CHAPTER VI

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.[40]

In this research we studied the cytogenetic and clinical profiles of primary amenorrhea patients who referred to Molecular and Cytogenetic Laboratory of Center for Biomedical Research CEBIOR, Faculty of Medicine Diponegoro University Semarang, from the period January 2004 until January 2017. Cytogenetic profiles of the referred patients 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,[38] in Indian population it accounted 27.8%,[16] 20% In Iranian population[41] and 41% in Mexican population.[42] 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.[42]

The previous result revealed that classical Turner syndrome detected in 30% (16/52) of primary amenorrhea patients in Turkish population,[38] and 26.9% (7/ 26) of Indian patients.[16] 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.[43] 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.[38] 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.[48] However, consuming an adequate and balanced healthy diet during all phases of growth is necessary both for proper growth and normal pubertal development. Girls begin puberty at an earlier age compared to past decades. Excessive eating of many processed, high-fat foods, may be the cause of this phenomenon.[49]

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.[28] while patients with mosaicism for 46,XY cell line or structural rearrangement of the Y chromosome mostly have masculinized external genitalia and are at increased risk for having gonadoblastoma and other gonadal tumor.[12,25,43]

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,[38] 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.[16,38]

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 genitali and no dysmorphic features. The high number of MRKH patients needs molecular study to detect the associated gene mutations. [17] Although causes of mullerian ageneis is unknown, but can be due to a mutation in the gene of the anti mullerian hormone or the AMH receptor. The underlying mechanism would be exposure to AMH acivity. No activating mutation is reported, in contrast to inactivating mutations which cause persistence of mullerian structures.[18]

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 patients for

karyotype analysis. Most patients came with female gender athough the karyotype 46, XY. Furthermore, conventional cytogenetic method missed the Y component up to 9.3%.[42] Detection of Y chromosome complement and their compostion is important in genetic counseling, because of the association with risk of gonadoplastoma.[9,12.41]

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.[17] 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).[44] 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%).[16,43,44] 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 patient can be have 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 initially screened 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 be explained to the patient. If cytogenetic abnormalities are detected, a full explanation should be given to the patient by a geneticist or gynaecologist with experience in genetics. Counselling should include

the risk of premature menopause for patients with Turner's syndrome and the use of hormonal replacement therapy, the possibility of infertility in the future

children of patients with mosaic Turner, and the risk of gonadal malignancy for patients with XY gonadal dysgenesis. Counselling should be performed tactfully, bearing in mind that sensitive issues related to femininity are involved. An experienced counsellor and clinical psychologist would be helpful.