Premutation Allele Combined with Caregiver Distress Factor Increase the Risk of Depression in Fragile X Carriers: Indonesia Setting

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Abstract: This study was done to determine the risk of anxiety and depression symptoms among fragile X premutation carriers. Hamilton anxiety rating scale (HARS) and Hamilton depression rating scale (HDRS) was administered by trained physicians to measure the severity of anxiety and depression symptoms, respectively. Caregiver distress factors which directly contribute to caregiver burden in particular degree of relationship with the child, number of FXS child, child institutional/educational status, number of hours spent providing care per day, and the degree of illness severity were documented. Thirty-one fragile X carriers (27 females, 4 males), aged 32-77 years participated in this study. Only 3.2% had anxiety symptoms, while depression symptoms were identified in 35.5% carriers. Number of hours of providing care/day were significantly associated with depression symptoms (p<0.001). The prevalence ratio (PR) of depression among individuals who had a distress score above cut-off was 3.2 (95% CI= 1.2 to 8.5) compared to those who had a distress score below cut-off with p=0.02. Fragile X premutation carriers are at a greater risk to develop depression symptoms related to the hours spent in caring for children with fragile X syndrome (FXS).

Keywords: Anxiety, depression, fragile X-associated disorders, premutation carriers, caregiver burden.

INTRODUCTION

Fragile X syndrome (FXS) is an X-linked neurodevelopmental disorder associated with intellectual disability (ID) caused by a trinucleotide CGG repeats expansion (>200 repeats) within the promoter region of the fragile X mental retardation 1 (*FMR1*) gene, called the full mutation allele [1]. It is transmitted from female carriers who carry *FMR1* premutation alleles (55-200 CGG repeats) during premeiotic or meiotic division [2, 3]. The prevalence of fragile X premutation carriers varies in population-based studies. Song *et al*., [4] estimate premutation prevalence of 1 in 149 to 643 in females in the general population. Premutation carriers typically do not have ID but they have a risk of having children with FXS. Current studies have demonstrated a high risk for a variety of disorders such as fragile X-associated primary ovarian insufficiency (FXPOI) in females, learning problems such as ADHD, a neurodegenerative disorder (fragile X-associated tremor ataxia syndrome/ FXTAS), and fragile X associated neuropsychiatric disorders (FXAND) [5-7].

Reproductive, neurocognitive, and neurode-generative issues have been investigated in carriers and the cause is related to the elevated *FMR1* mRNA level leading to RNA toxicity [8, 9]. However, emotional and psychopathological phenotypes are still documented inconsistently in various studies, and pathological mechanisms may relate to a variety of factors [10]. Mood, depression and anxiety are the most common psychopathological disorders associated with premutation status and the psychosocial parenting burden [5, 11]. Contributing distress factors such as the size of the CGG repeat, raising a child with FXS, having a close relationship with FXS children, child emotional and behavioural problems, and socio-economic status is associated with the psycho-pathological phenotype [11]. Due to hormonal fluctuations and other factors, females have a greater risk for depression and anxiety compared to males, [12]; in addition the female : male ratio of global major depression is 1.7:1 [13].

