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Relationship between panic disorder and plasma neuropeptide-S level

Hayriye Baykan¹, Özgür Baykan¹, Emre C. Esen¹, Hayrettin Kara¹, Adnan A. Hişmioğullari¹, Tunay Karlidere¹

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Abstract

Background: Panic disorder has long been associated with the changes in various neurotransmitters, such as Neuropeptide-S (NPS). Objective: In this study we aimed to determine whether there is a relationship between blood NPS levels and panic disorder. Methods: Twenty nine patients with panic disorder and thirty two healthy control subjects who were age and gender matched were enrolled to the study. Blood samples were taken from participants and plasma NPS levels were quantified by using an ELISA kit. Results: In the study group, median NPS blood level was 16.7 pg/mL and in the control group it was 32.5 pg/mL. There was a statistically significant difference (p = 0.021). Using receiver operating characteristics (ROC) curve, sensitivity and specificity of NPS blood level, for diagnosing panic disorder was calculated, and it was found 79.3% and 56.25% respectively (AUC:0.672, 95% CI: 0.540-0.787). Discussion: Malfunction at the NPS modulatory system in the cortical areas (which is causing excitations in brain areas, such as amygdala and hypothalamus) does not only increase anxiety symptoms and risk of panic disorder but also causes panic disorder patients to have lower plasma NPS levels than the control group. Therefore it can be argued that such malfunction can be treated with a systemic treatment.

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Keywords: Panic disorder, neuropeptide-S, anxiety disorder.

Introduction

A panic attack is a sudden and unexpected rush of immense anxiety. It has a lifetime prevalence of 13.1%. Not every patient who had a panic attack can be diagnosed with panic disorder. According to Diagnostic and Statistical Manual; a patient experiencing panic attacks can only be diagnosed as the panic disorder if that person is experiencing persistent anxiety about having more panic attacks and/or if these attacks are causing significant maladaptive changes in the behavior. Although two-thirds of the people who have panic attacks, experience them recurrently; only around one-sixth of them are eligible for panic disorder diagnosis.

Panic disorder has long been associated with various neurotransmitter signaling changes. In brief, there is a reduction of inhibitory signaling in the central nervous system among panic disorder patients. This postulate is backed up by the studies showing an increased function in glutamatergic neurons and decreased function in GABAergic neurons. There are also other monoamines (serotonin and noradrenaline) and neuropeptides (cholecystokinin) which have been associated with panic disorder as well.

Neuropeptide-S (NPS) was discovered around two decades ago. NPS has been shown to have several physiological roles; such as sleep regulation, modulation of feeding behavior, activation of the hypothalamic-pituitary-adrenal axis and adrenocorticotrophic hormone (ACTH) release; extinguishing anxiety and conditioned fear responses⁵. Animal studies have shown that intranasal⁶-⁸ and intracerebroventricular⁹-¹⁰ NPS injections were able to reduce symptoms associated with anxiety and panic disorder. Also, Neuropeptide-S Receptor (NPSR) polymorphism has been shown to increase the risk of panic disorder both in animal and human studies⁵,¹⁰-¹⁸. However, there has been no study that examines the relationship between blood NPS levels and panic disorder up till now.

In this study, we aimed to determine whether there is a relationship between blood NPS levels and panic disorder. To examine this hypothesis, we quantified the plasma NPS levels of panic disorder patients and healthy controls.

Methods

Subjects

We have designed our research as a case-control study. After getting an ethics committee approval we have prospectively enrolled participants to our study from January 2015 until December 2015. All the patients admitted to the Balıkesir University Psychiatry outpatient service during 2015, who were diagnosed with panic disorder (either with or without agoraphobia) for the first time and who were not using any psychotropic or beta blocker drugs at the time of their admission, were asked to enroll to our study.

All the subjects, both the participants of study and control group, underwent structured interview of SCID (Structured Clinical Interview for DSM-IV). Medical history of all the participants were self declared. Their drug history were verified with the Republic of Turkey Social Security Institution’s online pharmaceutical database.

Participants of the study group were excluded if they were given any other psychiatric diagnosis other than panic disorder. Additionally, participants of the control group were excluded from the study if any psychiatric diagnosis was given. Using any psychotropic or beta blocker drug was an exclusion criterion for all subjects.

Twenty-nine panic disorder patients who met criteria mentioned above agreed to participate in our study and gave a written informed consent. Thirty-two, age and gender matched healthy volunteers were enrolled to the study for the control group. This research was approved by the Balıkesir University School of Medicine Clinical Research Ethics Committee on 10/01/2014 with the approval number 2014/03.

Psychiatric scales

All participants were asked to fill the Panic and Agoraphobia Scale (PAS). PAS is a self-rated Likert type scale in which total scores can vary between 0 and 52. PAS measures panic attacks,
agoraphobia, anticipatory anxiety, disability, and worries among panic attack patients, either with or without agoraphobia. Even though PAS is not intended as a diagnostic instrument, it is a convenient tool to separate panic disorder patients from healthy individuals. Validity and reliability of the Turkish version of PAS has been established19.

Blood samples

Blood samples were taken from participants to determine the blood level of NPS. Fasting venous blood samples were extracted and were collected in EDTA tubes (Becton, Dickinson, USA). Samples were centrifuged at 1300 ×g for 10 minutes for plasma separation. Plasma samples and 1% concentrated protease inhibitor cocktail (Sigma Aldrich product number: P8340) were mixed in Eppendorf tubes. These mixtures were stored at -20°C while waiting for the further processing. NPS was quantified by using a commercial Human NPS (Neuropeptide S) ELISA kit (Wuhan Fine Biological Technology Company Ltd., China).

Statistics

All statistic analyses were made on SPSS version 15.0 (SPSS, Chicago, IL, USA). To determine the sensitivity and specificity of the blood level of NPS for diagnosing panic disorder ROC curve was made an area under the curve was calculated.

Results

Mean age of the study and control groups were 38.6 ± 9.0 years and 39.3 ± 9.3 years respectively. There was no statistically significant difference between the two groups (p = 0.768).

The panic disorder group consisted 71.9% of females whereas control group consisted 86.2% of females. There was no statistically significant difference regarding the gender between two groups (p = 0.172).

In the panic disorder group, median NPS plasma level was 16.7 pg/mL, with a minimum of 3.7 and a maximum of 99.3. In the control group, median NPS blood level was 32.5 pg/mL, with a minimum of 3 and maximum of 156.1. A statistically significant difference was observed between the two groups (p = 0.021) (Figure 1). There was not a statistically significant correlation between PAS scores and blood NPS levels (rs = -0.232, p = 0.072). Optimal cut off score for NPS blood level was found 18.28 with the receiver operating characteristics (ROC) curve. Using that cut-off score; sensitivity and specificity of NPS blood level were calculated for diagnosing panic disorder. The results were 79.3% and 56.25% respectively (AUC: 0.672, specificity of NPS blood level were calculated for diagnosing panic disorder ROC curve was made an area under the curve was calculated.

Discussion

NPS is a, recently discovered, neurotransmitter which consists of 20 amino acids. Since NPS has been uncovered, it has been associated with different physiological systems and attributed to various functions such as sleep regulation, modulation of feeding behavior, activation of the hypothalamic-pituitary-adrenal axis and release of adrenocorticotropic hormone (ACTH); extinguishing anxiety and conditioned fear responses3-5. Even though expression of NPS is mostly limited to brainstem areas, mRNA of the NPS receptor (NPSR) has been demonstrated to be widely expressed in various brain regions, including olfactory regions and amygdala, hippocampal formation, and hypothalamus.

At the first glance, the data on the relationship between NPS and panic disorder can give the impression that it is contradictory. On the one hand, NPS has crucial functions in extinguishing anxiety and conditioned fear responses3-5, and administration of NPS (both intranasally and intracerebroventricularly) has anxiolytic properties6-10. On the other hand, NPS has been shown to activate hypothalamic-pituitary-adrenal axis and increases the release of ACTH15. NPSR is mainly found on top of investigating similar polymorphisms mentioned earlier.

The modulatory function of the NPS and NPSR explains the findings mentioned in the previous paragraphs3-5. NPSR is mainly found on glutamatergic neurons, which are excitatory. However, these glutamatergic neurons mostly innervate GABAergic interneurons, especially in the amygdala. Overall NPSR activation causes inhibition in brain areas such as the amygdala. Therefore NPS has a modulatory role rather than a straightforward inhibitory or excitatory function3,5,10,11.

The neuroimaging studies support the discussions mentioned above. Human neuroimaging studies done by IMRI demonstrated that subjects who carried a mutated T allele for the rs324981 polymorphism in NPSR-1 gene showed increased activity in especially amygdala and prefrontal cortex. This increased activity is thought to cause a higher risk for anxiety and panic disorders13-17.

So far there has not been any study which investigated the relationship between plasma NPS levels and panic disorder. Most of the human studies on the subject looked into polymorphisms in the genes which are coding NPS and NPSR. Moreover, animal studies quantified NPS and NPSR mRNA in the brain tissue of mice and rats, on top of investigating similar polymorphisms mentioned earlier. However, our study brings forward a new approach to the subject.

Our hypothesis was, there would be a statistically significant difference in plasma NPS levels between panic disorder and control group. As hypothesized, possibly due to a malfunction at the NPS
modulatory system in the cortical areas, panic disorder patients had lower plasma NPS levels than the control group. It can be argued that such malfunctions can be treated with a systemic treatment. Hence intranasal administration of NPS was found to have anxiolytic properties, as it was mentioned previously.

Although there was a statistically significant difference between two groups in plasma NPS level, there was no relevant relationship between the PAS scores and blood NPS levels. These results can be caused by the size of our study. Moreover, PAS has five sub-scores but a total of 14 questions, which decreases the reliability of the test and caused the results reached.

Major limitation of our study was our small sample size which may have resulted in decreased statistical significance in our results. Moreover, small sample size limited our ability to obtain gender specific results. Although among female participants there was a statistically significant difference of plasma NPS levels between subject and control groups, there was no such difference among male participants. From the total of 61 participants, only 13 being men may have caused this result.

Furthermore, one blood sample was taken at the first admission to our outpatient service. Obtaining more samples from participants, including after sufficient treatment, can give us a better opinion on the trajectory of NPS throughout the progress and resolution of the disorder. Even though PAS is an established measurement of various sub-scales mentioned in the methods section; future researches can enhance study the design by including other panic or anxiety scales, such as Hamilton Anxiety Rating Scale and Panic Disorder Severity Scale.

In conclusion, NPS plasma levels of the panic disorder patients are found to be significantly lower than the control group. This result can be interpreted as a possible malfunction at the modulatory NPS system in panic disorder patients which causes NPS levels to be lower than the healthy population. Impaired NPS production and therefore function are producing excitations in brain areas, such as the amygdala, which results in anxiety symptoms and increased risk of panic disorder. Our findings are in line with the previous studies and take us a step closer to comprehending the roles of NPS in panic disorder and its possible use for the treatment of the disease.

Acknowledgements

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References


Living with the elderly is related to a better performance in the recognition of facial expressions of emotion among older individuals

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Abstract

Background: Cohabitation with the elderly may bring benefits to social relationships and exert an influence on the recognition of facial expressions of emotion.

Objective: Compare emotion recognition skills between a cohabitation group (CHG) of older adults who live with a dependent elderly individual and a non-cohabitation group (NCHG) of older adults who do not live with an elderly individual.

Methods: Interviews were conducted with 62 older adults in the CHG and 56 in the NCHG. The two groups were similar in terms of gender, age, schooling, degree of dependence, cognitive performance, and depressive symptoms. A dynamic task with six emotions (anger, disgust, happiness, surprise, sadness, and fear) and four levels of intensity was administered to evaluate the recognition of facial emotions.

Results: The CHG performed better than the NCHG regarding the correct identification of emotions, specifically surprise (60%), disgust (60%, 80%, and 100%), fear (80%), and sadness (80% and 100%).

Discussion: Cohabitation with an elderly individual seems to offer benefits to older adults in terms of recognizing facial expressions of emotion.

Keywords: Facial recognition, emotions, cognition, aged.
compare emotion recognition skills between older adults who live with an elderly individual and older adults who did not live with an elderly individual.

We hypothesized that the cohabitation group would recognize facial expressions of emotion more accurately than the non-cohabitation group, regardless of the emotion. When an elderly person lives with another elderly person, especially when care is required, the ability to recognize facial expressions of emotion is necessary more often.

It is important to identify groups that are more likely to have exhibit greater accuracy in terms of the recognition of emotions based on facial expressions in order to design interventions aimed at enhancing this ability in more disadvantaged groups.

Methods

Participants

The sample consisted of older adults registered at primary healthcare services in the city of São Carlos, which is located in the southeastern region of Brazil. The city has an estimated population of 243,765 residents (2016 estimate).

Two groups were formed: a cohabitation group (CHG) of older adults who lived with elderly individuals and a non-cohabitation group (NCHG) of older adults who did not live with other elderly individuals. The inclusion criteria for the CHG group were 1) 60 years of age and older, 2) registry at a primary healthcare service in the city, 3) normal or corrected-to-normal vision, 4) living with an elderly individual at home, and 5) being the most independent elderly person living at home. Independence was defined as requiring less assistance on basic or instrumental activities of daily living, which was assessed using the Katz Index15 and Lawton and Brody’s Scale16, respectively. For the NCHG group, the inclusion criteria were 1) 60 years of age and older, 2) registry at a primary healthcare service in the city, 3) normal or corrected-to-normal vision, 4) not living with other elderly person (age ≥ 60 years) in the same home, and 5) being as independent as the individuals selected for CHG. The exclusion criteria for both groups were uncorrected self-reported visual deficits or a self-reported neurological disorder. We chose the most independent elderly person in the household considering that elderly people in the NCHG would be comprised of more independent individuals. This was confirmed by the similar scores on the scales of instrumental and basic activities of daily living.

