JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

A Cohort Study of Intellectual Disability Focusing on Fragile X Syndrome in Indonesia

Tri Indah Winarni[1], Farmaditya EP Mundhofir[1], Sultana MH Faradz[2]

1. Department of Anatomy, Faculty of Medicine Diponegoro University
2. Center for Biomedical Research (CEBIOR), Faculty of Medicine, Diponegoro University, Semarang, Indonesia

Article Info
History:
Received 8 June 2016
Accepted 24 June 2016
Available 30 July 2016

ABSTRACT

Background: Intellectual disability (ID) is a major public health problem because the defect, treatment and rehabilitation require long life both medical and socioeconomic assessment. Fragile X syndrome (FXS) is the most common cause of inherited X-linked intellectual disabilities (ID) with reduced penetrance. With regard to behavioral and emotional phenotype, FXS commonly mixed up with idiopathic autism. The prevalence is found higher in males compared to females. In accordance with development of various diagnosis techniques, the prevalence of FXS is defining worldwide including Indonesia using the simplest techniques

Objectives: This study was aimed to diagnose genetic cause of ID and to establish the prevalence of FXS in the ID population in Central Java, and Yogyakarta Province.

Method: Screening has been performed since 1994 continuously in high risk population (special school with and without autism) using clinical, cytogenetic, and *FMR1* gene PCR-based molecular approach. Cascade testing was subjected to the family members with positive result of FXS and many new cases were disclosed in our cohort study.

Results: The prevalence of FXS among ID population was calculated to be 1.9% (5/262) in 1994 and 1.7% (9/527) in 2011. Among autism population it was determined to be 6.15% (4/65). Trisomy 21 was found in 14% (74/527) as a major cause of ID.

Conclusion: The prevalence of FXS among screened ID population overtime is comparable.

Keywords: intellectual disability; cohort study; fragile X syndrome; Indonesia

INTRODUCTION

ID is a public health problem and becoming important consideration due to some reasons: 1) high prevalence in general population, 2) lifelong disorder, 3) expensive cost of management, include direct cost of long term care, rehabilitation, special education and indirect cost of increased morbidity and mortality since individual with ID is more vulnerable in health problem[1], 4) High economic and society dependences, and 5) Prevention of ID for improving prognosis outcome is absolutely needed to be implemented even it is very challenging in low and middle income

countries.[2] The prevalence of ID across the world is around 1% and almost two times higher rates in low and middle income countries.[3] Fragile X syndrome (FXS) is the most common cause of inherited mental retardation and a single gene disorder associated with autism spectrum disorders (ASD).[4-6] The frequency of FXS due to full mutation allele is approximately 1 in 2500 in males and 1 in 4000 in females.[7-9] The FXS clinical phenotype is caused by imbalance of excitatory glutamatergic and inhibitory GABAergic neurotransmitters.[10] It is mostly due to the expansion of CGG repeats more than 200 (full mutation) in the promotor region of the *FMR1* gene that leads transcriptional silencing and results in lack of fragile X mental retardation protein (FMRP).[11] Fragile X syndrome is the first pathogenic mutations that affect trinucleotide repeat expansions (TREs) diseases involving non-coding repeats sequences.[12,13]

Until the late 1990s, fragile X site cytogenetic analysis was used to diagnose FXS. Subsequently, molecular techniques has been used to identify the *FMR1* alleles since the sequence of *FMR1* gene has been discovered in the beginning of 1990s.[14] However, in developing countries where advanced laboratory equipment and services are very minimal and unaffordable, cytogenetic techniques are still an option to accomplish the FXS diagnosis,[15] more overly, clinical symptoms of FXS especially in younger age is quite challenging to be differentiated with other non-syndromic intellectual disabilities (ID).

The prevalence of FXS varies across ethnic groups,[16] it is important to provide fragile X allele frequencies for all race and across the world, so that general (low-risk) and high-risk population screening the incidence of *FMR1* gene mutation is considered necessarily. Population-based screening is the most favorable method to identify new FXS cases both clinically and laboratory assessment. General population screening to determine the diseases and carriers prevalence in both male and female have been reported worldwide.[17-21] Based on the American College of Medical Genetics (ACMGs) guideline, screening for FXS in high-risk population such as ID and autism population-based FXS screening have also been done to established FXS prevalence.[5,22-24] Various simple and cost-effective DNA-based assay are developing with higher sensitivity and specificity to meet the requirement of population screening study.[25-28] Recently, prevalence studies were also reported from other developing countries although mostly done by scientist in Saudi Arabia, Malaysia, and Sri Lanka.[25,29,30] This study was aimed to define the prevalence of FXS in the high-risk population in Indonesia.