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E-ISSN: 2292-2598/19

Diverse pathological mechanisms underlying psychopathology of premutation carriers have been proposed and there are complex interactions among biological, behavioural, and environmental risk factors [14]. First, an abnormal elevation of *FMR1* mRNA was significantly associated with increased psychopathological symptoms, consistent with an RNA toxic gain-of-function mechanism [15]. The *FMR1* mRNA toxicity leads to neuronal cell dysregulation and eventually to intranuclear inclusions formation in the neurons and astrocytes throughout the central nervous system in individuals with FXTAS [16]. The inclusions have been found in the anterior pituitary gland reflect the neuropathological findings that the cortisol response is involved in the psychological problems in premutation carriers [17]. Alterations of hypothalamic-pituitary-adrenal (HPA) axis regulation has also been proven in the premutation mouse [18]. Second, depression and anxiety are complex disorders wherein both genetic and environmental factors play a role simultaneously, but the premutation may influence the onset and severity of depression [19] so that there is a gene-by-environment interaction [5]. Single nucleotide polymorphisms (SNPs) of corticotrophin-releasing hormone receptor 1 (*CRHR1*), which mediates regulation of expression and release of adrenocorticotrophic (ACTH) from the anterior pituitary, will facilitate the stimulation of cortisol release from the adrenal cortex. Cortisol can interact with stress to influence mood and anxiety-related outcomes [20]. In addition to compromising psychological well-being, stressful events such as caring for children with FXS will activate the HPA axis and cortisol release, so it is important to adapt individuals for engagement with the external stressful activity [21]. However, prolonged exposure to the stressor subsequently down-regulates HPA axis [22]. Third, the effect of premutation allele can decrease the size of the hippocampus associated with severity of anxiety experienced by females with a medium-sized premutation allele [23].

This study was aimed to determine the risk of depression and anxiety symptoms among fragile X carriers in the Indonesian population, those carriers who live in a developing country. Our contribution will be valuable to characterize the prevalence of psychopathological symptoms among carriers in Indonesia with a unique environmental background such as strong cultural and religious belief, lower level of educational background, and strong family/social support.

MATERIAL AND METHODS

Participants

Premutation individuals who carry FXS gene were included. Subjects were 31 fragile X premutation carriers (17 females, 4 males) aged 32 – 77 years (Mean 52.5 years, SD 13.4 years). Informed consent was obtained from all subjects who agreed to participate in our study and this study was approved by the Faculty of Medicine Diponegoro University/Dr. Kariadi Hospital Institutional Review Board. A blood sample was obtained from each subject and the premutation status was analyzed as previously described by Southern Blot DNA analysis [24]. Premutation alleles were subdivided into those with low premutation alleles (59–79 repeats), medium premutation alleles (80–100 repeats), and high premutation alleles (>100 repeats) [2]. The caregiver was a family member who had been living with an individual with FXS and closely involved in daily living activities, health care, social interaction for more than a year [25].

Procedures

Premutation individuals fulfilled a demographic questionnaire covered sex, age, marital status, occupational status, and formal education level. Caregiver distress factors were obtained using a semi-structured interview. Relationship with the individual with FXS was defined as a first-degree relative (children, parents and siblings), second-degree relative (aunts, uncles, grandparents, half-siblings, nieces, and nephews), and third-degree relative (cousins, and great-grandparents). Clinical Global Impression-Severity (CGI-S) was used to assess the clinical severity of individuals with FXS. Psychopathological symptoms were assessed using structured interview scales: Hamilton anxiety rating scale (HARS) and Hamilton depression rating scale (HDRS). HARS and HDRS were administered by trained physicians to scale the severity of anxiety and depression symptoms respectively [26, 27].

Psychopathological Assessment

Hamilton Anxiety Rating Scale

HARS is a semi-structured interview to measure the severity of anxiety symptoms and is widely used today in both clinical and research settings. The scale consisted of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental

agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). Each item was scored on a scale of 0 (not present) to 4 (severe). A total score range of 0-56, where 0-17 was indicated normal, 18-24 was indicated mild, 25-29 was indicated moderate, and 25-30 was indicated severe anxiety [26].

Hamilton Depression Rating Scale

HDRS is a semi-structured interview to measure the severity of depression symptoms and is widely used clinician-administered depression assessment scale. This original version was contained 17 items about symptoms of depression experienced over the past week. A total score range of 0-68, where 0-7 was indicated normal, 8-13 was indicated mild, 14-18 was indicated moderate, 19-22 was indicated severe, and >22 was indicated very severe depression [27].