The sample size was calculated based on a pilot study conducted with nine individuals meeting the criteria for the CHG and nine meeting the criteria for the NCHG registered at primary healthcare services. Considering mean and standard deviation values in the pilot sample, a 5% level of significance (alpha = 0.05), and 80% power, a minimum of 102 participants (n = 51 in the CHG and n = 51 in the NCHG) would be representative for comparisons between the two groups. To compensate for a possible 20% dropout rate, a convenience sample of 65 participants was selected for each group. The participants were from different areas of the city. Three were excluded due to visual deficits, two were excluded due to neurological disorders and seven did not complete the task. Thus, final sample consisted of 118 community-dwelling older adults (n = 62 in the CHG and n = 56 in the NCHG). Figure 1 displays the flowchart of the sample selection process.

Data collection

All participants signed a statement of informed consent. This study received approval from the ethics committee of the Federal University of São Carlos, Brazil.

The following data were collected between May 2016 and March 2017:

- Socio-demographic characteristics: gender, age (continuous), marital status (with or without a partner), schooling (continuous), personal income (in Brazilian currency, continuous), and number of residents in the home (continuous).
- Dependence level: the Katz Index15 was used to evaluate the performance on basic activities of daily living (possible range 0-6, with higher scores indicating greater dependence) and the Lawton and Brody Scale16 was used to evaluate the degree of dependence with regard to instrumental activities of daily living (possible range 7-21, with higher scores indicating less dependence).
- Cognitive status: Addenbrooke’s Cognitive Examination-Revised (ACE-R) was used for the assessment of cognitive status, which addresses five domains – attention & orientation, memory, fluency, language and visuospatial ability. The total score ranges from zero to 100, with higher scores representing better cognitive status (continuous)17.
- Depressive symptoms: the 15-item Geriatric Depression Scale (GDS)18 was administered. The final score ranges from zero to 15, with higher scores indicating more depressive symptoms (continuous).
- Recognition of emotions: the Emotion Recognition Task (ERT) was employed, which is a computer-assisted test with video clips of images that morph from a neutral face to different intensities of a given facial expression. The participant sees four Caucasian young adults (two men and two women) expressing six emotions (anger, disgust, happiness, surprise, sadness, and fear) at four different emotional intensities (0-40%, 0-60%, 0-80%, and 0-100%) and chooses from among the six response options offered. The length of each video depends on the emotional intensity and ranges from approximately 1 second (40%) to 3 seconds (100%). The face remains on the screen until the respondent chooses an answer. The instructions and verbal labels of the six emotions were read to illiterate participants. The participant enunciated the emotion and the examiner clicked on the corresponding response. There was no time limit for responding (the next face only appeared when the previous response was given). The presentation starts with lower intensities and then proceeds to higher intensities. Three practice trials were given to each participant before the test. The ERT was displayed on a 14-inch computer screen. The sum of correct answers determines the final score and ranges from 0 to 96, which corresponds to the number of faces displayed. It is also possible to calculate the score for each emotion (range: 0 to 16) and each level of intensity (range: 0 to 24)19.
- Cohabitation characteristics (only for the CHG): the question focused on who was the other elderly individual at home (spouse or other). We also asked whether the participant cared for the elderly person who lived in the same home and, if so, how many hours were spent per day on care-related activities regarding the other individual.

Figure 1. Flowchart of sample selection, São Carlos, Brazil, 2017.
Data analysis

The SAS program (version 9.2 for Windows) was used for the analysis. The data were expressed as absolute frequency, percentage, mean, standard deviation, and median. The chi-square test was used to compare the categorical variables between the two groups and the Mann-Whitney test was used to compare the ranking on numerical variables, which did not exhibit normal distribution. Repeated-measures ANOVA was used to compare the ERT scores between the two groups (CHG X NCHG), six emotions (anger x disgust x happiness x sadness x surprise x fear) and four intensities (40% x 60% x 80% x 100%). Tukey’s post hoc test was used for comparisons between groups and profile tests by contrasts were used for comparisons within subjects for the six emotions and four intensities. For such, the variables were transformed into ranks due to the non-normal distribution. The significance level was set at 5% (p ≤ 0.05).

Results

Table 1 shows the characteristics of the participants. The groups were similar regarding gender, age, schooling, degree of dependence, ACE-R and GDS. The CHG had more participants living with a partner, lower personal income and more residents living in the home, which was expected due to the inclusion criteria.

Table 2 displays the mean and standard deviation values of correct responses for each level of intensity and each of the six emotions.

---

Table 1. Descriptive characteristics of older caregivers (n = 62) and older non-caregivers (n = 56), São Carlos, Brazil, 2016/2017

<table>
<thead>
<tr>
<th></th>
<th>CHG (n = 62)</th>
<th>NCHG (n = 56)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (Female)</strong></td>
<td>91.9%</td>
<td>82.1%</td>
<td>0.11</td>
</tr>
<tr>
<td>Age, years (mean ± SD)**</td>
<td>69.7 ± 5.5</td>
<td>70.1 ± 6.7</td>
<td>0.88</td>
</tr>
<tr>
<td>Schooling, years (mean ± SD)**</td>
<td>4.1 ± 3.5</td>
<td>3.2 ± 2.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Marital status (with partner)**</td>
<td>85.5%</td>
<td>12.5%</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Personal income, R$ (mean ± SD)**</td>
<td>1020.5 ± 897.6</td>
<td>1312.1 ± 671.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Number of residents in home (mean ± SD)**</td>
<td>3.1 ± 1.6</td>
<td>2.4 ± 1.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Katz Index (mean ± SD)**</td>
<td>0.11 ± 0.3</td>
<td>0.16 ± 0.4</td>
<td>0.45</td>
</tr>
<tr>
<td>Lawton and Brody’s Scale (mean ± SD)**</td>
<td>19.9 ± 1.5</td>
<td>19.2 ± 2.5</td>
<td>0.19</td>
</tr>
<tr>
<td>ACE-R**</td>
<td>64.5 ± 15.2</td>
<td>63.1 ± 17.8</td>
<td>0.66</td>
</tr>
<tr>
<td>GDS**</td>
<td>3.8 ± 2.6</td>
<td>3.0 ± 2.7</td>
<td>0.88</td>
</tr>
<tr>
<td>Care recipient (spouse)</td>
<td>85.5%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hours/day in care activities (mean ± SD)</td>
<td>5.8 ± 4.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Chi-square test; ** Mann-Whitney test.
SD: standard deviation; CHG: cohabitation group; NCHG: non-cohabitation group; ACE-R: Addenbrooke’s Cognitive Examination-Revised; GDS: Geriatric Depression Scale.

Table 2. Mean and standard deviation values of correct responses for each level of intensity, each of the six emotions, and total score according to group, São Carlos, Brazil, 2016/2017

<table>
<thead>
<tr>
<th></th>
<th>CHG (n = 62)</th>
<th>NCHG (n = 56)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Level of intensity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40%</td>
<td>9.7 ± 2.9</td>
<td>8.7 ± 2.4</td>
<td>0.03</td>
</tr>
<tr>
<td>60%</td>
<td>13.2 ± 2.6</td>
<td>11.1 ± 3.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>80%</td>
<td>14.4 ± 2.5</td>
<td>12.3 ± 3.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>100%</td>
<td>14.7 ± 2.7</td>
<td>12.8 ± 3.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Emotion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Happiness</td>
<td>13.6 ± 2.1</td>
<td>13.4 ± 2.3</td>
<td>0.91</td>
</tr>
<tr>
<td>Surprise</td>
<td>6.6 ± 3.1</td>
<td>5.1 ± 3.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Disgust</td>
<td>11.0 ± 3.0</td>
<td>9.0 ± 4.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Fear</td>
<td>4.0 ± 2.6</td>
<td>3.0 ± 2.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Anger</td>
<td>11.4 ± 2.7</td>
<td>10.5 ± 2.9</td>
<td>0.14</td>
</tr>
<tr>
<td>Sadness</td>
<td>5.4 ± 3.2</td>
<td>3.9 ± 3.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>52.0 ± 8.7</td>
<td>45.0 ± 10.3</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

CHG: cohabitation group; NCHG: non-cohabitation group; SD: standard deviation.
A significant interaction was found between the type of emotion and level of intensity \( [F(15,1740) = 14.75; p < 0.001] \), as the difference between the levels of intensity depended on the type of emotion and vice versa. For example, for the intensity of 100%, differences were found among fear, happiness, sadness, and surprise, with poorer results regarding the recognition of fear. On this intensity level, no differences were found between fear and anger or fear and disgust. No significant differences among intensity levels were found regarding fear. On the other hand, differences among intensity levels were clear regarding fear (40% < 60% < 80% < 100%).

**Discussion**

The purpose of this study was to investigate whether older adults living with a dependent elderly individual \((n = 62)\) differ from older adults not living with an elderly individual \((n = 56)\) on a dynamic emotion recognition task with six emotions and four levels of intensity. The findings demonstrated the following: 1) the CHG performed better than the NCHG regarding the correct identification of emotions (52 ± 8.7 and 45 ± 10.3, respectively); 2) the differences were specifically with regard to surprise (60%), disgust (60%, 80%, and 100%), fear (80%), and sadness (80% and 100%); 3) the two groups performed similarly with regard to recognizing happiness and anger; 4) no significant interaction among group, type of emotion, and intensity was found; 5) happiness was identified the most accurately and fear was identified the least accurately in both groups; 6) emotions expressed at 40% intensity were more difficult to recognize; and 7) a significant interaction was found between type of emotion and level of intensity.

Previous research has shown that the recognition of facial expressions of emotion is more accurate with regard to happiness\(^{5,2,4,19,20}\). The emotion least accurately recognized varies among studies, but the most cited are fear\(^{5,2,21}\), sadness\(^{19,20}\), and anger\(^{21}\). Moreover, investigations have demonstrated that lower levels of intensity are more difficult to identify\(^{2,19,22}\). These findings are consistent with the present results. The fact that expressions of lower intensity are more difficult to identify may be the reason why the difference between the groups only appeared for emotions of greater intensity.
The majority of studies evaluating emotion recognition have sought to identify differences in accuracy between adults and elderly groups or across ages. The aim of some studies was to identify differences between groups with and without neurological diseases. Only older adults were included in the present study and were divided into those who lived with an elderly individual (cohabitation group) and those who did not (non-cohabitation group).

A normative study involving 373 healthy participants aged eight to 75 years used the ERT and found the following mean scores for each emotion in the elderly group (65 to 75 years of age): happiness (13.7), disgust (18.9), anger (9.8), surprise (7.7), sadness (5.3), fear (4.6), and total (51.9). These scores are similar to the scores achieved in the CHG, but the individuals in the present study had a lower level of schooling and the number of participants was larger.

Studies show that older adults are less accurate with regard to the recognition of some emotions, specifically negative emotions, such as fear, sadness, and anger. In the present study, the CHG was better at recognizing fear and sadness than the NCHG, which may mean that living with an elderly individual at home leads to more opportunities for an older adult to recognize emotions based on facial expressions.

Ageing is often associated with a smaller social network. However, interactions with remaining social partners are rated as more satisfying than in younger adults. This is explained by the optimization of positive relationships, avoidance of potential conflicts, social expertise, and other aspects, such as the contributions of the social partners. Even when negative social exchanges occur, older adults tend to have ways to minimize the consequences of such exchanges. Living with a dependent elderly person is a situation that can cause stress and confrontations with negativity. Therefore, the individuals in the CHG may be required to perceive negative emotions more often than those in the NCHG. Therefore, one possible explanation for the present findings is that cohabitation leads to an increased ability to recognize negative emotions in order to minimize their consequences. Furthermore, a caregiver’s sense of self-efficacy contributes to the development of meaning in the experience of care, reinforces the positive aspects of caregiving, and is associated with enhanced motivation. A previous study found that motivation is an important aspect of the perception of emotions, that is, experimenter-provided motivation was found to eliminate age differences in the recognition of facial expressions of emotion.

Disgust is an emotion that is reported to be recognized better with age and may be even better recognized by older adults in the role of caregiver, as demonstrated by the present results. Cohabitation and providing care may lead to an improvement in the recognition of this specific emotion. Evidence suggests that older adults who receive care experience feelings of self-disgust, which can be expressed in the face. However, it seems that this feeling diminishes over time and is related to the use of coping strategies and caregiver characteristics. Daily contact can enhance the ability to recognize the facial expression of disgust and then employ strategies to control the situation. This can help older adults, who become more accurate in recognizing this specific emotion, and also the care recipient, who experiences lower levels of self-disgust.

No difference between groups was found regarding anger, which suggests that cohabitation with elderly individuals does not help to recover deficits in the recognition of this emotion. The same occurred for happiness, which was the emotion that both groups correctly identified the most. According to previous studies, the recognition of happiness has a ceiling effect.

Despite the results, we should highlight the significant correlation found in the overall sample between the performance on the ERT and the number of residents in the home as well as the significant difference in the comparison between ERT scores and marital status.

The mean number of residents in the home differed between groups (3.1 in the CHG and 2.4 in the NCHG) and this could be a possible explanation for the differences found. Studies show that deficits in the recognition facial expressions of emotion are associated with social isolation and difficulties in social interactions. Therefore, living with more people may have influenced the effect found in the CHG. However, a previous study found that one’s social network as well as exercise, smoking, and a healthy diet had no influence on emotion recognition among older adults. It is possible that the number of people in the social network is not the only aspect that matters and that age group and the exchange of support between individuals are equally important. These interactions should be tested in future studies.

Marital status also differed between groups. This may be explained by the inclusion criteria, as most of the individuals in the CHG were the spouses of the elderly individuals living in the same home. This difference should be evaluated in future studies involving married elderly people who live in the same house, but are both independent. While this is a limitation of the present study, we advocate the hypothesis that living, being married, and care may have more benefits in terms of social interactions than what is found in married couples who do not exchange care. The benefits of caring in couples have been studied previously. Researchers have found that providing care to a spouse for 14 hours or more/week was a predictor of reduced mortality for the caregiver and that wives who provided care to a husband with a disability had higher levels of happiness than those who only carried out chores.

