CYTOGENETIC AND CLINICAL STUDY OF PRIMARY AMENORRHEA IN INDONESIAN PATIENTS

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ABSTRACT

Background Primary amenorrhea is a symptom that can be caused by different disorders such as gonadal, endocrinal, physiological and genetic disorders.
Aim of study This study provide the clinical and cytogenetic profile of Indonesian primary amenorrhea patients and detect the clinical criteria of those patients and match it with their karyotype results using score system.
Method A retrospective descriptive study of 79 PA patients, whom referred to molecular and Cytogenetic unit of Center For Biomedical Research CEBIOR. We made a scoring system that consists of four scores, all the patients had been distributed to match the scores according to their clinical criteria and then confirmed with the karyotype results.
Results The karyotype results of 79 patients of PA revealed 55 (69.6%) patients with female karyotype 46,XX, 6 (7.6%) patients with male karyotype 46,XY, 8(10.1%) patients with monosomy X, 3 (3.8%) patients with 45, X/46,XX, 3 (3.8%) patients with Isochromosome 45 X/46, X,iXq. Mosaicism with Y constitution 45,X/46,XY was seen in 2(2.5%) patients, marker chromosome 45,X/46,X+mar2% in one patient (1.3%) and chromosome 1 and X translocation 46,XX,t(1;X)(p34;q25) detected in one(1.3%) patient. Scoring system results showed that all patients with normal karyotype (46,XX/46,XY) matched score 1 and 2 while 17 patients with chromosomal abnormalities matched score 3 and 4, only one patient with mosaic Turner syndrome 45,X(10%)/46,XX(90%) matched score 1.
Conclusion Turner syndrome was the most common cause of primary amenorrhea which attests the importance of cytogenetic analysis for diagnosis of primary amenorrhea patients. The scoring system needs further tests for measuring reliability and validity.

Key words: Primary amenorrhea, karyotype, clinical examination, score system

INTRODUCTION

Primary amenorrhea defined as failure of menarche, associated with undeveloped secondary sexual signs by age of 13 years, or failure of menarche with well-developed secondary sexual signs by the age of 16 years.[1]It is a symptom that caused by different disorders.[2,3] The worldwide incidence estimated to be 1%,[4,5] and based on research results from different countries, there was no evidence for higher frequency in a specific population or ethnic group.[3]World Health Organization(WHO) ranked the primary amenorrhea as the sixth most common cause of infertility, hence the amenorrhea account 20% of all cases of infertility.[6]

A number of studies estimated the frequency of primary amenorrhea based on the causes including gonadal dysgenesis due to chromosomal abnormality as the largest cause accounting 45%, followed by 15% due to Mayer Rokitanski Kuser Hauser syndrome (MRKH), Hypothalamic Idiopathic HypogonadotropicHypogonadism (IHH) estimated to be account 15% of the primary amenorrhea causes. Complete androgen insensitivity syndrome (CAIS) assessed for 10% of all the cases,[7]imperforated hymen and transverse septum hymen reported to be 5%,[8]while the remaining 5% is distributed among congenital adrenal hyperplasia(CAH) and ovarian insensitivity syndrome.[3]

This is the first study in Indonesia to provide the profiles of primary amenorrhea patients including the karyotype results and their clinical profiles. The new of this study that we made a scoring system and the patients had been distributed to match the scores according to their clinical criteria and then confirmedd with their karyotype results. This scoring system needs further studies to measure validity and reliability, and whether it could be used as a clinical tool for rough prediction of karyotype results.

METHODS

Population and Sample

The sampling method was purposive sampling that patient had been selected according to the purpose of the study. Primary amenorrhea patients whom referred to CEBIOR in Semarang from the period January 2004 to January 2017 included in this study. In this study we made a scoring system consists of 4 scores, score 1 for primary amenorrhea symptom only, score 2 for primary amenorrhea and poor secondary sexual signs, score 3 for primary amenorrhea, poor secondary sexual signs and short stature, score 4 for primary amenorrhea, poor secondary sexual signs, short stature and webbed neck. All the cases had been distributed to match the scores according to their clinical profiles and then confirmed with the karyotype results.