Clinical Global Impression-Severity (CGI-S)

An observer-rated scale was used to measures illness severity (CGI-S) in both clinical and research settings. CGI-S was rated by trained physicians who are dealing with FXS individuals regularly. The illness severity was rated on a 7-point scale from normal-1, borderline ill -2, mildly ill -3, moderately ill -4, markedly ill -5, severely-6, extremely ill-7 [28].

Number of Hours Providing Care/Day

The length of caring was determined by the hours the caregiver spent in charge of caring the children with FXS. The length of caring was divided into two categories: 1) 15 hours/day if the children with FXS was not attending the special school and/or the caregiver did not have other work or a job; 2) 9 hours/day if the children with FXS were educated in a special school daily program and/or the caregiver was occupied by other work.

Caregiver Distress Factors

Five factors that may considerably confer caring burden were: 1) degree of relationship with child; 2) number of FXS child; 3) child institutional/educational status; 4) number of hours spent providing care per day; and 5) the degree of illness severity (assessed using CGI-S) were scored and categorized into above and below cut-off.

Statistical Analysis

The association between sex, marital status, educational background, occupational status, caregiver distress factor, depression, and anxiety symptoms were analyzed by χ^2 test. Fisher's exact test was used if the table contained cells with an expected frequency of less than 5 was more than 25 per cent of total cells. The difference of caregiver distress factor between depression and not depression symptoms was analyzed using the Mann-Whitney test. Since anxiety was only found in 1 subject, the difference was not analyzed. Because the cut off value of caregiver burden score was not available, therefore Receiver Operating Characteristics (ROC) analysis was used to determine the cut-off value. The smallest of distance value was considered as cut off. The accuracy of the ROC analysis was measured by the area under the curve (AUC) which was classified: 90-100 was excellent; 80-90 was good; 70-80 was fair; 60-70 was poor, and 50-60 failed [29]. The risk of depression was expressed as a prevalence ratio according to the cut-off of caregiver distress score. Statistical analysis was performed using SPSS version 16.

RESULTS

Psychopathological symptoms and *FMR1* genotype data for 31 individuals (27 females, 4 males) were included in these analyses. The average of age was 52.4 years (SD=13.42), the range was 32-77 years. Among all premutation individuals who participated in this study, one (3.2%) had anxiety and 11 (35.5%) had depression symptoms. Both anxiety and depression symptoms were identified only in females at reproductive age (41 years and 50.4 years, respectively). The majority of females who had depression were married (63%) Although, occupational status did not have a significant association with depression (p=0.4), the majority of females carrier who had depression were unemployed (90.9%) and one female carrier who had both anxiety and/or depression was also unemployed. There were no significant associations between sex, marital status, educational background and occupational status of fragile X premutation carriers and depression symptoms.

There was no significant association between caregiver distress factors such as premutation size, degree of relationship with the child, number of FXS child, child institutional/educational status and depression symptoms (see Table **1**). Only one distress factor, number of hours providing care/day was significantly associated with depression symptoms (p<0.001). The severity of behaviour problems in children with FXS or CGI-S per se did not significantly contribute to the depression. However, all premutation

Table 1: Contributing Distress Factors Characteristics of the 31 Fragile X Premutation Carriers Associated with Depression

Contributing Distress Factors	Psychiatric Symptoms		*p* value
	Depression (%)	Non Depression (%)	
Premutation Size			
Small Size	4 (36.4)	2 (10)	0.2
Middle Size	3 (27.3)	6 (30)	
Large Size	4 (36.4)	12 (60)	
Degree of relationship with FXS Child:			
First Degree Relatives	9 (81.8)	12 (60)	0.3
Second Degree Relatives	2 (18.2)	4 (20)	
Third Degree Relatives	0 (0.0)	4 (20)	
Number of FXS children:			
No	3 (27.3)	8 (40)	0.4
1	4 (36.4)	9 (45)	
≥2	4 (36.4)	3 (15)	
Child Institutional/educational Status*			
- Not Institutionalized	8 (88.9)	7 (58.3)	0.2
- Institutionalized	1(11.1)	5 (41.7)	
Number of hours providing care /day:**			
15 hours/day	8 (88.9)	1 (9.1)	<0.001
9 hours/day	1 (11.1)	10 (90.9)	