At the very least, we evaluated two groups that were similar with regard to a range of variables. We may conclude that living with and providing care for an older person can help recover the skill of perceiving emotions that is lost with ageing, specifically with regard to fear, sadness, and surprise, and may also enhance the identification of disgust. These differences can be explained by the opportunities for social interactions that emerge from cohabitation and care activities. Older adults in this situation are in constant contact with another elderly person. The demands of such a relationship require specific skills that may mitigate the decline in identifying emotions and may even improve emotion recognition.

Our results also suggest that it is important to continue to examine the positive influence of cohabitation. The loss or decline in the ability to recognize emotions is associated with greater psychosocial costs to patients, family members, and healthcare teams. The early identification of such deficits is important and can enable the development of proper interventions. Therefore, older people who do not live with an elderly individual should be encouraged to participate in social activities and engage in care activities, such as volunteer work. Furthermore, interventions with elderly populations aimed at training the recognition of emotions are required to enable better functioning in this specific cognitive domain, which is essential to social life.

The present study has limitations that should be addressed. The use of a convenience sample does not enable the generalization of the results, despite the calculation of a representative sample. The cross-sectional design does not allow the establishment of temporal associations among the variables. Moreover, the comparison groups were extreme. Thus, other groups should be investigated, such as older adults who live with an elderly individual, but do not have a dependency relationship and also younger adults who live with and self-care for elderly individuals. Future studies should perform a more in-depth analysis of other aspects of the cohabitation context that can contribute to understanding emotion recognition, such as the number of hours of daily contact, quality of the relationship between the caregiver and elderly care recipient, and the evaluation of other individuals in the home.

The present findings offer new insights regarding the recognition of facial expressions of emotion in older adults who live with other elderly individuals. The cohabitation group demonstrated greater accuracy than the non-cohabitation group, especially with regard to surprise, disgust, fear, and sadness. The data show that the cohabitation can offer benefits to older adults, which may mitigate losses related to the ageing process and enhance social interaction skills.
Acknowledgments

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Association of synaptosomal-associated protein 25 (SNAP-25) gene polymorphism with temperament and character traits in women with fibromyalgia syndrome

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Abstract

Background: Synaptosomal-associated protein 25 (SNAP-25) may be contribute to the pathogenesis of fibromyalgia Syndrome (FMS) by affecting the release of neurotransmitters. Objectives: We aimed to investigate the relationship between the SNAP-25 gen (Ddel = rs1051312 and MnlI = rs3746544) polymorphism and the temperament and character traits. Methods: A total of 85 female patients diagnosed with FMS and 70 age-matched healthy female subjects were enrolled into the study. The Temperament and Character Inventory (TCI) were performed on all the patients. SNAP-25 gene polymorphism was determined in the patients group and controls group. Results: No significant difference between groups was found regarding the distribution of SNAP-25 MnlI polymorphism (p > 0.05), but it was seen that the frequency of TC genotype for Ddel gene was higher in the patients group (p < 0.05). Increased hazard avoidance was found in the patients group (p < 0.05). When TCI scores were assessed in terms of SNAP-25 gene polymorphism, no statistically significant relationship was detected between the TT, TG, GG genotypes for MnlI gen and TCI scores (p > 0.05). However, increased hazard avoidance was detected in patients with TC genotype for Ddel gene compared to patients without such genotype. Discussion: SNAP-25 might be an etiological factor in FMS pathogenesis and might affect personality traits of FMS patients by mediating neurotransmitter release.

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Keywords: Fibromyalgia syndrome, SNAP-25 gene polymorphism, temperament and character inventory.

Introduction

Fibromyalgia syndrome (FMS) is a common chronic musculoskeletal disorder characterized with widespread pain and multiple sensitive points in physical examinations, the etiology of which is not completely known. It is a syndrome accompanied by many systemic disorders. Although it is an illness which can be seen in every age group, its frequency increases with age. It is most common between the ages 20 and 55. It is mostly seen in women.

Co-diagnosis of psychiatric disorders in FMS is common. When previous studies are considered, the most common psychiatric disorders that coexist with FMS are depression, somatization disorder, bipolar disorder, anxiety and obsessive compulsive disorder. Assuming that some personality and temperament traits may predispose to FMS, character evaluation studies have been conducted, and various disorders have been found in personality inventory profiles. Although symptoms that involve many systems can be seen, the symptom that bothers patients most is widespread pain. Unfortunately, there is no effective treatment of FMS. This is an important problem not only for patients, but also for clinicians. Therefore, studies for etiopathogenesis usually target the enlightenment of pathogenesis of pain. While the etiology and mechanisms of FMS are still not thoroughly understood, however, pain mechanisms and central sensitization in addition to neuroendocrine dysfunctions seem to be the most important factors in occurrence of FMS. Many factors can trigger or modulate neuroendocrine anomalies in FMS. Because psychiatric disorders frequently accompany to FMS, studies performed to enlighten its etiopathogenesis usually focus on psychiatric disorders and pain mechanisms. As known, serotonin and dopamine are the neurotransmitters, the roles of which have been widely discussed in the etiology of depression and anxiety disorders. Recent studies have found that levels of serotonin, noradrenalin and dopamine are distinctly low in FMS patients.

SNARE (soluble N-ethylmaleimide-sensitive factor activating protein receptor) proteins are proteins that have basic role in fusion between eukaryotic cells and organelles, and between organelles and plasma membrane. Synaptosomal-associated protein 25 (SNAP-25) is a SNARE protein found in plasma membrane. These proteins play a very important role in regulation of voltage-gated calcium channels and transmission of neurotransmitters between nerve cells. Neurotransmitters are found in synaptic vesicles, and are sent to the other synapse by way of exocytose. It is essential for normal brain functions. Several studies have reported that abnormalities in structure and expression of SNAP-25 are associated with various neurological disorders. For this reason, SNAP-25 may be contributing to the pathogenesis of FMS by mediating the release of neurotransmitters such as serotonin and dopamine. In a study carried out by Balkarlı et al. which was the preliminary of our study, it was reported that SNAP-25 gene polymorphism was more frequently detected in FMS. Depression and pain score was higher in patients with Ddel T/C genotype. Therefore, we intend to investigate the relationship between SNAP-25 gen (Ddel = rs1051312 and MnlI = rs3746544) polymorphism and the temperament and character traits.

Methods

Patients and evaluation

A total of 85 female patients diagnosed with FMS according to the ACR 2010 FMS diagnostic criteria, and 70 age-matched healthy female subjects were enrolled in the study. Postmenopausal or climacteric female patients were not enrolled in the study. Here, the objective was to reduce the effect of hormonal changes, and to reduce...
the effect of osteoporosis which usually accompanies to FMS in the postmenopausal period. Because depression affects personality and temperament traits, all the patients were evaluated with the Beck depression inventory (BDI) during the screening phase. The patients, whose BDI scores were ≥11, were excluded from the study. We tried to minimize the contribution of depression to personality traits. In order to obtain accurate data about the symptoms of the patients, subjects graduated at least from 8th class or attending 8th class were enrolled to the study, since more educated patients explain their symptoms better. All patients and healthy subjects were informed on the study. After informed consents from the patients group and controls were obtained, their blood samples were collected into 10 cc EDTA tubes, and were kept at –20°C.

Genetic analyses were performed on the samples in the Medical Genetics Department. The Tempeartament and Character Inventory (TCI) consisting of 240 questions was performed and assessed on both groups. Approval of the local ethics committee was obtained for the study.

**Tools**

**Socio-demographic Information Form**

This form was developed by the investigators, and data such as age, gender, educational background, socioeconomic status, place of residence, marital status and duration of illness were recorded on the form.

**BDI**

This inventory was used to determine the depression risk of subjects, and to measure the changes in level and severity of depressive symptoms. It was developed by Beck et al., and the studies for validation, reliability testing and adaptation of it into Turkish were conducted by Hisli.

**TCI**

It is a self-report inventory that evaluates four temperament and three character traits, which is filled out as "wrong/correct", which consists of 240 items, which can be completed in 30-45 minutes, and which can be applied to people 17 and above years old. All dimensions except for the persistence were divided into three and five subscales. In the temperament dimension, novelty seeking (NS) was divided into 4 subscales (NS1: exploratory excitability, NS2: impulsiveness, NS3: extravagenza, NS4: disorderliness); and harm avoidance (HA) was divided into 4 subscales (HA1: anticipatory worry, HA2: fear of uncertainty, HA3: shyness, HA4: fatigability); and reward dependence (RD) was divided into 3 subscales (RD1: sentimentality, RD2: attachment, RD4: dependence). In the character dimension, self-directedness (SD) was divided into 5 subscales (SD1: responsibility, SD2: purposefulness, SD3: resourcefulness, SD4: self-acceptance, SD5: enlightened second nature); and cooperativeness (C) was divided into 5 subscales (C1: social acceptance, C2: empathy, C3: helpfulness, C4: compassion, C5: pure-hearted conscience); and transcendence (ST) was divided into 3 subscales (ST1: self-forgetfulness, ST2: transpersonal identification, ST3: spiritual acceptance).

**Molecular analysis**

Genetic analyses were performed at the Medical Genetics Department. Genomic DNAs of the patients and the controls were isolated from peripheral blood by using QuickGene DNA whole blood kit (Kurabo, Japan). In order to multiply the UTR region of the 8th exon, where the Ddel (rs1051312) and MnlI (rs3746544) polymorphisms of the SNAP-25 gene are found, the primary sequences predefined in the literature as forward 5’- TTC TCC TTC TCC AAA TGC TGT CG-3’ and reverse 5’- CCA CGG AGG AGA GAA AAT G-3’ were used.

In the PCR reaction, which was formed by using these primary sequences, 10X PCR Buffer, 5 μl dNTP mixture containing 0.2 mM of every nucleotide, and Taq polymerase enzyme were also used. The PCR reaction conditions performed: After the first 2-minute denaturation at 95°C, following 35 cycles consisting of 1 minute at 58°C and 2 minutes at 72°C, a final extension of 7 minutes at 72°C. 10 U Ddel and 10 U MnlI enzymes were separately added to the PCR products of 261 base pairs obtained, and they were left to fragmentation at 37°C for 14 hours. For separation of the fragments that were formed after fragmentation, 3.5% ultra-pure agarose gel was prepared. Then, the PCR products were subjected to gel electrophoresis for 40-50 minutes, and divided into fragments. The allele band sequencings expected for Ddel polymorphism after electrophoresis for the T allele: an uncut band of 261 base pairs; for the C allele: two separate bands of 228 base pairs and 33 base pairs. The allele band sequencings expected for MnlI polymorphism for the T allele: two separate bands of 256 base pairs and 5 pairs, for the G allele: three separate bands of 210 base pairs, 46 base pairs and 5 base pairs.

**Statistical analysis**

Statistical package for the Social Sciences (SPSS) (IBM Corp., Armonk, New York, USA) 20.0 software was used for statistical evaluation. Descriptive statistics included mean, standard deviation and percentage. The confidence interval of the study was determined as 95%. First of all, Kolmogorov-Smirnov normality test was conducted for analysis of the differences between the ages, number of births, and number of children of the patients group and the controls group. At the end of the normality test, it was decided to use Mann-Whitney U test for the nonparametric tests. For analysis of marital status of the patients group and control group, Fischer’s exact test was used. For analysis of the differences between the patients group and the controls group in terms of the places they live in, occupational status, and MnlI, Ddel genes; Pearson chi-square test was used. Allele frequencies were calculated with the Hardy-Weinberg equation. p, q: allele frequency, p2, q2, 2pq: genotype frequency. p+q=1

\[(p^2)+(2pq)+(q^2)=1\]

Spearman correlation analysis was used to identify associations between the parameters. p < 0.05 was considered statistically significant.

**Results**

Of the patients, the mean age was 41.2 ± 5.3 years, and the mean durations of the symptoms was 7.43 ± 1.9 years, and the mean level of hemoglobin was 12.97 ± 0.81 g/dL. Eighty one (95.2%) patients were married, and 4 (4.8%) patients were single. Sixty two (72.9%) patients lived in the city, and 23 (23%) patients lived in the country. Twenty eight (32.9%) patients were working, and 57 (67.1%) patients were unemployed. The patients group and the controls group were similar with regards to their ages, marital status, the places they live in and occupational status.

When the MnlI (rs3746544) gene polymorphism distribution in the patients group and the controls group were reviewed, there were 38 subjects (44.70%) with TT genotype, 39 subjects (45.88%) with TG genotype, and 8 subjects (9.41%) with GG genotype. In the control group, there were 32 subjects (45.71%) with TT genotype, 23 subjects (32.86%) with TG genotype, and 15 subjects (21.42%) with GG genotype. When the Ddel (rs1051312) genotype distribution was reviewed, there were 35 subjects (41.1%) with TT genotype, 47 subjects (55.2%) with TC genotype, and 3 subjects (3.5%) with CC genotype in the patients group. In the control group, there were 46 subjects (65.7%) with TT genotype, 19 subjects (27.1%) with TC genotype, and 5 subjects (7.1%) with CC genotype. No difference between the groups was found regarding the distribution of SNAP-25 MnlI polymorphism, but statistically significant difference was found in the patients group and the controls group.
between the groups with regards to DdeI polymorphism (p = 0.004). When a separate matchings review was done to find out the source of the difference, it was seen that the difference was caused by the higher number of TC genotype in the patients group. No difference was found between the two groups with regards to allele frequency (Table 1).

When the TCI scores of the patients group and controls were compared, NS, NS4 and RD4 scores of the patients were found lower than those of the control group (p values 0.002, < 0.001, < 0.001 respectively), and HA, HA1, HA2, HA4, S5 scores were found higher in the patients compared to controls (p < 0.05), (Table 2).

