skin, cutaneous and soft tissue abscess.... 100% of the patients accompanied one of the warning signs of primary immunodeficiency disease.

Laboratory features: A total of patients who suffered from congenital neutropenia, had chronically reduced absolute neutrophil counts with severe neutropenia (≤ 0.5G/l), even very severe neutropenia (≤ 0.2G/l). Whereas, other leucocyte cell lines were not change.

Genetic analysis results of five severe congenital neutropenia patients showed that: There were 4 *ELANE* gene mutations and a novel frameshift mutation (c.423_424insG, p.Gly143fs) of *HAX1* gene. The *HAX1* gene mutation is also the first reported in the word.

RECOMMENDATIONS

The patients who suffer from early, recurrent and severe infections in their prehistories with chronic severe neutropenia, should be examined by Hematologist and Immunologist. Since then, these patients can receive necessary screening tests to help early and timely diagnosis of congenital neutropenia.