Table 1. Scoring system for primary amenorrhea patients

Score	Clinical craiteria
1	PA
2	PA, poor secondary sexual development
3	PA, poor secondary sexual development and short stature
4	PA, poor secondary development, short stature, webbed neck.

PA= primary amenorrhea

Poor secondary sexual signs = Tanner stage less than 4 with spare or abscent axillary hair

RESULTS

The distribution of patients according to the karyotype results revealed that 55 (69.6%) of patients had 46, XX karyotype, six (7.6%) patients had 46, XY karyotype and 18 (22.8%) patients with chromosomal abnormalities as showed in Figure 1.

In this study the Karyotype results of 79 patients revealed 55 (69.6%) patients with female karyotype 46, XX and six (7.6%) patients with male karyotype 46, XY. the most frequent chromosomal abnormality was monosomy X in 10.1% followed bymosaic cell line 45, X/46,XX by 3.7% . Isochromosome 45 X/46, X,iXq accounted 3.8%. Mosaicism with Y constitution 45,X/46,XY was seen in 2 patients 2.5%, marker chromosome 45,X/46,X +mar2% in one patient and chromosome 1 and X translocation 46,XX,t(1;X)(p34;q25) detected in one patient. The Karyotype results of all the patients showed in Table 2.

The distribution of diagnosis among 55 patients with female karyotype 46, XX revealed 14 patients with MRKH, two patients with CAH and one patient with pure gonadal dysgenesis, while the remaining 33 patients still with unknown causes.

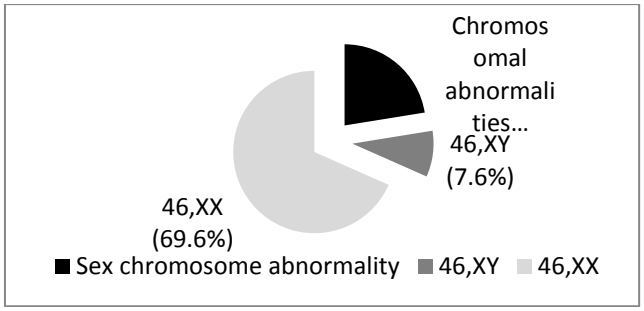

Figure 1. Distribution of karyotype results in primary amenorrhea patients

According to the cytogenetic results of 18 patients with chromosomal abnormalities the diagnosis were Classical Turner syndrome in 8 patients, mosaic Turner in 9 patients and one patient with autosomal X translocation. The distribution of diagnosis among all patients showed in Figure 4. The diagnosis of 6 patients with male karyotype 46, XY revealed CAIS in 2 patients, PAIS in one patient, 3 patients with pure gondal dysgenesis of undetermined cause.

Table 2. The karyotype results of 79 patients with primary amenorrhea.

Chromosomal categories	***Karyotype***	***Number of cases***	
		n	***%***
Female Karyotype	46,XX	55	69.6%
Male karyotype	46, XY	6	7.6%
Numerical Abnormality			
Monosomy	X 45,X	8	10.1%
Turners Mosaic	45,X/46,XX	3	3.8%
Presence of XY constitution	45,X/46,XY	2	2.5%
Structural Abnormality			
Marker chromosome	45,X/46,X +mar2%	1	1.3%
Isochromosome	45,X/46,X,iXq	3	3.8%
Translocation 46,XX,t(1;X)(p34;q25)	X;1	1	1.3%