**Table 1. SNAP-25 gene allele and genotype distribution in the patients group and controls group**

<table>
<thead>
<tr>
<th>Mnl1 gene genotype/allele</th>
<th>Patients n, %</th>
<th>Controls n, %</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>38 (44.70%)</td>
<td>32 (45.71%)</td>
<td>0.248</td>
</tr>
<tr>
<td>TG</td>
<td>39 (45.88%)</td>
<td>23 (32.86%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>9 (10.41%)</td>
<td>15 (21.42%)</td>
<td></td>
</tr>
<tr>
<td>T allele</td>
<td>115 (67.6%)</td>
<td>87 (62.14%)</td>
<td></td>
</tr>
<tr>
<td>G allele</td>
<td>55 (32.4%)</td>
<td>53 (38.5%)</td>
<td>0.500 (Pearson chi-square)</td>
</tr>
</tbody>
</table>

**DdeI gene**

<table>
<thead>
<tr>
<th></th>
<th>Patients n, %</th>
<th>Controls n, %</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>35 (41.1%)</td>
<td>46 (65.7%)</td>
<td>0.004</td>
</tr>
<tr>
<td>TC</td>
<td>47 (55.2%)</td>
<td>19 (27.1%)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>3 (3.5%)</td>
<td>5 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>T allele</td>
<td>117 (68.92%)</td>
<td>111 (79.28%)</td>
<td></td>
</tr>
<tr>
<td>C allele</td>
<td>53 (31.17%)</td>
<td>29 (20.71%)</td>
<td></td>
</tr>
</tbody>
</table>

HWE p = 0.004

<table>
<thead>
<tr>
<th></th>
<th>HWE p = 0.330</th>
</tr>
</thead>
<tbody>
<tr>
<td>HWE p = 0.037</td>
<td></td>
</tr>
</tbody>
</table>


**Table 2. TCI values of Patients Group and Control Group**

<table>
<thead>
<tr>
<th>TCI parameter</th>
<th>Patients n = 85</th>
<th>Controls n = 70</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>15.73 ± 4.78</td>
<td>18.16 ± 4.780</td>
<td>0.002</td>
</tr>
<tr>
<td>NS1</td>
<td>5.38 ± 2.17</td>
<td>5.67 ± 1.835</td>
<td>0.299</td>
</tr>
<tr>
<td>NS2</td>
<td>4.88 ± 7.06</td>
<td>4.05 ± 2.125</td>
<td>0.998</td>
</tr>
<tr>
<td>NS3</td>
<td>3.46 ± 1.586</td>
<td>3.46 ± 1.908</td>
<td>0.115</td>
</tr>
<tr>
<td>NS4</td>
<td>2.64 ± 1.445</td>
<td>4.47 ± 1.872</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HA</td>
<td>20.48 ± 6.81</td>
<td>16.63 ± 5.811</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HA1</td>
<td>6.62 ± 1.769</td>
<td>5.58 ± 2.095</td>
<td>0.003</td>
</tr>
<tr>
<td>HA2</td>
<td>4.25 ± 1.796</td>
<td>3.37 ± 1.665</td>
<td>0.003</td>
</tr>
<tr>
<td>HA3</td>
<td>3.98 ± 2.569</td>
<td>3.98 ± 2.569</td>
<td>0.441</td>
</tr>
<tr>
<td>HA4</td>
<td>5.46 ± 2.181</td>
<td>3.89 ± 2.177</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RD</td>
<td>13.14 ± 2.964</td>
<td>14.23 ± 3.123</td>
<td>0.71</td>
</tr>
<tr>
<td>RD1</td>
<td>7.32 ± 1.53</td>
<td>7.16 ± 1.73</td>
<td>0.546</td>
</tr>
<tr>
<td>RD2</td>
<td>3.76 ± 1.549</td>
<td>4.19 ± 1.586</td>
<td>0.065</td>
</tr>
<tr>
<td>RD3</td>
<td>1.94 ± 1.165</td>
<td>2.88 ± 1.196</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RD4</td>
<td>5.29 ± 1.541</td>
<td>5.19 ± 2.057</td>
<td>0.983</td>
</tr>
<tr>
<td>P</td>
<td>26.05 ± 7.669</td>
<td>25.02 ± 6.86</td>
<td>0.227</td>
</tr>
<tr>
<td>SD1</td>
<td>3.98 ± 2.43</td>
<td>3.95 ± 2.191</td>
<td>0.917</td>
</tr>
<tr>
<td>SD2</td>
<td>5.42 ± 2.213</td>
<td>5.04 ± 1.70</td>
<td>0.099</td>
</tr>
<tr>
<td>SD3</td>
<td>2.61 ± 1.66</td>
<td>3.21 ± 1.436</td>
<td>0.144</td>
</tr>
<tr>
<td>SD4</td>
<td>5.2 ± 2.75</td>
<td>5.6 ± 2.423</td>
<td>0.315</td>
</tr>
<tr>
<td>SD5</td>
<td>8.94 ± 2.62</td>
<td>7.23 ± 2.291</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C</td>
<td>26 ± 5.684</td>
<td>25.46 ± 6.459</td>
<td>0.373</td>
</tr>
<tr>
<td>C1</td>
<td>5.54 ± 1.793</td>
<td>5.49 ± 1.872</td>
<td>0.800</td>
</tr>
<tr>
<td>C2</td>
<td>3.8 ± 1.471</td>
<td>4.11 ± 1.472</td>
<td>0.401</td>
</tr>
<tr>
<td>C3</td>
<td>4.32 ± 1.592</td>
<td>4.44 ± 1.376</td>
<td>0.738</td>
</tr>
<tr>
<td>C4</td>
<td>6.51 ± 1.892</td>
<td>6.07 ± 2.034</td>
<td>0.170</td>
</tr>
<tr>
<td>C5</td>
<td>5.74 ± 1.729</td>
<td>5.28 ± 1.677</td>
<td>0.067</td>
</tr>
<tr>
<td>ST1</td>
<td>18.2 ± 4.64</td>
<td>19.32 ± 4.958</td>
<td>0.176</td>
</tr>
<tr>
<td>ST2</td>
<td>5.68 ± 2.196</td>
<td>6.09 ± 2.081</td>
<td>0.350</td>
</tr>
<tr>
<td>ST3</td>
<td>5.10 ± 1.74</td>
<td>5.11 ± 2.102</td>
<td>0.701</td>
</tr>
<tr>
<td>ST4</td>
<td>7.43 ± 1.341</td>
<td>8.12 ± 2.376</td>
<td>0.410</td>
</tr>
</tbody>
</table>

TCI: Character and Temperament Inventory; NS: novelty seeking; HA: harm avoidance; RD: reward dependence; P: persistence; SD: self-directedness; C: cooperativeness; ST: self-transcendence.

Values are presented as mean ± standard deviation.

p < 0.05 is significant; NS: non significant.
Discussion

The etiology of FMS is still unknown, but it seems to be multifactorial. FMS is classified as a functional somatic syndrome. Co-diagnosis of psychiatric disorders in FMS is common. In previous studies; depression, somatization disorder, bipolar disorder, anxiety and obsessive compulsive disorder have been found to be the most common coexisting disorders with FMS. Cloninger's personality that accounted for both normal and abnormal variations of personality traits and sub-dimensions of FMS patients. We found that NS and ST3 were significantly low, whereas; HA, HA1, HA2 and C5 were significantly high (p < 0.05, Table 3). It is assumed that personality also plays an important role in the etiology of psychosomatic illnesses. It is stated that there are specific personality traits for each psychosomatic illness.

Table 3. TCI scores of Patients Group and Control Group based on genotypes and alleles

<table>
<thead>
<tr>
<th>TCI Parameter</th>
<th>TC Genotype (+) patients n = 47</th>
<th>TC Genotype (–) patients n = 38</th>
<th>p</th>
<th>C allele (+) patients</th>
<th>C allele (–) patients</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>14.64 ± 4.086</td>
<td>16.61 ± 4.753</td>
<td>0.042</td>
<td>14.72 ± 3.99</td>
<td>18.85 ± 4.78</td>
<td>0.025</td>
</tr>
<tr>
<td>NS1</td>
<td>5.41 ± 2.81</td>
<td>5.47 ± 2.58</td>
<td>0.898</td>
<td>5.44 ± 1.78</td>
<td>5.44 ± 2.64</td>
<td>0.992</td>
</tr>
<tr>
<td>NS2</td>
<td>3.81 ± 1.989</td>
<td>6.19 ± 10.436</td>
<td>0.176</td>
<td>3.77 ± 1.87</td>
<td>4.52 ± 1.97</td>
<td>0.062</td>
</tr>
<tr>
<td>NS3</td>
<td>3.3 ± 1.525</td>
<td>3.53 ± 1.52</td>
<td>0.520</td>
<td>3.18 ± 1.57</td>
<td>3.88 ± 1.43</td>
<td>0.162</td>
</tr>
<tr>
<td>NS4</td>
<td>2.27 ± 1.217</td>
<td>2.97 ± 1.5</td>
<td>0.031</td>
<td>2.28 ± 1.19</td>
<td>3.00 ± 1.53</td>
<td>0.035</td>
</tr>
<tr>
<td>HA</td>
<td>22.11 ± 6.244</td>
<td>18.64 ± 7.68</td>
<td>0.038</td>
<td>21.77 ± 6.48</td>
<td>18.82 ± 7.65</td>
<td>0.040</td>
</tr>
<tr>
<td>HA1</td>
<td>7.08 ± 1.6</td>
<td>6.19 ± 1.399</td>
<td>0.037</td>
<td>7.05 ± 1.66</td>
<td>6.18 ± 1.89</td>
<td>0.048</td>
</tr>
<tr>
<td>HA2</td>
<td>4.89 ± 1.449</td>
<td>3.5 ± 1.905</td>
<td>0.001</td>
<td>4.79 ± 1.47</td>
<td>3.53 ± 1.95</td>
<td>0.003</td>
</tr>
<tr>
<td>HA3</td>
<td>4.49 ± 2.388</td>
<td>3.19 ± 2.776</td>
<td>0.056</td>
<td>4.38 ± 2.43</td>
<td>3.24 ± 2.78</td>
<td>0.111</td>
</tr>
<tr>
<td>HA4</td>
<td>5.46 ± 2.317</td>
<td>5.56 ± 2.223</td>
<td>0.857</td>
<td>5.56 ± 2.36</td>
<td>5.58 ± 2.14</td>
<td>0.521</td>
</tr>
<tr>
<td>RD</td>
<td>12.95 ± 2.613</td>
<td>13.36 ± 3.339</td>
<td>0.555</td>
<td>13.3 ± 2.62</td>
<td>13.5 ± 3.35</td>
<td>0.362</td>
</tr>
<tr>
<td>RD1</td>
<td>7.3 ± 1.45</td>
<td>7.44 ± 1.681</td>
<td>0.690</td>
<td>7.23 ± 1.45</td>
<td>7.53 ± 1.67</td>
<td>0.404</td>
</tr>
<tr>
<td>RD3</td>
<td>3.57 ± 1.346</td>
<td>3.83 ± 1.444</td>
<td>0.418</td>
<td>3.54 ± 1.31</td>
<td>3.88 ± 1.47</td>
<td>0.386</td>
</tr>
<tr>
<td>RD4</td>
<td>2.08 ± 1.09</td>
<td>1.81 ± 1.238</td>
<td>0.316</td>
<td>2.08 ± 1.08</td>
<td>1.79 ± 1.25</td>
<td>0.214</td>
</tr>
<tr>
<td>P</td>
<td>5.54 ± 1.574</td>
<td>5.19 ± 1.498</td>
<td>0.338</td>
<td>5.56 ± 1.55</td>
<td>5.15 ± 1.50</td>
<td>0.133</td>
</tr>
<tr>
<td>SD</td>
<td>25.43 ± 6.743</td>
<td>25.5 ± 8.484</td>
<td>0.970</td>
<td>25.87 ± 8.85</td>
<td>25.08 ± 8.45</td>
<td>0.628</td>
</tr>
<tr>
<td>SD1</td>
<td>3.81 ± 2.158</td>
<td>4 ± 2.64</td>
<td>0.738</td>
<td>3.85 ± 2.13</td>
<td>3.97 ± 2.69</td>
<td>0.858</td>
</tr>
<tr>
<td>SD2</td>
<td>5.22 ± 2.275</td>
<td>5.56 ± 2.298</td>
<td>0.528</td>
<td>5.31 ± 2.26</td>
<td>5.47 ± 2.32</td>
<td>0.542</td>
</tr>
<tr>
<td>SD3</td>
<td>2.57 ± 1.537</td>
<td>2.83 ± 1.92</td>
<td>0.515</td>
<td>2.69 ± 1.59</td>
<td>2.71 ± 1.89</td>
<td>0.826</td>
</tr>
<tr>
<td>SD4</td>
<td>5.05 ± 2.677</td>
<td>4.86 ± 2.779</td>
<td>0.763</td>
<td>5.18 ± 2.66</td>
<td>4.71 ± 2.78</td>
<td>0.332</td>
</tr>
<tr>
<td>SD5</td>
<td>8.78 ± 3.152</td>
<td>8.94 ± 3.641</td>
<td>0.802</td>
<td>8.85 ± 3.130</td>
<td>8.29 ± 1.60</td>
<td>0.110</td>
</tr>
<tr>
<td>C</td>
<td>26.54 ± 6.09</td>
<td>25.39 ± 7.762</td>
<td>0.483</td>
<td>26.84 ± 5.98</td>
<td>25.21 ± 7.93</td>
<td>0.314</td>
</tr>
<tr>
<td>C1</td>
<td>5.54 ± 1.865</td>
<td>5.56 ± 1.843</td>
<td>0.973</td>
<td>5.56 ± 1.81</td>
<td>5.53 ± 1.89</td>
<td>0.864</td>
</tr>
<tr>
<td>C2</td>
<td>3.84 ± 1.385</td>
<td>3.96 ± 1.676</td>
<td>0.949</td>
<td>3.97 ± 1.39</td>
<td>3.42 ± 1.67</td>
<td>0.669</td>
</tr>
<tr>
<td>C3</td>
<td>4.14 ± 1.601</td>
<td>4.39 ± 1.695</td>
<td>0.513</td>
<td>4.13 ± 1.62</td>
<td>4.41 ± 1.67</td>
<td>0.812</td>
</tr>
<tr>
<td>C4</td>
<td>6.73 ± 2.023</td>
<td>6.39 ± 1.817</td>
<td>0.463</td>
<td>6.74 ± 1.98</td>
<td>6.35 ± 1.96</td>
<td>0.301</td>
</tr>
<tr>
<td>C5</td>
<td>6.05 ± 1.508</td>
<td>5.22 ± 1.987</td>
<td>0.047</td>
<td>6.10 ± 1.48</td>
<td>5.12 ± 1.89</td>
<td>0.025</td>
</tr>
<tr>
<td>ST</td>
<td>18.41 ± 4.862</td>
<td>18.67 ± 6.641</td>
<td>0.815</td>
<td>18.31 ± 4.78</td>
<td>18.79 ± 4.70</td>
<td>0.739</td>
</tr>
<tr>
<td>ST1</td>
<td>6 ± 2.273</td>
<td>5.64 ± 2.27</td>
<td>0.499</td>
<td>5.90 ± 2.26</td>
<td>5.74 ± 2.28</td>
<td>0.750</td>
</tr>
<tr>
<td>ST2</td>
<td>5.3 ± 1.762</td>
<td>5.17 ± 1.699</td>
<td>0.748</td>
<td>5.33 ± 1.78</td>
<td>5.12 ± 1.86</td>
<td>0.593</td>
</tr>
<tr>
<td>ST3</td>
<td>7.05 ± 1.747</td>
<td>7.92 ± 2.089</td>
<td>0.059</td>
<td>7.03 ± 1.73</td>
<td>8.00 ± 2.08</td>
<td>0.029</td>
</tr>
</tbody>
</table>