MATERIALS AND METHODS

These were cross sectional study designs which were done in special school in Central Java and Yogyakarta provinces. Individuals who were diagnosed with ASD using the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM IV-TR) for ASD and ID by experienced pediatricians were recruited.[31] This research was approved by the Institutional Review Board of the Faculty of Medicine, Diponegoro University and dr. Kariadi Hospital Semarang, Indonesia. All participants gave their consent in written/signed a consent form to participate in this study. This study was conducted in six special schools in Central Java and Yogyakarta Province.

Heparinized peripheral blood vein was collected for cytogenetic analysis of the fragile site at Xq27.3 and chromosomal abnormalities as done previously.[32] Deoxyribonucleic acid (DNA) was extracted from ethylene diamine tetra acetic acid (EDTA) blood using modified salting out method.[33] The fragile site was identified using solid staining followed by Giemsa staining for fragile X site confirmation. The *FMR1* gene was analyzed using a PCR-based method to determine the CGG repeat length in the promotor region as described previously.[34] Males with no bands and females with only one band were assumed to have alleles consisting of high pre-mutation or full mutation alleles, therefore, Southern blot analysis was used to confirm the diagnosis.[34]

RESULTS

This study was dedicated to determine the prevalence of FXS in the high risk population (ID and ASD). The first population study in 1997 among ID population in special school**,** the overall prevalence of was 5/262 (1.9%). In all, five children from three unrelated families were found to have FMR1 CGG-repeat expansions. All came from the population attending type C (ID) special schools.[22]

The second population study in 2007-2010 among ID population in special school, of the 527 individuals with ID, chromosomal abnormalities were found in 87 (16.5%). Trisomy 21 was the major chromosomal abnormality, identified in 74 patients (14%). In the remaining of 445 individuals (162 females and 283 males), 607 alleles were reported (male has 1 allele, female has 2 alleles) i.e. 593 alleles were within the normal range (15-44 CGG repeats), 3 alleles in the intermediate range (45-55 CGG repeats), 2 alleles in the premutation range (between 55 and 200 CGG

repeats) and 9 alleles in the full mutation range (> 200 CGG repeats). The prevalence of FXS is 9/527 (1.7%). The prevalence in males and females is 1.5% (5/329) and 2% (4/198), respectively.[35]

The study in high risk (autism) population study, the fragile X site and *FMR1* full mutation allele were identified in 3 out of 65 (4.6%) and 4 out of 65 (6.15 %) children aged 3 to 17 years (57 boys, 8 girls) respectively.[36] Impairment of social communication and interaction domains have been found to be dominant in four FXS children with ASD.

Table 1. Prevalence of FXS among ID and Autism Population in Indonesia.

Author	Year	Institution	Population	Σ Sample	Prevalence
Faradz SMH, et al	1999	Faculty of Medicine Diponegoro University	ID	262	1.9%
Mundhofir FEP, et al	2012	CEBIOR, Faculty of Medicine Diponegoro University	ID	527	1.7%
Winarni TI, et al	2013	CEBIOR, Faculty of Medicine Diponegoro University	Autism	65	6.15%

The level of cognitive impairment in FXS usually ranging from learning disabilities to severe ID is associated with the FMRP level.[37] In addition to the cognitive impairment, behavioral and emotional impairment have also been observed as a major problem in individuals with FXS.[38-42] Early intervention has been reported in many centers worldwide shows efficacy decreasing the risk of severe cognitive impairment, improving behavioral problems, emotional, and psychosocial abilities in later life. There are numerous psycho-pharmacological agents that being used to improve behavioral and emotional disorders in FXS whose may have impact on cognitive function[43] ,behavioral and emotional abilities.[44,45] Psychopharmacological agents may support the effectiveness of non-psychopharmacological intervention such as behavior, physical, and speech therapy; and both intervention result the improvement of cognitive function. The similarity and overlapping clinical phenotype, especially in behavioral impairments, and neuroanatomical background between FXS and ASD encouraging the same approach in FXS targeted treatment.[46,47]