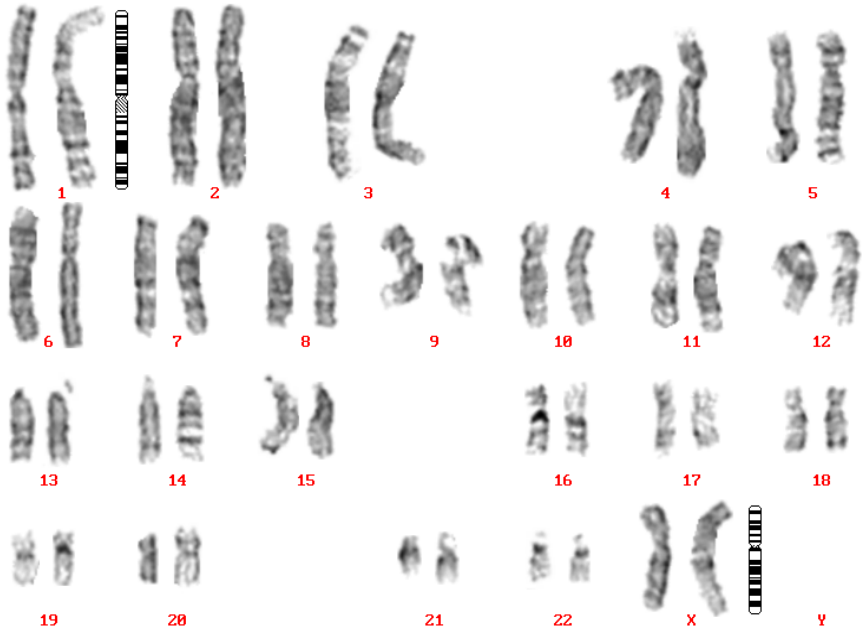

Figure 3. Karyotype result 46,XX,t(1;X)(p34;q25) of patient no. 69
The karyotype result showed translocation between X chromosome and chromosome 1, the patient present with PA, short stature, Tanner stage (1) absent axillary hair and no other dysmorphic features.

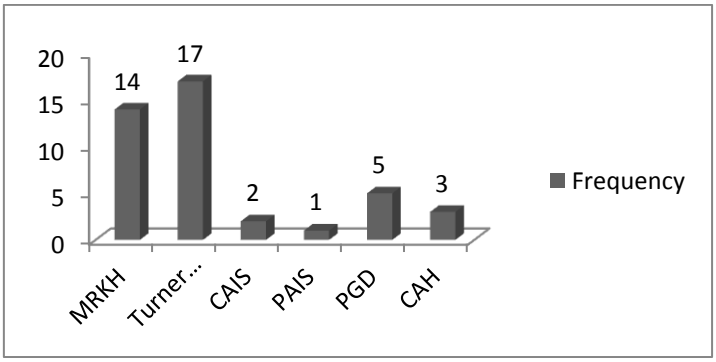

Figure 3. Frequency of diagnosis among 42patients with primary amenorrhea

The result of scoring system and their related karyotype results revealed that under score 1 which is presented with primary amenorrhea only, there were 30 patients with female karyotype 46,XX, one patient with male karyotype 46,XY and one patient of mosaic Turner syndrome45,X(1%)/46,XX(99%).

There were 25 patients with female karyotype 46,XX and 5 patients with male karyotype 46,XY matched score 2. Under Score 3 we demonstrated 4 patients with classical Turnersyndrome(45,X), 4patients with mosaic Turner and one patient with karyotype 46,XX,t(1;X)(p34;q25).

This study demonstrated 4 patients with classical Turner syndrome(45,X), three patients with is chromosme 46,X,iX(q10)()/45X(), 46,X,Ix(q10)()/45,X(), 46,X,Ix()() and one patient with marker chromosome 45,X(98%) /46,X,+mar(2%). Matched score 4 spaces.

Table 8. Scoring system and the related karyrotype results.

Score	Frequency of cases	karyotype results
1	30	46,XX
	1	45,X(1%)/46.XX(99%)
	1	46,XY
2	25	46,XX
	5	46,XY
3	4	45,X
	1	45,X(20%)/46,XX(80%)
	1	45,X(2%)/46,XX(98%)
	1	45,X(90%)/46,XY(10%)
	1	45,X(80%)/46,XY(20%)
	1	46,XX,t(1;X)(p34;q25)
4	4	45,X
	1	46,X,iX(q10)(10%)/45X(90%)
	1	46,X,iX(q10)(24%)/45X(76%)
	1	46,X,iX(q10)(20%)/45X(80%)
	1	45,X(98%)/46,X,+mar(2%)

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Table 8. Scoring system and the related karyrotype results.