TCI: Character and Temperament Inventory; NS: novelty seeking; HA: harm avoidance; RD: reward dependence; P: persistence; SD: self-directedness; C: cooperativeness; ST: self-transcendence.

Values are presented as mean ± standard deviation.

p < 0.05 is significant; NS: non significant.
found between low SD scores and depression scores. It has been reported that there is an increase in HA scores and decrease in SD scores in emotional pathologies associated with depressive state. Considering the likelihood of depression, we screened our patients with BDI and did not include the patients whom we found to have BDI scores higher than 17 in the study. Thus, we minimized the effect of depression on character and temperament. Therefore, SD score in FMS patients may have found to be normal, unlike in other studies.

Patients with high HA behavior are defined as careful, meticulous, passive, fearful and insecure. Similar findings have been found in all these studies including our study, suggesting that these personality traits cause more susceptibility to developing mood and pain disorders.

One of the hypotheses emphasized with respect to FMS etiopathogenesis is the serotonin (5-hydroxytryptophan) hypothesis. Serotonin is found both in peripheral and in central serotonergic neurons. As known, serotonin and dopamine are the neurotransmitters, the roles of which have been widely discussed in the development of pain and etiology of depression and anxiety disorders. Serum levels of tryptophan, which is the precursor of serotonin, have been found to be low in patients with FMS. In another study, decrease has been detected in 5-hydroxyindoleacetic acid levels, which is the serotonin metabolite, in cerebrospinal fluid of FMS patients. SNARE proteins are proteins that have basic role in fusion of synaptic vesicles and released at the synapse by way of membrane. These proteins play a very important role in formation of protein receptor complex which are responsible for the transmission of neurotransmitters between nerve cells. Neurotransmitters are found in synaptic vesicles and released at the synapse by way of calcium-dependent exocytosis. For this reason, SNAP-25 is important in the release of neurotransmitters such as serotonin and dopamine. Changes in the SNAP-25 functions may cause changes in the levels of serotonin and other neurotransmitters. Cloninger's personality model makes it possible to correlate behavior appearances with neurotransmitters. In a study carried out by Peirson and Heuchert, it was reported that HA tendency is linked to serotonin activation, and there is a negative correlation between serotonergic activation and HA tendency of the individuals. Individuals with high HA respond to stressful events with high levels of depressive signs. These individuals expect danger even when there is no danger. This expectation leads to an inappropriate adaptation. Such individuals may have the tendency to wait for the pain to begin. No common personality profile has been established for psychosomatic illnesses. However, the common result is that the HA scores are high. Cloninger described that people with chronic anxiety difficulty calm down, get tired easily, and display specific signs based on specific anticipatory anxiety. The high scores of HA, which we found in FMS patients, suggest that these patients may have a temperament with a tendency to develop pain symptoms as a response to stress.

In a study of Balkarlı et al., it was found that SNAP-25 gene polymorphism was more frequently detected in FMS. Depression and pain score was higher in patients with DdeI T/C genotype. In our study, we also found lower NS, lower NS4, higher HA, high HA1, high HA2, and high C5 in the patients with this genotype. We compared the TCI scores of patients with and without C allele, and we found difference in their TCI parameters (p < 0.005, Table 3).

There is limited research on character and temperament of FMS patients. Different inventories have been used in these studies. Personality models other than TCI have not taken into account the underlying social and biological identifiers, have failed in distinguishing types of memory. Cloninger's psychobiological personality model (TCI) evaluates the responses of a person to novelty, danger and various types of reward with four basic personality dimensions. This way, it has been determined that basic emotions mediate perceptive information processes, and form early learning patterns such as unconscious responses to conditioned stimuli, and inherited tendencies of information processed by memory systems have been reflected. Using the TCI to evaluate character and temperament in our study is supremacy of our study. Furthermore, most of these patients are also diagnosed with depression. Yet, higher HA, lower SD and lower persistence have been found in subjects with depression compared to healthy individuals. It has been reported that these personality traits may also be related to the severity of depressive symptomatology. For this reason, we did not include patients with depression into our study. Therefore, we minimized the contribution of depression to personality traits. This is the supremacy of our study.

Our study had various restrictions. First of all, the number of patients was limited, and we tried to explain pathology with only one gene. Serotonin and dopamine levels were not measured. The use of BDI instead of Structured clinical interview for DMS-IV diagnosis for the diagnosis of depression was another limitation. The fact that there was no psychiatric evaluation or screening test for other common diseases (such as bipolar disorder) was another shortcoming of this study. Although it was stated that patients with higher educational background were included in order to fill these form more accurately, level of education may an effect on severity of disease symptoms and temperament.

In conclusion, SNAP-25 might be an etiological factor in FMS pathogenesis and might affect personality traits of FMS patients by mediating neurotransmitter release. The findings of our study may be considered as preliminary data, and further studies are required to be conducted. We think that studies with larger patient populations, in which serotonin and serotonin receptors will also be evaluated, will be beneficial for enlightening of the subject matter.

Acknowledgement

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Conflict of interest

No conflict of interest was declared by the authors.

References


Persistent and multisite homophobic harassment during childhood and adolescence and its association with school difficulties in gay and bisexual men in Taiwan

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Abstract

Background: Homophobic harassment can compromise mental health of sexual minority youths. Objectives: This study examined the rates of persistent and multisite homophobic harassment and their associations with school difficulties during childhood and adolescence among gay and bisexual men in Taiwan. Methods: Participants were recruited through advertisements on the Facebook, Bulletin Board Systems, and the home pages of health promotion and counseling centers for the gay, lesbian, and bisexual community. The experiences of traditional and cyber harassment based on gender role nonconformity and sexual orientation of 500 gay or bisexual men were examined. The associations of multisite and persistent harassment victimization with school difficulties were evaluated. Results: A total of 239 (47.8%) and 131 (26.2%) participants experienced persistent and multisite harassment victimization, respectively. Harassment victimization was significantly associated with low satisfaction with academic performance in any stage of study. Moreover, the participants who were harassed in senior high schools were more likely to miss classes or be truant than those who were not harassed. The victims of multisite harassment at senior high schools were more likely to miss classes or be truant than those of school-only harassment. Discussion: Prevention and intervention programs are warranted to reduce homophobic harassment in sexual minority youths.

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Keywords: Sexual minority, homophobia, harassment, gender role nonconformity, sexual orientation.

Introduction

Homophobia is defined as negative beliefs, attitudes, stereotypes, and behaviors directed toward sexual minorities, including lesbian, gay, bisexual, transgender, and questioning (LGBTQ) individuals1. Homophobic harassment of sexual minority children and adolescents based on gender role nonconformity and sexual orientation is a major global concern for mental health and educational professionals. The 2015 National School Climate Survey of the Gay, Lesbian, and Straight Education Network on secondary students from all 50 states and the District of Columbia in the United States reported that 85% of sexual minority students experienced verbal harassment based on a personal characteristic and 66% experienced sexual or gender-related discrimination at schools2. Furthermore, a survey on harassment among adolescents in Canada revealed that sexual minority students consistently reported higher victimization rates than heterosexual peers over time3. A 3.5-year follow-up study observed that prior experiences of homophobic harassment predicted subsequent psychological distress in LGBTQ adolescents, and higher victimization rates resulted in higher distress4. A 6-month follow-up study proved that homophobic harassment victimization significantly mediated the effects of sexual minority status on depressive symptoms and suicidality5. Therefore, the aforementioned study findings support public policy initiatives that reduce homophobic harassment and prevent victimization-related effects on the health and well-being of sexual minority youths. In addition to mental health concerns and psychological distress, homophobic harassment may be associated with school difficulties in sexual minority youths. The minority stress hypothesis6 suggested that homophobic harassment may partially account for disparities in sexual minority youths. Studies have observed that sexual minority youths with prior harassment experiences are at an increased risk of low educational aspiration7, missing classes8, and truancy due to fear9. Low educational aspiration is a critical indicator of the ineffective school life of students. Moreover, high rates of missing classes may endanger students’ school performance and increase the risk of deviant behaviors outside schools such as alcohol and drug abuse, aggression, and criminal behaviors. Therefore, efforts to prevent homophobic harassment-caused school difficulties are urgently required to ameliorate social and behavioral problems in sexual minority youths.

Several concerns regarding homophobic harassment and its association with school difficulties in sexual minority youths warrant additional investigation. Data on the prevalence of homophobic harassment and its adverse effects on the school life and mental health of sexual minority youths in Asia are limited compared with data from Western societies. A study found that people in completely industrialized Asian countries, such as Japan and South Korea, show much less tolerance for homosexuality than people in industrialized European and North American countries do10. A study on sexual minority men in Japan revealed that 83% and 60% of the men experienced school bullying and verbal harassment, respectively, because of their perceived homosexuality. In addition, a history of verbal harassment was significantly associated with the risk of attempted suicide11. Therefore, homophobic harassment victimization during childhood and adolescence and its association with school difficulties in sexual minority youths in Asian countries warrant further investigation.

Compared with the traditional forms of homophobic harassment, including teasing, social exclusion, and physical assault11, cyber harassment in sexual minority youths has been less investigated.
Cyber harassment is a new mode of harassment that has emerged in the digital age. Almost one in two sexual minority youths experience online peer victimization compared with one in six heterosexual youths. Because adolescent victims of cyber harassment are more likely to experience psychological problems, including depression, anxiety, suicidality, and adjustment difficulties in schools, they are more likely to experience psychological problems, including depression, anxiety, suicidality, and adjustment difficulties in schools. However, data on the adverse effects of persistent homophobic harassment on sexual minority youths are limited. Sexual minority youths may experience homophobic harassment not only at schools but also in other environments. Because most parents in Taiwan have long working hours and can only take care of their children after work during weekdays, their children are sent to afterschool classes to receive care and complete their homework after their daily study at primary schools. Just as in other East Asian countries with Confucian roots, people in Taiwan encourage children to pursue academic success, and therefore, many students continue studying in tutoring schools after finishing classes in primary (grades 1 to 6), junior high (grades 7 to 9), and senior high (grades 10 to 12) schools. Moreover, some senior high school students may start part-time work. According to our review of the relevant literature, no study has evaluated the experiences of homophobic harassment occurring at schools, afterschool classes, tutoring schools, and part-time workplaces simultaneously. However, whether the place of homophobic harassment (i.e., school-only or outside school [multisite] harassment) has variable effects on school difficulties requires further investigation.

The present study examined the victimization rates of traditional and cyber harassment, particularly persistent and multisite homophobic harassment, based on gender role nonconformity and sexual orientation of gay and bisexual men in Taiwan during their childhood and adolescence. In addition, we investigated the associations between homophobic harassment (any form, persistent, and multisite) victimization and school difficulties, including dissatisfaction with academic performance and the tendency of missing classes or truancy. We hypothesized that receiving homophobic harassment is significantly associated with school difficulties in gay and bisexual men. Furthermore, sexual minority youths who experience persistent and multisite homophobic harassment are more likely to have school difficulties than those who experience nonpersistent and school-only homophobic harassment.

Methods

Participants

Participants were recruited through advertisements on the Internet, including Facebook, Bulletin Board Systems, and the home pages of five health promotion and counseling centers for the gay, lesbian, bisexual, and transgender (LGBT) community. Individuals who exhibited any deficits (e.g., intellectual disability or substance use) that prevented them from understanding the study purpose or completing the questionnaires were excluded. A total of 500 gay or bisexual men aged between 20 and 25 years were recruited in this study. Informed consent was obtained from all participants prior to assessment. This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-F(I)-20150026) and meets ethical guidelines in Taiwan.