CGG repeat size: Small (55-79); Middle (80-99); Large (100-200).
*Percentage was calculated only on the family (caregiver) who has FXS children.
**Percentage was calculated only on the family (caregiver) who was caring for FXS children. Maximum of caring was 15 hours/day and minimal caring was 9 hours/day. A number of FXS children was not considered in this calculation.

individuals who were caring for FXS children with extremely ill-7, the highest illness severity rate, experienced depression (See Table **2**).

The caregiver distress score was calculated from, 1) degree of relationship with FXS child, 2) number of FXS child, 3) child institutional/educational status, 4) number of hours providing care/day, and 5) illness severity, found significantly (p=0.04) higher in individuals with depression compared to those of without depression (See Figure **1**).

Receiver operating characteristics (ROC) curve analysis shown area under the curve (AUC) of distress

Table 2: The CGI-Severity of FXS Children Related to the Depression Symptoms in Caregivers

CGI-Severity of FXS child	Σ FXS Children	Depression among caregivers		*p*-value
		No	Yes	
Normal-1	0	0 (0.0%)	0 (0.0%)	0.6
Borderline-2	10	6 (16.2%)	4 (10.8%)	
Mildly-3	6	4 (10.8%)	2 (5.4%)	
Moderately-4	2	1 (2.7%)	1 (2.7%)	
Markedly-5	12	8 (21.6%)	4 (10.8%)	
Severely-6	5	3 (8.1%)	2 (5.4%)	
Extremely-7	2	0 (0.0%)	2 (5.4%)	
Total	37*	21	16	

*Four caregivers have ≥ 2 children with FXS.

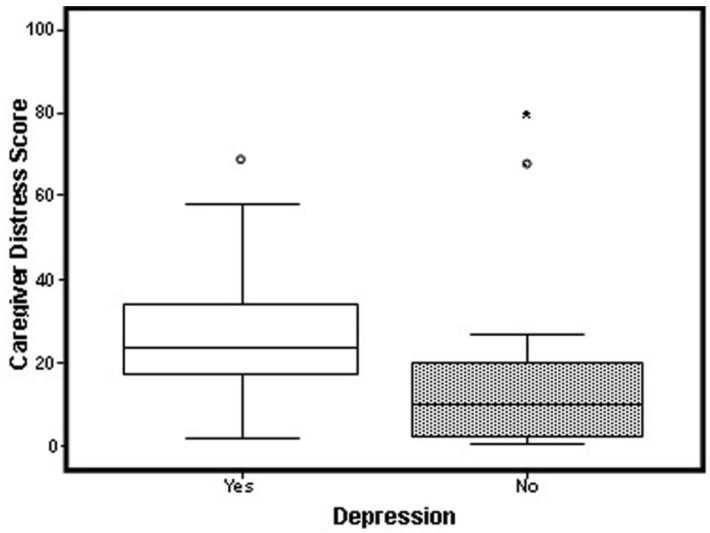

Figure 1: Boxplot diagram the risk of caregiver distress score and depression with cut off value of 21.

The caregiver distress score among premutation carriers who had depression (above 21) and without depression (below 21). SD depression group (no-fill boxplot); SD non-depression group (pattern boxplot).