Score	Frequency of cases	karyotype results
1	30	46,XX
	1	45,X(1%)/46.XX(99%)
	1	46,XY
2	25	46,XX
	5	46,XY
3	4	45,X
	1	45,X(20%)/46,XX(80%)
	1	45,X(2%)/46,XX(98%)
	1	45,X(90%)/46,XY(10%)
	1	45,X(80%)/46,XY(20%)
	1	46,XX,t(1;X)(p34;q25)
4	4	45,X
	1	46,X,iX(q10)(10%)/45X(90%)
	1	46,X,iX(q10)(24%)/45X(76%)
	1	46,X,iX(q10)(20%)/45X(80%)
	1	45,X(98%)/46,X,+mar(2%)

DISCUSSION

Primary amenorrhea is failure to start menstruation in female in reproductive age.[8] Attainment of menarche is important for female confidence and feminism. Primary amenorrhea can be a cause of psychological trauma in any female in reproductive age group.[2] The causes of primary amenorrhea are different and the role of genetic factors are significant, Several studies for cytogenetic analysis of primary amenorrhea patients have been done in aim to understand the frequency and chromosomal constitution in those patients.[15]

In this sturdy we demonstrated that 22.8% (18/79) patients with chromosomal abnormalities either numerical or structural. In earlier studies the chromosomal abnormalities among primary amenorrhea patients reported to be account 20.63% in Egyptian patients,[16] in Indian population it accounted 27.8%,[17] 20% In Iranian

population[18] and 41% in Mexican population.[19] However, our study results is coordinate to the world wide estimated range for chromosomal abnormality among primary amenorrhea patients which is between 15.9% and 63.3%.[5]

All the patients with sex chromosomal abnormalities in our study was Turner syndrome either classical Turner syndrome or Mosaic. This result was agree with the previous studies which reported that the Turner syndrome is the most common observed chromosomal abnormality in primary amenorrhea and also strengthened the role of sex chromosome in the reproduction of female.[19]

The previous result revealed that classical Turner syndrome detected in 30% (16/52) of primary amenorrhea patients in Turkish population,[16] and 26.9% (7/ 26) of Indian patients.[17] The high percentage of Turner syndrome in our study could be due to selection of patients, lack of facilities and refusal of some patients to cytogenetic analysis.

The clinical profile of Turner syndrome patients showed that all patients with classical Turner syndrome had short stature. Despite short stature, which seems to be the general clinical characteristic of TS, all other clinical stigmata are inconsistent, even in individuals with non-mosaic 45,X.Possible explanation for this fact is that the physical manifestations of TS patients largely depends on the karyotype.[20] The cause of short stature in Turner syndrome suggested to be that the homeobox gene, *SHOX*, in the pseudoautosomal region is the major player and that haploinsuffiency of this gene leads to the growth failure.[16] The other possible cause of short stature in Turner syndrome is inadequate production of Estrogen, many of those with Turner syndrome develop osteoporosis. This can decrease height further, as well as exacerbate the curvature of the spine.[14] The short sature considered if the height of adult female lower than 148 cm which is the mean height for adult Indonesian females according to the WHO range.

Mosaic Turner syndrome with XY constitution seen in 11.7%(2/17) of the patients, detection of Y chromosome and its componenet is very important due to the risk of gonadoblastoma since the risk is quite high 10 to 20%. So, early intervention should be done for orchidectomy.[21] while patients with mosaicism for 46,XY cell line orstructural rearrangement of the Y chromosome mostlyhave masculinized external genitalia and are at increasedrisk for having gonadoblastoma and other gonadal tumor.[12,20,22]

In this study we observed one patient with marker chromosome 45,X(98%)/46,X mar(2%),the clinical profile of this patient showed short stature and no other dysmorphic features, compared to the previous study which reported sever phenotype manifestations in those type of Turner variants,[16] this could be due to the low percentage of marker X , However the conventional cytogenetic can not detect the nature and origin of marker chromosome, the molecular cytogenetic techniques, FISH, can accurately detect it.[16,17]

While most of the studies in primary amenorrhea patients reported the involvement of sex chromosome abnormality, we observed a translocation between X and chromosome 1. However, further study is needed to explain the role of autosomal translocations with X chromosome and primary amenorrhea.