Measures

Chinese version of the School Bullying Experience Questionnaire

We used six items from the self-reported Chinese version of the School Bullying Experience Questionnaire (C-SBEQ) to evaluate participants’ retrospective experiences of traditional harassment in primary (grades 1 to 6), junior high (grades 7 to 9), and senior high (grades 10 to 12) schools based on their gender role nonconformity and sexual orientation at schools, afterschool classes, tutoring schools, and part-time workplaces. Two forms of traditional harassment victimization were evaluated, including verbal ridicule and relational exclusion (three items for experiencing social exclusion, mean name-calling, and ill-speaking; for example: "How often have others spoken ill of you because they thought of you as a sissy [they found you homosexual or bisexual]?" and physical aggression and theft of belongings (three items for experiencing physical abuse, forced work, and confiscation of money, school supplies, and snacks; for example: "How often have others beaten you up because they thought of you as a sissy [they found you homosexual or bisexual]?").

The responses for these six items were graded on a 4-point Likert scale ranging from 0 = never, 1 = just a little, 2 = often, and 3 = all the time. A previous study on C-SBEQ psychometrics revealed that the C-SBEQ has acceptable reliability and validity. The Cronbach’s α of the scale for evaluating two forms of harassment based on gender role nonconformity and sexual orientation ranged from 0.70 to 0.86. In the present study, the participants who answered 1 on any item were identified as self-reported victims of mild traditional harassment and those who reported 2 or 3 on any item were identified as self-reported victims of traditional bullying. Furthermore, the place and time of harassment of all participants who did not answer 0 on any item were investigated. Harassment that occurred both inside and outside schools was defined as multisite harassment and that persisted from primary to high schools or from junior high to senior high schools was defined as persistent harassment.

Cyberbullying Experiences Questionnaire

We used three items of the Cyberbullying Experiences Questionnaire to assess participants’ retrospective experiences of cyber harassment in primary, junior high, and senior high schools based on their gender role nonconformity and sexual orientation. The three items addressed the experiences of posting mean or hurtful comments; posting upsetting pictures, photos, or videos; and online rumor-spread through emails, blogs, social media (Facebook/Twitter/Plurk), and pictures or videos; for example: "How often have other students posted mean or hurtful comments on you through e-mails, blogs, or social media because they thought of you as a sissy (they found you homosexual or bisexual)?" The responses to these items were graded using a 4-point Likert scale, ranging from 0 (never) to 3 (all the time). The Cronbach’s α values of the scales for evaluating cyber harassment victimization due to gender role nonconformity and due to sexual orientation were 0.71 and 0.86, respectively. In the present study, the participants who answered 1 on any item were identified as self-reported victims of mild cyber harassment and those who reported 2 or 3 on any item were identified as self-reported victims of cyberbullying. Furthermore, the time of cyber harassment or cyber bullying of all participants who did not answer 0 on any item was investigated. Cyber harassment that persisted from primary to high schools or from junior high to senior high schools was defined as persistent cyber harassment.

School difficulties

We invited the participants to label retrospectively their subjective satisfaction with their academic performance in primary, junior high, and senior high schools using an item on the 4-point Likert scale, ranging from 0 (very satisfied) to 3 (not satisfied at all). In the present study, participants who answered 2 or 3 were classified as dissatisfied with their academic performance. The tendencies of missing classes or truancy in primary, junior high, and senior high schools were evaluated using an item on the 4-point Likert scale, ranging from 0 (never) to 3 (very frequent). In the present study, all
participants who did not answer as 0 on any item were classified as having a tendency of missing classes or truancy.

Procedure and statistical analysis
Research assistants explained the procedures and methods for completing the research questionnaires to the participants individually. The research assistants resolved any difficulties encountered by the participants while completing the questionnaires. Data analysis was performed using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA).

The ratios of the participants with victimization of two types (verbal ridicule and relational exclusion and physical aggression and theft of belongings) of mild traditional harassment and traditional bullying, mild cyber-harassment and cyber-bullying due to gender non-conformity and sexual orientation were calculated. The proportion of the participants who were dissatisfied regarding their academic performance and who had missed classes or engaged in truancy were also calculated.

The associations between harassment victimization during primary, junior high, and senior high schools and dissatisfaction with academic performance and the tendency of missing classes or truancy were examined using the chi-squared test. In addition, the associations between persistent and multisite harassment victimization and dissatisfaction with academic performance and the tendency of missing classes or truancy were investigated using the chi-square test. A p value of 0.05 was considered statistically significant for all tests.

Results
A total of 500 gay (n = 129) or bisexual (n = 129) men participated in this study. Their mean age was 22.9 years (standard deviation: 1.6 years, range: 20–25 years).

Forms of harassment
Table 1 presents the rates of verbal ridicule and relational exclusion, physical aggression and theft of belongings, and cyber harassment based on gender role nonconformity and sexual orientation of the participants. A total of 438 (87.6%) participants experienced traditional harassment (n = 435, 87%) or cyber harassment (n = 201, 40.2%) during their childhood and adolescence. Of the 414 (82.8%) participants who experienced traditional harassment based on gender role nonconformity, 408 (81.6%) experienced verbal ridicule and relational exclusion, and 183 (36.6%) experienced physical aggression and theft of belongings. Of the 266 (53.2%) participants who experienced traditional harassment based on sexual orientation, 262 (52.4%) experienced verbal ridicule and relational exclusion, and 73 (14.6%) experienced physical aggression and theft of belongings. Of the 201 (40.2%) participants who experienced cyber harassment, 162 (32.4%) experienced harassment based on gender role nonconformity, and 142 (28.4%) experienced harassment based on sexual orientation.

Time and place of harassment
Table 2 presents the time and place of harassment and school difficulties. In this study, 199 (39.8%), 309 (61.8%), and 230 (46%) participants experienced harassment during primary, junior high, and senior high schools, respectively. Of the participants who experienced harassment at primary schools, 166 (33.2%) and 84 (16.8%) experienced persistent harassment in junior high and senior high schools, respectively. Of the participants who experienced harassment at junior high schools, 147 (29.4%) experienced persistent harassment in junior high and senior high schools, respectively. Of the participants who experienced harassment at senior high schools, 141 (28.2%) experienced persistent harassment.

Of the 435 participants who reported traditional harassment, 58 (11.6%), 68 (13.6%), and 56 (11.2%) experienced harassment at afterschool classes, tutoring schools, and part-time workplaces, respectively. Furthermore, 92 (18.4%), 27 (5.4%), and 12 (2.4%) participants experienced traditional harassment at one, two, and three places outside schools, respectively. A total of 131 (26.2%) participants reported multisite harassment victimization.

<table>
<thead>
<tr>
<th>Table 2. Time and place of harassment and school difficulties (N = 500)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time of traditional or cyber harassment</strong></td>
</tr>
<tr>
<td>Primary school</td>
</tr>
<tr>
<td>Persistent up to junior high school</td>
</tr>
<tr>
<td>Persistent up to senior high school</td>
</tr>
<tr>
<td>Junior high school</td>
</tr>
<tr>
<td>Persistent up to senior high school</td>
</tr>
<tr>
<td>Senior high school</td>
</tr>
<tr>
<td>Persistent harassment victimization</td>
</tr>
<tr>
<td><strong>Place of traditional harassment</strong></td>
</tr>
<tr>
<td>School</td>
</tr>
<tr>
<td>Afterschool class</td>
</tr>
<tr>
<td>Tutoring school</td>
</tr>
<tr>
<td>Workplace</td>
</tr>
<tr>
<td><strong>Number of places outside school at which harassment occurred</strong></td>
</tr>
<tr>
<td>One</td>
</tr>
<tr>
<td>Two</td>
</tr>
<tr>
<td>Three</td>
</tr>
<tr>
<td>Multisite harassment victimization</td>
</tr>
<tr>
<td><strong>Low satisfaction with academic performance</strong></td>
</tr>
<tr>
<td>Primary school</td>
</tr>
<tr>
<td>Junior high school</td>
</tr>
<tr>
<td>Senior high school</td>
</tr>
<tr>
<td><strong>Tendency of missing classes or truancy</strong></td>
</tr>
<tr>
<td>Primary school</td>
</tr>
<tr>
<td>Junior high school</td>
</tr>
<tr>
<td>Senior high school</td>
</tr>
</tbody>
</table>
Associations between harassment and school difficulties

Table 3 shows the associations between harassment and satisfaction with academic performance and the tendency to miss classes or to engage in truancy. Harassment victimization was significantly associated with low satisfaction with academic performance in primary (p < 0.01), junior high (p < 0.01), and senior high (p < 0.001) schools. Moreover, the participants who were harassed in senior high schools were more likely to miss classes or be truant than those who were not harassed (p < 0.01).

Associations between multisite and persistent harassment and school difficulties

Table 4 presents the associations between multisite and persistent harassment and dissatisfaction with academic performance and the tendency of missing classes or truancy. The senior high school students victimized by multisite harassment were more likely to miss classes or engage in truancy than the senior high school students who were harassed only at schools (p < 0.01), whereas no significant association was observed between multisite harassment and the tendency of missing classes or truancy at primary and junior high schools. Furthermore, persistent harassment from primary to junior high schools and school difficulties at junior high schools. Furthermore, no significant association was found between persistent harassment from junior high to senior high schools and the tendency of missing classes or truancy at senior high schools. However, the participants who experienced persistent harassment from junior high to senior high schools were more likely to be satisfied with their academic performance at senior high schools than those who experienced harassment only at senior high schools but not at junior high schools.

Discussion

In the present study, a high proportion (87.6%) of gay and bisexual men experienced homophobic harassment during their childhood and adolescence. Almost half (47.8%) and more than one-fourth (26.2%) of the gay and bisexual men experienced persistent and

Table 3. Associations between harassment and satisfaction with academic performance and the tendency of missing classes or truancy

<table>
<thead>
<tr>
<th>Harassment in primary school</th>
<th>High n (%)</th>
<th>Low n (%)</th>
<th>χ²</th>
<th>No n (%)</th>
<th>Yes n (%)</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (n = 301)</td>
<td>254 (84.4)</td>
<td>47 (15.6)</td>
<td>8.343**</td>
<td>280 (93.0)</td>
<td>21 (7.0)</td>
<td>3.133</td>
</tr>
<tr>
<td>Yes (n = 199)</td>
<td>147 (73.9)</td>
<td>52 (26.1)</td>
<td></td>
<td>176 (88.4)</td>
<td>23 (11.6)</td>
<td></td>
</tr>
<tr>
<td>Harassment in junior high school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 191)</td>
<td>146 (76.4)</td>
<td>45 (23.6)</td>
<td>11.971**</td>
<td>166 (86.9)</td>
<td>25 (13.1)</td>
<td>3.668</td>
</tr>
<tr>
<td>Yes (n = 309)</td>
<td>190 (61.5)</td>
<td>119 (38.5)</td>
<td></td>
<td>248 (80.3)</td>
<td>61 (19.7)</td>
<td></td>
</tr>
<tr>
<td>Harassment in senior high school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 270)</td>
<td>189 (70)</td>
<td>81 (30)</td>
<td>15.944***</td>
<td>209 (77.4)</td>
<td>61 (22.6)</td>
<td>9.115**</td>
</tr>
<tr>
<td>Yes (n = 230)</td>
<td>121 (52.6)</td>
<td>109 (47.4)</td>
<td></td>
<td>150 (65.2)</td>
<td>80 (34.8)</td>
<td></td>
</tr>
</tbody>
</table>

** p < 0.01; *** p < 0.001.

Table 4. Associations between multisite and persistent harassment and satisfaction with academic performance and the tendency of missing classes or truancy

<table>
<thead>
<tr>
<th>Multisite harassment</th>
<th>High n (%)</th>
<th>Low n (%)</th>
<th>χ²</th>
<th>No n (%)</th>
<th>Yes n (%)</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 134)</td>
<td>95 (70.9)</td>
<td>39 (29.1)</td>
<td>1.880</td>
<td>117 (87.3)</td>
<td>17 (12.7)</td>
<td>0.511</td>
</tr>
<tr>
<td>Yes (n = 65)</td>
<td>52 (80)</td>
<td>13 (20)</td>
<td></td>
<td>59 (90.8)</td>
<td>6 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Junior high school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 257)</td>
<td>159 (61.9)</td>
<td>98 (38.1)</td>
<td>0.093</td>
<td>206 (80.2)</td>
<td>51 (19.8)</td>
<td>0.010</td>
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<tr>
<td>Yes (n = 52)</td>
<td>31 (59.6)</td>
<td>21 (40.4)</td>
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<td>42 (80.8)</td>
<td>10 (19.2)</td>
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</tr>
<tr>
<td>Senior high school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 166)</td>
<td>88 (53.0)</td>
<td>78 (47.0)</td>
<td>0.039</td>
<td>117 (70.5)</td>
<td>49 (29.5)</td>
<td>97.289**</td>
</tr>
<tr>
<td>Yes (n = 64)</td>
<td>33 (51.6)</td>
<td>31 (48.4)</td>
<td></td>
<td>33 (51.6)</td>
<td>31 (48.4)</td>
<td></td>
</tr>
<tr>
<td>Persistent harassment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>From primary school to junior high school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 143)</td>
<td>90 (62.9)</td>
<td>53 (37.1)</td>
<td>0.150</td>
<td>103 (72.0)</td>
<td>40 (28.0)</td>
<td>0.167</td>
</tr>
<tr>
<td>Yes (n = 168)</td>
<td>100 (65.1)</td>
<td>58 (34.9)</td>
<td></td>
<td>123 (74.1)</td>
<td>43 (25.9)</td>
<td></td>
</tr>
<tr>
<td>From junior high school to senior high school</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 83)</td>
<td>36 (43.4)</td>
<td>47 (56.6)</td>
<td>4.442*</td>
<td>54 (65.1)</td>
<td>29 (34.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Yes (n = 147)</td>
<td>85 (57.8)</td>
<td>62 (42.2)</td>
<td></td>
<td>96 (65.3)</td>
<td>51 (34.7)</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05; **p < 0.01.
multisite harassment, respectively. Harassment victimization was significantly associated with dissatisfaction with academic performance at all stages of school life. Furthermore, harassment victimization was significantly associated with the tendency of missing classes or truancy in senior high schools. The victims of multisite harassment at senior high schools were more likely to miss classes or be truant than those of school-only harassment, whereas the victims of persistent harassment were more likely to be satisfied with their academic performance than those of nonpersistent harassment at senior high schools.