Table 3: The Association between Caregiver Distress Score and Depression among Premutation Carriers

Category of caregiver distress score	Depression		Total (%)
	Yes n (%)	No n (%)	
≥21	7 (63.6)	4 (20)	11 (35.5)
≤21	4 (36.4)	16 (80)	20 (64.5)
Total	11 (100)	20 (100)	31 (100)

p=0.02; χ^2 test.
Prevalence ratio= 3.2 (95% CI= 1.2 to 8.5).

factor to determine the cut-off of the risk to develop depression was 0.73 (95% CI: 0.55 to 0.91, p=0.04), was categorized fair. ROC curve analysis found the cut-off value of distress score was 21. Caregiver distress factors were categorized and analyzed to determine the risk of depression. There was a significant (p=0.02) association between caregiver distress score category (above or below cut-off) with the presence of depression (see Table **3**). The prevalence ratio of individuals who had a caregiver distress score above cut-off was 3.2 (95% CI= 1.2 to 8.5).

DISCUSSIONS

Anxiety Symptoms

In this study, anxiety symptoms were not prevalent in female carriers (3.2%). This result is similar to the previous well-matched premutation and non-premutation controlled group study [30]. In contrast, several more recent studies have found an increased risk of anxiety symptoms in premutation carriers [11]. Bourgeois *et al*. report an increased prevalence of anxiety and mood disorders among premutation especially in individual with FXTAS, become prominent when movement symptoms become noticeable [31]. This finding supports the evidence that anxiety is associated with acute factors and life stressors such as anticipation of threat and integrity of body or self [32]. Anxiety has also been reported more frequent in several chronic medical illnesses [33]. Interestingly, when compared to those of in developing countries, our result is similar to WHO World Mental Health (WMH) epidemiological surveys, lifetime social anxiety disorders (SAD) was found only 2.1% in 11 developing countries [34]. Therefore, we conclude that there is no increased risk of anxiety symptoms among fragile X premutation carriers in Indonesia in this study.

Religiosity and spiritual factors are increasingly being considered in psychiatric disorders. Recent studies have identified that religiosity may serve as a psychological and social resource for coping with

stress. Religious beliefs and practices can represent powerful sources of comfort, hope, and meaning, beneficent preventing and supportive treatment in anxiety and other psychiatric disorders [35]. According to the Asian Development Bank, Indonesia is a middle-income developing country with the highest religious involvement among the population in Asia-Pacific countries [36]. This factor may influence the prevention of anxiety in Indonesia. Another privileged factor for people who live in a rural area, where our study participants mostly live, is a supportive social atmosphere compared to people who live in an urban area. Social/family support has generally been found to be inversely related to depression and anxiety [37].

Depression Symptoms

The prevalence of depression among the general population varies widely worldwide; in developed countries, the number is higher compared to a developing country [38]. The prevalence of depression symptoms seems relatively more common compared to clinical depression and females are at greater risk (1.5 to 2 times) than males [39]. The prevalence of depression in a developing country such as India (12.1%), China (16.5%), Malaysia (17.5%), Korea (19.4%), and Taiwan (19.9%) is relatively lower compared to developed countries [40]. Depression symptoms in this study were found in 35.5% of fragile X premutation carriers, it is higher compared to the general population and similar to previous reports of premutation carriers [11].

Elevated *FMR1* mRNA is one of the pathological mechanisms that may responsible for the genetic vulnerability of premutation carriers to develop psychopathological conditions. The association of premutation size with psychopathological disorders shows varying results, Johnston *et al.* reported there is positive association between depression and higher repeat size (>100 CGG repeats) in which translation of the FMRP level is less efficient, [41] although Seltzer and colleagues found a curvilinear association between CGG repeat length (mid-size repeat in a higher risk) and depression [32]. In another study by Hunter and colleagues, an association between psychopathology and CGG repeat size was not found, [42] which is the case in our study. Further investigation is warranted to explore specific markers associated with the risk of psychological disorders including the level of FMRP as suggested by Hessl *et al.* and the level of elevated *FMR1* mRNA [43].