The first FXS population-based study on 262 subjects at schools with special need in Indonesia showed a prevalence of 1.9%.[22] New cohort of high-risk population screening of FXS has been conducted among ASD and ID population using fragile X site method combine with molecular one.[48,49] The fragile X site expression has been able to detect 3 out of 4 FXS cases among autism population that eventually confirmed using PCR-based method followed by Southern blot method. The prevalence of FXS is 6.15% in ASD population in Indonesia.[36] Similar result also reported from a number of studies that have been carried out in ASD population varying from 4-7%.[5,23] The prevalence of FXS is 1.7% (considered equal in males and females) in ID population. Surprising result had been reported in the prevalence of FXS in male and female is equal in Indonesia, suggested that the prevalence of FXS females had been underestimated.[49] The FXS is an X-link disorders with reduced penetrance (79% in males, 35% in females), females usually less affected due to the X-inactivation.[50,51] This study was a continuation of the first FXS population-based study with broader approach to identify FXS cases, cascade testing to identify carriers, and finally, to offer early intervention among FXS children. Population-based screening method in FXS has been developed related to Indonesian condition which is contained more than 17.000 islands indweled by 237.6 million populations with very minimal access to genetic facilities and centers. In Java island, the most populated area where 58 percent of Indonesian population live, only one FXS center conducts fragile X diagnosis both cytogenetic and molecular diagnosis that is Center for Biomedical Research (CEBIOR) Faculty of Medicine Diponegoro University.

With regards to the prevalence and the mode of inheritance, FXS screening high-risk population program should be performed routinely and initiate by the government in Indonesia. On the contrary, health care provider, government, and public awareness is inadequate with regard to identify FXS and to provide basic special need services in order to get better outcome. Seminar, workshops, dissemination information of fragile X syndrome should also be conducted regularly using/ leaflet distribution and/or online media to educate clinicians, health care professionals, families, researchers and broader community to increase awareness the role of genetic especially FXS in ID. Using a comprehensive approach to whole Indonesian data of FXS overtime and other cause of ID, our studies will give a great contribution to science, medical community, and stake holders in increasing awareness of FXS in Indonesia. In a contrary, in developed countries such as European and Unites Stated, clinical and molecular guidelines have

been established and revised continuously for fragile X and fragile X-associated disorders in order to identify FXS cases.[52,53] With regard to the prevalence of FXS, similar study is extremely needed to be done in other area of Java Island where the rest of 40 percent Indonesian population live.

ACKNOWLEDGEMENT

We thank all of the participants and their families for their contributions. This work was supported by Risbin IPTEKDOK grant from the Ministry of Health Republic Indonesia in 1998-1999 and 2006 – 2010. We thank Lusi Suwarsi, Wiwik Lestari, Dwi Kustiani, Rita Indriati, Dina Aprilani, Intus Apriasa, and Evi Nurwulan from CEBIOR Faculty of Medicine Diponegoro University for their help in laboratory processing.

REFERENCES

1. van Schrojenstein Lantman-de Valk HM, van den Akker M, Maaskant MA, et al. Prevalence and incidence of health problems in people with intellectual disability. J Intellect Disabil Res. 1997;41 (Pt 1):42-51.
2. Durkin MS, Schneider H, Pathania VS, et al. Learning and Developmental Disabilities. In: Jamison DT, Breman JG, Measham AR, et al., eds. Disease Control Priorities in Developing Countries. 2nd ed. Washington (DC)2006.
3. Maulik PK, Mascarenhas MN, Mathers CD, Dua T, Saxena S. Prevalence of intellectual disability: A meta-analysis of population-based studies. Research in developmental disabilities. 2011;32(2):419-436.
4. Garber KB, Visootsak J, Warren ST. Fragile X syndrome. Eur J Hum Genet. 2008;16(6):666-672.
5. Reddy KS. Cytogenetic abnormalities and fragile-X syndrome in Autism Spectrum Disorder. BMC Med Genet. 2005;6(1):3.
6. Raymond FL. X linked mental retardation: a clinical guide. Journal of medical genetics. 2006;43(3):193-200.
7. Song FJ, Barton P, Sleightholme V, Yao GL, Fry-Smith A. Screening for fragile X syndrome: a literature review and modelling study. Health Technol Assess. 2003;7(16):1-106.
8. Hagerman PJ. The fragile X prevalence paradox. J Med Genet. 2008;45(8):498-499.
9. Mundhofir FE, Winarni TI, Nillesen W, et al. Prevalence of fragile X syndrome in males and females in Indonesia. World J Med Genet. 2012;2(3):15-22.
10. Paluszkiewicz SM, Martin BS, Huntsman MM. Fragile X syndrome: the GABAergic system and circuit dysfunction. Developmental neuroscience. 2011;33(5):349-364.
11. Coffee B, Zhang F, Warren ST, Reines D. Acetylated histones are associated with FMR1 in normal but not fragile X-syndrome cells. Nature Genetics. 1999;22:98-101.
12. Verkerk AJ, Pieretti M, Sutcliffe JS, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell. 1991;65(5):905-914.
13. Cummings CJ, Zoghbi HY. Trinucleotide repeats: mechanisms and pathophysiology. Annual review of genomics and human genetics. 2000;1:281-328.
14. Wang Q, Green E, Barnicoat A, et al. Cytogenetic versus DNA diagnosis in routine referrals for fragile X syndrome [see comments]. Lancet. 1993;342(8878):1025-1026.
15. Bhowmik DA, Dutta A, Chatterjee A, Sinha AK, Chattopadhyay A, Mukhopadhyay K. Screening for fragile X syndrome among neurobehavioral patients from Kolkata, Eastern India. J Clin Diagnostic Res. 2009;3(1):1266-1273.
16. Crawford DC, Meadows KL, Newman JL, et al. Prevalence of the fragile X syndrome in African-Americans. Am J Med Genet. 2002;110(3):226-233.
17. Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. Prevalence of carriers of premutation-size alleles of the FMRI gene--and implications for the population genetics of the fragile X syndrome. American journal of human genetics. 1995;57(5):1006-1018.
18. Hantash FM, Goos DM, Crossley B, et al. FMR1 premutation carrier frequency in patients undergoing routine population-based carrier screening: insights into the prevalence of fragile X syndrome, fragile X-associated tremor/ataxia syndrome, and fragile X-associated primary ovarian insufficiency in the United States. Genetics in medicine : official journal of the American College of Medical Genetics. 2011;13(1):39-45.
19. Dombrowski C, Levesque ML, Morel ML, Rouillard P, Morgan K, Rousseau F. Premutation and intermediate-size FMR1 alleles in 10 572 males from the general population: loss of an AGG interruption is a late event in the generation of fragile X