The present study observed that nearly 9 in 10 gay and bisexual men experienced harassment based on gender role nonconformity or sexual orientation during their childhood and adolescence, which is similar to the results of a study in the Unites States. Taiwan is considered to be one of the most LGBTQ-friendly countries in Asia. An analysis of attitudes toward homosexuality revealed that the overall social tolerance toward homosexuality among people in Taiwan has increased progressively from 1995 to 2012. Moreover, Taiwan has made the most progress in terms of attitudinal shifts toward a higher acceptance of homosexuality between 1995 and 2012 than China, Japan, and South Korea. However, the high rate of homophobic harassment in sexual minority youths in the present study indicates that homophobic harassment remains prevalent and warrants intervention of educational and health professionals.

The present study revealed that harassment in the form of verbal ridicule and relational exclusion was more common than physical aggression and theft of belongings in gay and bisexual men. These results are consistent with the experiences of general but not homophobic harassment in general populations. Compared with physical aggression, verbal ridicule and relational exclusion are less detectable by school personnel and parents. Similar to physical aggression, verbal ridicule and relational exclusion can also compromise victims’ mental health. Therefore, parents and school personnel should focus on the active and early detection of verbal and social harassment in sexual minority youths.

Notably, in this study, harassment based on gender role nonconformity was more prevalent than that based on sexual orientation. Studies have reported that the negative effects of victimization based on the sexual minority status are not limited to individuals who actually identify as a sexual minority. Cross-sectional and longitudinal studies have observed that boys can be bullied for being perceived as gay because of their gender role nonconformity, regardless of their actual sexual identity; in addition, bullying victimization increases the severity of psychosocial stress. The aforementioned results indicate that challenging gender-stereotyped perceptions and promoting gender equality concepts are fundamental to prevent homophobic harassment.

In the present study, 40% of the sexual minority men experienced cyber harassment based on gender role nonconformity or sexual orientation during their childhood and adolescence. According to Urie Bronfenbrenner’s ecological system perspective, homophobic bullying is established and perpetuated gradually due to complex interactions between individuals and multilevel social systems, whereas social support is a protective factor for homophobic harassment in schools. Because of the presence of numerous peers who have hostile attitudes in schools and communities, the Internet is an important source of social support for sexual minority youths. However, adolescent victims of highly severe traditional bullying are more likely to experience cyberbullying. The results of the present study support the claim that both in-person harassment and cyber harassment should be the targets of prevention and intervention programs for homophobic harassment in sexual minority youths.

The present study found that harassment victimization was significantly associated with low satisfaction with academic performance in all stages of school life. According to the psychological mediation model, peer harassment increases psychological distress in sexual minority individuals and negatively influences cognitive, regulatory, and social mechanisms associated with the development of psychopathology. Furthermore, studies have observed that homophobic harassment victimization predicts psychological distress, depressive symptoms, and suicidality in sexual minority individuals. Moreover, mental health concerns further compromise academic outcomes in bullying victims. The study participants who were harassed in senior high schools were more likely to miss classes or be truant than those who were not harassed. Missing classes and truancy may be coping strategies adopted by sexual minority youths to avoid persistent homophobic harassment as senior high school students. However, low school attendance may further jeopardize their performance. The present results indicate that parents and educational and mental health professionals should routinely investigate the experiences of homophobic harassment in sexual minority youths who miss classes frequently, have low academic success, or are truant.

Multisite homophobic harassment at senior high schools was significantly associated with the tendency of missing classes or truancy in the present study. According to the minority stress hypothesis, sexual minority youths who experience multisite harassment must endure continuous homophobic harassment outside schools, which may adversely affect their morale and aggravate psychological distress. Victims of multisite harassment may run away from schools to ameliorate the source of homophobic harassment. The present results support that prevention and intervention programs for homophobic harassment should be established not only at schools but also in other environments, such as tutoring schools and part-time workplaces.

In contrast to our hypothesis, sexual minority youths who experienced persistent homophobic harassment from junior high to senior high schools were more likely to be satisfied with their academic performance than those who were harassed only at senior high schools. Persistent bullying can result in more severe mental health and school-related problems than nonpersistent bullying. One possible explanation for discrepancies in the present and previous study results is that pursuing academic success might be a method adopted by sexual minority youths to earn respect from others and develop self-esteem, which may mitigate the distress caused by harassment. However, additional studies are warranted to replicate the present results and examine the possible mechanisms.

The present study has several limitations. First, this study obtained data on participants’ homophobic harassment victimization and school difficulties retrospectively, and therefore, recall bias might have been introduced. However, bullying victims may have strong emotional reactions to such events and develop vivid and lasting memories of such experiences, reducing the possibility of recall bias. Second, the study could not determine the causal relationship between homophobic harassment victimization and school difficulties. Third, study data were exclusively self-reported. Therefore, the use of only a single data source could have influenced our findings and may have resulted in shared-method variances.

Conclusion
A high proportion of gay and bisexual men experience homophobic harassment, in the forms of verbal, social, physical, and cyber harassment, during their childhood and adolescence. Furthermore, homophobic harassment victimization, particularly multisite harassment, is significantly associated with school difficulties. The present findings support public policy initiatives that curtail homophobic harassment because decreasing harassment victimization may reduce school difficulties in sexual minority youths.

Acknowledgment
This study was supported by the Ministry of Science and Technology, Taiwan, R.O.C. (grant no. MOST 104-2314-B-037-024-MY3) and the Kaohsiung Medical University Hospital (grant nos. KMUH104-4R60, KMUH105-5R59 and KMUH106-6R67).
Ethical approval

This study was approved by the Institutional Review Board of Kaohsiung Medical University (KMUHIRB-F(I)-20150026).

Competing interests

None declared.

References

The number of war-related traumatic events is associated with increased behavioural but not emotional problems among Syrian refugee children years after resettlement

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Abstract

Background: Turkey is the leading refugee-hosting country in the world. However, there are few studies which investigate mental wellbeing of refugee children in Turkey. Objective: The paper aims to examine the prevalence of emotional and behavioural problems and associated risk factors among Syrian refugee minors in Turkey. Methods: The research involved 85 students from 2nd to 8th grades. We investigated emotional and behavioural problems with parent-reported Arabic form of Strength and Difficulties Questionnaire (SDQ). Socio-demographical findings and children’s war-related experiences were also examined. Results: The study sample consisted of 49 (63.6%) girls, and 28 (36.4%) boys (total 77) from age 7 to 17. Average time after resettlement was 29.8 ± 11.2 (5 to 50 months) months. 66 (85.7%) children reported to had lost at least one familiar person due to the war. The mean experienced war-related traumatic events were calculated as 2.92 ± 1.86. Total difficulty scores of 30 (39.0%) children were above the cut-off values. The rates of children whose SDQ problem scores exceeded the cut-off values were as high as 45.5% (35) for Emotional problems, 64.9% (50) for Peer, 27.3% (21) for conduct and 19.5% (15) for Hyperactivity problems. Discussion: Results indicate high prevalence rates of severe traumatic experiences and possible psychiatric disorders among child survivors of Syrian war which in its seventh year now.


Keywords: Syria, children, war, mental health, depression, SDQ, emotional problems, PTSD.

Introduction

The word of ‘disaster’ derived from its Latin roots ‘dis’ and ‘astrum’ which mean ‘The stars are against us’ to describe the despair of the early humans after the massive destruction of natural disasters. Today, child survivors of Syria civil war that have caused deaths of hundreds of thousands of people, also feel similar kind of despair in the face of the civil war which is in its eighth year now. Evidence suggests that war-related traumatic events may have adverse consequences on the mental well-being of children. However, war-related traumatic events are not the only adversities that threat psychological well-being of refugee children. There is a growing body of literature that recognises the importance of other adversities on the psychosocial development of children which may have benefits on mental well-being and, could also contribute the psychosocial development. However only one out of three school-age refugee children could attend school in Turkey. "Transient Education Centers" which are established to give education for refugee children in Arabic language and, national public schools where provides training to both Turkish and refugee children in Turkish language systematic education. Due to the lack of integration policies that expected to support refugee children attending public schools and the situation of the public schools which are already overcrowded, only 19% of refugee children are attending to public schools. What means, most of the refugee children who have enrolled in school are going to Transient Education Centers which are not providing systematic education.

Prior to the work of Kinzie et al., which showed high rates of PTSD and depression among child survivors of concentration camps, the role of traumatic experiences on the mental well-being of children was largely unknown. The research to date has been designed to determine whether refugee minors are at higher risk for development of psychiatric disorders in Iraq, Bosnia, Syria, Palestine, and Lebanon have shown increased rates of post-traumatic stress disorder (PTSD), depression, anxiety disorders and enuresis among refugee children. It is now well established from a variety of studies that children’s psychosocial well-being is adversely affected by the war and related adverse events. However, the most of the studies that aimed to assess the mental wellbeing of refugee children have been conducted in high-income countries while most of the refugees shelter in low-income countries. And even though, Syria civil war has uprooted more than ten million people from their home and was recognised as one of the biggest humanitarian crisis that humankind has faced in the 21st century. Up to now, very few studies have been carried out on the mental well-being of child survivors. This paper aims to contribute to the understanding of the effect of war and related traumatic events on the psychological well-being of child survivors. The research seeks to address the prevalence of
emotional and behavioural problems and explore differences in the prevalence rates of psychological problems and exposure to war-related traumatic events between genders and age groups in a school sample of Syrian refugee children in Turkey. We also aimed to investigate risk and protective factors which are associated with higher or lower rates of psychiatric symptoms among Syrian refugee kids in Turkey.

Methods

Participants and procedure
The study was conducted in autumn of 2015, in Hatay, a southern city of Turkey which borders with Syria at the east and south side. The municipality sheltered 377,198 refugees from Syria in September 2015 what means that almost one-quarter of Hatay’s inhabitants were refugees at the time. The participants in this study were recruited from a ‘Transient Education Center’ in Türkmenadağı province. At the time of the contact 147 children and adolescent were attending school. It was planned to reach all of the children between ages 7 to 17. Teachers informed children and their parents and invited them to participate. Both children and their parents/guardians gave informed consent for the participation in the study. Children and their parent’s anonymity preserved. Research documents were sent to parents by teachers. From 104 students who were studying from 2nd to 8th grades only 85 brought reports back. We excluded eight forms because of missing data. The research was approved by the Ethics Council of the medical faculty of Sakarya University.

Measures
The participants were asked to fill strengths and difficulties questionnaire (SDQ) which is a widely used scale to screen for psychosocial problem among children23-25. SDQ has 25 items and five distinct subscales that aim to evaluate hyperactivity, peer problems, emotional symptoms, conduct problems and prosocial behaviours26. The total difficulties score (TDS) reveals sum of subscales except for prosocial subscale27. The scale has been translated, and validated in the Arabic language28 and has been used in various Arabic spoken countries29. Although it is planned to gather information both from children and their parents using children and parent-reported SDQ, we could not do that because of the differences in age requirements between parent-reported SDQ and children and their parents using children and parent-reported SDQ, we could not do that because of the differences in age requirements. The participants were asked to fill strengths and difficulties questionnaire (SDQ) which is a widely used scale to screen for psychosocial problem among children23-25. SDQ has 25 items and five distinct subscales that aim to evaluate hyperactivity, peer problems, emotional symptoms, conduct problems and prosocial behaviours26. The total difficulties score (TDS) reveals sum of subscales except for prosocial subscale27. The scale has been translated, and validated in the Arabic language28 and has been used in various Arabic spoken countries29. Although it is planned to gather information both from children and their parents using children and parent-reported SDQ, we could not do that because of the differences in age requirements. The participants were asked to fill strengths and difficulties questionnaire (SDQ) which is a widely used scale to screen for psychosocial problem among children23-25. SDQ has 25 items and five distinct subscales that aim to evaluate hyperactivity, peer problems, emotional symptoms, conduct problems and prosocial behaviours26. The total difficulties score (TDS) reveals sum of subscales except for prosocial subscale27. The scale has been translated, and validated in the Arabic language28 and has been used in various Arabic spoken countries29. Although it is planned to gather information both from children and their parents using children and parent-reported SDQ, we could not do that because of the differences in age requirements.

Data analysis
We analysed the emotional and behavioural data which was gathered by SDQ using two different methods. The first method was used to assess mean scores for each subscale of SDQ. The analysis focused on children whose scores were above the cut-off values according to the UK SDQ site in the second method of evaluation. We used Statistical Package for the Social Sciences (SPSS 20) for data analysis. We analysed the frequency of demographic variables and war-related traumatic events by descriptive statistics. The $\chi^2$ test was used to compare groups for categorical variables. We used Students’ t-test to analyse ordinarily distributed questionnaire scores and, Mann–Whitney U-test for non-ordinarily distributed scores. Multiple logistic and linear regression analyses were performed to explore the predictive values of independent variables on the having higher SDQ subscale scores than the cut off values. We used logistic regression analysis to examine significant differences between psychiatric cases and non-cases according to the cut-off values according to the SDQ UK site.