Caregiver distress factor was one of the main factors that correlated with and predicted caregiver depressive symptoms [44]. Caregiver distress factors including the degree of relationship with FXS child, number of FXS child, the child institutional/educational status, the number of hours providing care /day, and illness severity had been analyzed in association with the depression. Only a number of hours providing care/day was significantly associated with depression symptoms (p<0.001). Although, behaviour problems in children with FXS per se did not significantly contribute to the depression, however, all individuals who were caring for children with extremely ill-7 experienced depression. Wheeler *et al*. [45] showed child behavioral and emotional problems play a role in an increased stress level and a decreased caregiver quality of life. Further analysis had been done to explore the association between caregiver distress score category (cut-off point=21) with depression and found that the higher distress score was significantly associated with depression (p=0.02). Caregiver distress refers to the level of stress that may be experienced by premutation individuals who were caring FXS children. Experiences with stressful event in a daily life will activate the HPA axis to release cortisol in the morning (cortical awakening response/CAR) in order to help the body to adapt/face the stressful situation [21]. If the stressful event occurred in a chronic manner, the HPA axis will be down-regulated and may result in difficulty in responding to the ordinary stressful challenge, [46] hence, premutation carriers who experience significant levels of stress are more vulnerable to develop psychopathological symptoms compared to those who do not have a high level of stress [15].

Study Limitation

We did not measure FMRP levels, *FMR1* mRNA levels, nor the activation ratio (AR) for the premutation carriers which would have given us more depth in understanding the molecular variables leading to depression. The result did not take into account the socio-economic status (SES) directly that may play a role in the development of anxiety and depression disorders. However, those with a higher SES would have been more likely to have help in caring for their children which was considered in our analysis. The SES may have significant impact in this study especially in Indonesia (middle-income country) where SES is quite different compared to developed countries, where the emotional and psychological profile of fragile X premutation carriers have been reported. Social support, a supportive social

environment may also be an important protective factor in our lowered anxiety rates, although it did not protect from a significant rate of depression. An important limitation is the lack of a control group of age matched non carriers from the same regions as the premutation carriers. This control group would have helped us evaluate the cultural protections and whether psychopathology is indeed increased in premutation women compared to controls in Indonesia.

Future Directions

Fragile X premutation carriers are at a greater risk to develop depression vs. anxiety symptoms perhaps related to the stress of caring for a child with FXS. Based on this result, diagnosis of psychopathological morbidity especially depression among caregivers of FXS who carry premutation allele should be handled and supported by a healthcare professional because of depression among caregiver results in reduction caregiver ability and directly associated with a high level of child dysfunction.

ACKNOWLEDGEMENT

We thank the family who participated in this study. We all address our appreciation to Cytogenetic and Molecular Unit Centre for Biomedical Research, Faculty of Medicine Diponegoro University (FMDU), and Semarang, Indonesia especially Lusi, Wiwik, Nanik, Rita for their help with laboratory work. We thank Hardian, MD PhD from the Faculty of Medicine Diponegoro University for his help with data analyzing. We appreciate Costrie Gannes, MSc who give great support in the preparation of psychopathological tools and its interpretation.

This work was supported by Competitive Research-DIPA PNBP Diponegoro University No. 259.12/UN7.5/ 2012 and PUPT Kemenristek Dikti Grant No.176-13/UN7.5.1/PG/2016

CONFLICT OF INTEREST

All authors declare there is no conflict of interest.

ABBREVIATIONS

CAR = cortisol awakening response

CGI-S = clinical global impression-severity

ID = intellectual disabilities

FMRP = fragile X mental retardation protein

FXPOI = fragile X associated premature ovarian failure

FXS = fragile X syndrome

FXAND = fragile X associated neuropsychiatric disorders

FXTAS = fragile X associated tremor ataxia syndrome

HARS = Hamilton anxiety rating scale

HDRS = Hamilton depression rating scale

HPA = hypothalamus-pituitary adrenal

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Received on 05-08-2019 Accepted on 06-09-2019 Published on 18-11-2019

DOI: https://doi.org/10.6000/2292-2598.2019.07.04.1