syndrome alleles. Hum Mol Genet. 2002;11(4):371-378.
20. Toledano-Alhadef H, Basel-Vanagaite L, Magal N, et al. Fragile-X Carrier Screening and the Prevalence of Premutation and Full-Mutation Carriers in Israel. American journal of human genetics. 2001;69:351-360.
21. Coffee B, Keith K, Albizua I, et al. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. American journal of human genetics. 2009;85(4):503-514.
22. Faradz SMH, Buckley M, Tang L-P, Leigh D, Holden JJA. Molecular screening for fragile X syndrome among Indonesian children with developmental disability. American journal of medical genetics. Part A. 1999;83(4):350-351.
23. Li SY, Chen YC, Lai TJ, Hsu CY, Wang YC. Molecular and cytogenetic analyses of autism in Taiwan. Hum Genet. 1993;92(5):441-445.
24. Estecio M, Fett-Conte AC, Varella-Garcia M, Fridman C, Silva AE. Molecular and cytogenetic analyses on Brazilian youths with pervasive developmental disorders. J Autism Dev Disord. 2002;32(1):35-41.
25. Elias MH, Ankathil R, Salmi AR, Sudhikaran W, Limprasert P, Zilfalil BA. A new method for FMR1 gene methylation screening by multiplex methylation-specific real-time polymerase chain reaction. Genetic testing and molecular biomarkers. 2011;15(6):387-393.
26. Rajan-Babu IS, Law HY, Yoon CS, Lee CG, Chong SS. Simplified strategy for rapid first-line screening of fragile X syndrome: closed-tube triplet-primed PCR and amplicon melt peak analysis. Expert reviews in molecular medicine. 2015;17:e7.
27. Teo CR, Law HY, Lee CG, Chong SS. Screening for CGG repeat expansion in the FMR1 gene by melting curve analysis of combined 5' and 3' direct triplet-primed PCRs. Clinical chemistry. 2012;58(3):568-579.
28. Lyons JI, Kerr GR, Mueller PW. Fragile X Syndrome: Scientific Background and Screening Technologies. The Journal of molecular diagnostics : JMD. 2015;17(5):463-471.
29. Chandrasekara CH, Wijesundera WS, Perera HN, Chong SS, Rajan-Babu IS. Cascade Screening for Fragile X Syndrome/CGG Repeat Expansions in Children Attending Special Education in Sri Lanka. PloS one. 2015;10(12):e0145537.
30. Chaudhary AG, Hussein IR, Abuzenadah A, et al. Molecular diagnosis of fragile X syndrome using methylation sensitive techniques in a cohort of patients with intellectual disability. Pediatric neurology. 2014;50(4):368-376.
31. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th Edition-Text Revised. Washington DC: American Psychiatric Association; 2000.
32. Sutherland GR. Heritable fragile sites on human chromosomes I. Factors affecting expression in lymphocyte culture. American journal of human genetics. 1979;31(2):125-135.
33. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research. 1988;16:1215.
34. Fu Y-H, Kuhl DPA, Pizzuti A, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the Sherman paradox. Cell. 1991;67(6):1047-1058.
35. Mundhofir FE, Winarni TI, van Bon BW, et al. A cytogenetic study in a large population of intellectually disabled Indonesians. Genetic testing and molecular biomarkers. 2012;16(5):412-417.
36. Winarni TI, Utari A, Mundhofir FE, Hagerman RJ, Faradz SM. Fragile X syndrome: clinical, cytogenetic and molecular screening among autism spectrum disorder children in Indonesia. Clinical genetics. 2013;84(6):577-580.
37. Kaufmann WE, Abrams MT, Chen W, Reiss AL. Genotype, molecular phenotype, and cognitive phenotype: correlations in fragile X syndrome. Am J Med Genet. 1999;83(4):286-295.
38. Hagerman RJ, Amiri K, Cronister A. Fragile X checklist. Am J Med Genet. 1991;38(2-3):283-287.
39. Symons FJ, Clark RD, Hatton DD, Skinner M, Bailey DB, Jr. Self-injurious behavior in young boys with fragile X syndrome. American journal of medical genetics. Part A. 2003;118(2):115-121.
40. Wisbeck JM, Huffman LC, Freund L, Gunnar M, Davis EP, Reiss AL. Cortisol and social stressors in children with fragile X: A pilot study. Journal of developmental and behavioral pediatrics : JDBP. 2000;21:278-282.
41. Hessl D, Glaser B, Dyer-Friedman J, Reiss AL. Social behavior and cortisol reactivity in children with fragile X syndrome. J Child Psychol Psychiatry. 2006;47(6):602-610.
42. Budimirovic DB, Bukelis, I., Cox, C., Gray, R.M., Tierney, E., Kaufmann, W.E. Autism spectrum disorder in Fragile X syndrome:

Differential contribution of adaptive socialization and social withdrawal. American Journal of Medical Genetics Part A. 2006;140A(17):1814-1826.
43. Roberts JE, Weisenfeld LA, Hatton DD, Heath M, Kaufmann WE. Social approach and autistic behavior in children with fragile X syndrome. J Autism Dev Disord. 2007;37(9):1748-1760.
44. Hagerman RJ, Rathmell B, Wang P, et al. Treatment of Fragile X Syndrome with STX209 (arbaclofen): Open-Label Extension Experience. Paper presented at: International Society for Autism Research2012; Toronto, Canada.
45. Hagerman R, Lauterborn J, Au J, Berry-Kravis E. Fragile X Syndrome and Targeted Treatment Trials. In: Denman RB, ed. Modeling Fragile X Syndrome. Vol 54. Berlin Heidelberg: Springer-Verlag; 2012:297-335.
46. Hagerman R, Hoem G, Hagerman P. Fragile X and autism: Intertwined at the molecular level leading to targeted treatments. Mol Autism. 2010;1(1):12.
47. Wang LW, Berry-Kravis E, Hagerman RJ. Fragile X: leading the way for targeted treatments in autism. Neurotherapeutics. 2010;7(3):264-274.
48. Winarni TI, Utari A, Mundhofir FE, Hagerman RJ, Faradz SM. Fragile X Syndrome: Clinical, Cytogenetics and Molecular Screening among Autism Spectrum Disorder Children in Indonesia. Clinical genetics. 2013.
49. Mundhofir FEP, Winarni TI, Nillesen W, et al. Prevalence of fragile X syndrome in males and females in Indonesia. World Journal of Medical Genetics. 2012;2(3):15.
50. Crawford DC, Acuna JM, Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. Genetics in medicine : official journal of the American College of Medical Genetics. 2001;3(5):359-371.
51. Sherman SL, Jacobs PA, Morton NE, et al. Further segregation analysis of the fragile X syndrome with special reference to transmitting males. Hum Genet. 1985;69(4):289-299.
52. Biancalana V, Glaeser D, McQuaid S, Steinbach P. EMQN best practice guidelines for the molecular genetic testing and reporting of fragile X syndrome and other fragile X-associated disorders. European journal of human genetics : EJHG. 2015;23(4):417-425.
53. Monaghan KG, Lyon E, Spector EB, erican College of Medical G, Genomics. ACMG Standards and Guidelines for fragile X testing: a revision to the disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics and Genomics. Genetics in medicine: official journal of the American College of Medical Genetics. 2013;15(7):575-586.