In our study the most common cause of primary amenorrhea in female karyotype 46,XX patients was MRKH (14/55). The clinical profile of the patients show normal Tanner stage in all patients, normal external genital and no dysmorphic features. The high number of MRKH patients needs molecular study to detect the associated gene

mutations.[23] Although causes of mullerian ageneis is unknown, butcan be due to a mutation in the gene of the anti mullerianhormone or the AMH receptor. The underlying mechanismwould be exposure to AMH acivity. No activatingmutation is reported, in contrast to inactivating mutationswhich cause persistence of mullerian structures.[24]

Male karyotype presented in a significant percentage(7.6%) of patients with primary amenorrhoea although they appeared physically normal with some just appearing tall for their age. In our study y chromosome had been detected in 10% of PA patients Compare to previous results which demonstrate that Y chromosome constitution compromise 20% of patients that referred as complain of primary amenorrhea.[14] However, this study demonstrates lower percentage, this could be due to selection of patients, preservation of some cases for karyotype analysis. Furthermore, conventional cytogenetic methodmissed the Y component up to 9.3%.[21] Detection of Y chromosome complement and their compostion is important in genetic counseling, because of the association with risk of gonadoplastoma.[9,12,20]

In this study the most common cause of primary amenorrhea was gonadal dysgenesis Turner syndrome followed by MRKH, pure gonadal dysgenesis, CAH, CAIS and PAIS. The remaining 37(46.8%) patients still not diagnosed, it could be due to mutations and need molecular analysis for establishing the diagnosis. This study was agree with the American study which demonstarate that gonadal dysgenesis as the commonest cause of primary amenorrhea and MRKH as the second most common cause.[23] The same result had been reported from Korean study, which reported that the common causes of primary amenorrhea were gonadal dysgenesis (28.0%, 37/132), followed by Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome (20.0%, 27/132).[25] However, MRKH was the most prevalent cause in primary amenorrhea in Thailand,they reported that the three most common causes of primary amenorrhea were Müllerian agenesis (39.7%), gonadal dysgenesis (35.3%), and hypogonadotropic hypogonadism (9.2%).[17,20,25] This verified that racial and environmental factors played an essential part in the causes of primary amenorrhea.

In the present study we made a scoring system from the clinical criteria of the patients and we matched the patients to appropriate scores. This scoring system can help for distinguish roughly the possible karyotype results in situation of deficient genetic facilities before cytogenetic analysis hold out. So, presence of PA with normal secondary sexual signs predict that this case can be female or male karyotype results and can not be a chromosomal abnormality. In contrast PA with poor secondary sexual development and short stature can give you a prediction of chromosomal abnormalities. However, this scoring system needs more study with large sample population and measurement for validity and reliability.

A significant number of patients had sex chromosomal abnormalities, thus early cytogenetic investigation is prudent to guide further management.Patients with primary amenorrhoea should be initiallyscreened by primary physicians and gynaecologists for non-genetic causes. After exclusion of non-genetic causes, patients should receive prompt referral for genetic study. The reason for referral should beexplained to the patient. If cytogenetic abnormalitiesare detected, a full explanation should be given tothe patient by a geneticist or gynaecologist withexperience in genetics. Counselling should include the risk of premature menopause for patients withTurner's syndrome and the use of hormonal replacementtherapy, the possibility of infertility in the future children of patients with mosaic Turner, and the riskof gonadal malignancy for patients with XY gonadaldysgenesis. Counselling should be performed tactfully,bearing in mind

that sensitive issues related tofemininity are involved. An experienced counsellor and clinical psychologist would be helpful.

CONCLUSION

Turner syndrome was the most common cause of primary amenorrhea which attests the importance of cytogenetic analysis for diagnosis of primary amenorrhea patients. The scoring system needs further tests for measuring reliability and validity.

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