Results

Socio-demographical characteristics
The study sample consisted of 77 children and adolescents from age 7 to 17. Of the 77 participants, 49 (63.6%) were girls, and 28 (36.4%) were boys. There were 30 (39%) adolescents (ages 13 to 17) and 47 (61%) children (age 7 to 12). Almost all attendants were living with their parents, while one child was separated from his parents and was living with his relatives. The satisfaction of life was evaluated using multiple choice questions (Yes, No and Do not know) such as: are you satisfied with your life in Turkey. There was no statistically significant difference in socio-demographical features and war-related or daily life stressors between girls and boys as shown in Table 1. Similarly, no difference was observed in socio-demographical features, war-related or daily life stressors between child and adolescent age groups except that younger children were more pessimistic about the fate of the war ($\chi^2 = 4.053$, p = 0.044).

War related traumatic events
Children assessed for the history of exposure to common war-related events that were shown in Figure 1. Although, mean experienced traumatic events were higher among boys (m = 3.28) than girls (m = 2.71) the difference was not statistically significant.

Table 1. Socio-demographical variability and gender differences

<table>
<thead>
<tr>
<th></th>
<th>Girls n = 49</th>
<th>Boys n = 28</th>
<th>Total n = 77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>12.2 ± 2.33</td>
<td>11.8 ± 2.17</td>
<td>12.07 ± 2.27</td>
</tr>
<tr>
<td>Children (7-12</td>
<td>29 (59.2%)</td>
<td>18 (64.3%)</td>
<td>47 (61.0%)</td>
</tr>
<tr>
<td>Adolescent (13-17)</td>
<td>20 (40.8%)</td>
<td>10 (35.7%)</td>
<td>30 (39.0%)</td>
</tr>
<tr>
<td>Fathers Age</td>
<td>44.5 ± 6.98</td>
<td>41.9 ± 6.99</td>
<td>43.5 ± 7.05</td>
</tr>
<tr>
<td>Mothers Age</td>
<td>37.1 ± 7.3</td>
<td>35.5 ± 6.87</td>
<td>37.1 ± 7.30</td>
</tr>
<tr>
<td>Sibling</td>
<td>4.3 ± 1.73</td>
<td>3.5 ± 1.85</td>
<td>4.0 ± 1.81</td>
</tr>
<tr>
<td>Time after resettlement (Month)</td>
<td>28.9 ± 10.9</td>
<td>31.5 ± 12.1</td>
<td>29.8 ± 11.2</td>
</tr>
<tr>
<td>Traumatic Events</td>
<td>2.71 ± 1.89</td>
<td>3.28 ± 1.80</td>
<td>2.92 ± 1.86</td>
</tr>
<tr>
<td>Having relatives in Turkey</td>
<td>44 (98.9%)</td>
<td>24 (85.7%)</td>
<td>68 (88.3%)</td>
</tr>
<tr>
<td>Having Friends in Turkey</td>
<td>46 (93.9%)</td>
<td>26 (96.3%)</td>
<td>72 (94.7%)</td>
</tr>
<tr>
<td>Fathers Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary School</td>
<td>32 (85.3%)</td>
<td>18 (64.3%)</td>
<td>50 (64.9%)</td>
</tr>
<tr>
<td>High School</td>
<td>12 (24.5%)</td>
<td>6 (21.4%)</td>
<td>18 (23.4%)</td>
</tr>
<tr>
<td>University</td>
<td>5 (10.2%)</td>
<td>4 (14.3%)</td>
<td>9 (11.7%)</td>
</tr>
<tr>
<td>Mothers Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary School</td>
<td>28 (57.1%)</td>
<td>20 (71.4%)</td>
<td>48 (62.3%)</td>
</tr>
<tr>
<td>High School</td>
<td>14 (28.6%)</td>
<td>6 (21.4%)</td>
<td>20 (26.0%)</td>
</tr>
<tr>
<td>University</td>
<td>7 (14.3%)</td>
<td>2 (7.1%)</td>
<td>9 (11.7%)</td>
</tr>
<tr>
<td>Speaking Turkish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can’t Speak</td>
<td>11 (22.4%)</td>
<td>2 (7.1%)</td>
<td>13 (16.9%)</td>
</tr>
<tr>
<td>A bit</td>
<td>17 (34.7%)</td>
<td>14 (50.0%)</td>
<td>31 (40.3%)</td>
</tr>
<tr>
<td>Well</td>
<td>21 (42.9%)</td>
<td>12 (42.9%)</td>
<td>33 (42.9%)</td>
</tr>
<tr>
<td>Dissatisfaction with life in Turkey</td>
<td>17 (34.7%)</td>
<td>13 (46.4%)</td>
<td>30 (39.0%)</td>
</tr>
<tr>
<td>Lack of food or shelter</td>
<td>8 (17.0%)</td>
<td>9 (33.3%)</td>
<td>17 (23.0%)</td>
</tr>
<tr>
<td>Not Believing that</td>
<td>6 (12.2%)</td>
<td>4 (14.3%)</td>
<td>10 (13.0%)</td>
</tr>
<tr>
<td>The War will End</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wish to Turn Back to Hometown</td>
<td>45 (91.8%)</td>
<td>24 (85.7%)</td>
<td>69 (89.6%)</td>
</tr>
</tbody>
</table>
Emotional and behavioural problems

To assess the emotional and behavioural problems SDQ questionnaire was used. We analysed SDQ data with two sets of analysis. The first set of analyses examined the mean SDQ subscale scores as shown in Table 2. The second set of analysis was made according to the cut-off values that were proposed by the SDQ UK site to predict possible psychopathology as illustrated in Table 3.

As can be seen from the Table 2, the mean score for emotional problems was significantly higher among girls (t = 2.065, p = 0.043) and conduct problem score was significantly higher among boys (t = 2.980, p = 0.004). Childrens (7-12 years old) mean prosocial behaviour score was considerably higher than adolescents’ (13-17 years old) mean prosocial behaviour score (t = 2.054, p = 0.043). No more significant difference was observed between genders and child and adolescent age groups.

Prevalence of children and adolescents whom their SDQ subscale scores were above the cut-off values that have been proposed by SDQ UK site are shown in Table 3. From the data in Table 3, it is apparent that almost two-thirds of the participants (64.9%) reported having possible peer problems and around half of the participants (45.5%) have possible emotional problems.

Traumatic events and emotional or behavioral problems

Number of traumatic events were significantly correlated to TDS (r = 0.318, p = 0.005), conduct problems (r = 0.305, p = 0.07) and hyperactivity scores (r = 0.274, p = 0.016). No statistically significant correlation was found between the number of experienced traumatic events and other SDQ subscales.

SDQ subscale correlations

Analysis of correlations between SDQ subscores showed negative correlation of prosocial behaviour with emotional (r = -0.323, p = 0.04), conduct (r = -0.281, p = 0.01) and peer (r = -0.338, p = 0.00) problems. Hyperactivity was positively correlated to conduct (r = 0.450, p = 0.00) and peer (r = 0.263, p = 0.02) problems. Peer problems were also positively correlated to conduct problems (r = 0.335, p = 0.00).

Comparison of children above and below the cut-off values for SDQ problem scores

Children with higher TDS scores more commonly reported to have a parent with maltreatment or torture history during the war than children with lower TDS score (χ² = 4.014, p = 0.045).

Children with higher conduct problem scores more commonly reported to not feel satisfied in Turkey (χ² = 9.637, p = 0.008), and have a familiar person left behind (χ² = 5.331, p = 0.021). They also more commonly reported witnessing to insult of someone (χ² = 6.174, p = 0.013) and having a parent with maltreatment or torture history during the war (χ² = 6.027, p = 0.014).

Fathers of children with higher emotional problem scores were significantly less educated when compared to children with lower emotional problem scores (χ² = 9.052, p = 0.011).

Children with hyperactivity more commonly reported to be dissatisfied in Turkey (χ² = 6.132, p = 0.047) and, had lost a familiar person due to the war (χ² = 5.193, p = 0.023). They also more commonly reported to have a close person left behind (χ² = 5.713, p = 0.017), had seen corpses or body parts (χ² = 4.785, p = 0.029).

Table 2. SDQ subscale scores according to gender and age groups

<table>
<thead>
<tr>
<th>N: 77</th>
<th>Gender</th>
<th>Age Groups</th>
<th>Total Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Girls</td>
<td>Boys</td>
<td>7-12</td>
</tr>
<tr>
<td>Emotional problems</td>
<td>3.05 ± 2.3</td>
<td>2.64 ± 1.8</td>
<td>2.89 ± 2.0</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>3.75 ± 1.9</td>
<td>4.35 ± 2.3</td>
<td>4.23 ± 2.0</td>
</tr>
<tr>
<td>Peer problems</td>
<td>2.95 ± 1.4</td>
<td>3.14 ± 1.4</td>
<td>3.00 ± 1.5</td>
</tr>
<tr>
<td>Conduct problems</td>
<td>1.61 ± 1.3**</td>
<td>2.57 ± 1.4**</td>
<td>2.04 ± 1.4</td>
</tr>
<tr>
<td>Prosocial behaviour</td>
<td>7.73 ± 1.8</td>
<td>8.32 ± 1.3</td>
<td>8.25 ± 1.6*</td>
</tr>
<tr>
<td>Total difficulties</td>
<td>11.97 ± 4.6</td>
<td>12.71 ± 5.1</td>
<td>12.17 ± 4.5</td>
</tr>
</tbody>
</table>

Man-Whitney U test; * = p < 0.05; ** = p < 0.01.

Table 3. Rates of children above the cut-off values

<table>
<thead>
<tr>
<th>SDQ Subscale N(%)</th>
<th>Age groups</th>
<th>Genders</th>
<th>Total N:77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotional problems</td>
<td>20 (42.6)</td>
<td>15 (50.0)</td>
<td>7 (25.0)*</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>9 (19.1)</td>
<td>6 (20.0)</td>
<td>6 (21.4)</td>
</tr>
<tr>
<td>Peer problems</td>
<td>29 (61.7)</td>
<td>21 (70.0)</td>
<td>20 (71.4)</td>
</tr>
<tr>
<td>Conduct problems</td>
<td>14 (29.8)</td>
<td>7 (23.3)</td>
<td>15 (53.6)**</td>
</tr>
<tr>
<td>Prosocial</td>
<td>1 (2.1)</td>
<td>1 (3.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total difficulties</td>
<td>18 (38.3)</td>
<td>12 (40.0)</td>
<td>12 (42.9)</td>
</tr>
</tbody>
</table>

Chi square, * = p < 0.05; ** = p < 0.01.
and have a parent with maltreatment or torture history during the war ($\chi^2 = 5.831, p = 0.016$). Children whose prosocial scores were below the cut-off values more prevalently reported maltreatment in Syria ($\chi^2 = 4.853, p = 0.028$), have witnessed other people getting killed or injured around them ($\chi^2 = 6.717, p = 0.010$) and, have a parent with maltreatment or torture history during the war ($\chi^2 = 5.476, p = 0.019$).

Risk factors
Regression analysis was used to predict the possible risk factors. According to the analysis, history of parent’s maltreatment was the only predictor of higher TDS ($B = -1.035$, Sig = 0.045). Being girl ($B = 1.508$, Sig = 0.07) and having less educated father ($B = 1.151$, sig = 0.08) were predicting the development of emotional problems while not being satisfied in resettlement was predicting conduct problems ($B = -0.981$, Sig = 0.025).

Discussion
Our findings indicate very high rates of emotional and behavioural problems among refugee children and adolescents when compared to previous study findings that were carried out with refugee children28,32,35,36. Similarly, our findings showed high prevalent rates for exposing to severe traumatic events such as loss of a familiar person, being confronted with body parts and witnessing blasts and clashes24,32,35,36. Although first studies that investigated if mental wellbeing of children is adversely affected by violence and related experiences were carried out only 30 years ago today it is a well-known fact that war-related adversities have pervasive adverse effects on mental health of child survivors27. Results also indicated high rates of traumatic exposure which may have an everlasting effect on mental health of children. Exposure to traumatic events and inability to reach essential life requirements are prevalent among refugee children34. Although first studies that investigated if mental wellbeing of children is adversely affected by violence and related experiences were carried out only 30 years ago today it is a well-known fact that war-related adversities have pervasive adverse effects on mental health of child survivors27. Our findings also pointed out increased emotional and behavioural problems among child survivors of the Syrian war by showing very high rates of possible peer, emotional and conduct problems.

Previous studies which were carried out with Syrian refugee children reported high rates of severe traumatic experiences such as loss of a familiar person, being confronted with body parts and witnessing blasts and clashes24,32,35,36. Similarly, our findings showed high prevalent rates for exposing to severe traumatic events such as loss of a familiar person due to the war, witnessing of clashes or blasts and exposed to corpses or body parts. Despite United Nations (UN) agreement to protect children during wars, our findings, like previous study results7,20,34,37, indicate very high rates of traumatic events among child survivors of wars.

Lenore Terr, who showed that traumatic events adversely affect the mental well-being of children for the first time48, pointed out everlasting effects of childhood traumatic events on mental health49. Other studies which investigated effects of traumatic events on psychosocial wellbeing of children also have shown the adverse results of such experiences on mental health1,2,40,41. Beyond the traumatic events to which refugee children had been exposed before the flight, they commonly face various stressors in the resettlement42. Accordingly, the most of the refugees who live in Turkey do not live in camps41. What means compromised conditions, inability to access medical and education services, lack of sheltering and food43 that may lead to worst mental consequences among refugees in Turkey. Although we observed very high rates of emotional and behavioural problems, we can not generalise our findings to all refugee children because of the sample selection. Indeed, our study sample consisted of school children while only one out of three school-age children could attend school in Turkey5.

Both pre and post flight traumatic events and stressors may lead mental disorders in refugee children11,44. In a comparative study which was carried out with refugee, ethnic minority and indigenous children in the UK, results pointed out higher SDQ scores for refugee children than both ethnic minority and native children50. Various recent studies have been carried out using questionnaires also indicated increased emotional and behavioural problems among refugee children24,40,46. Results of a recent study which was carried out with Syrian refugee children who were accepted to Germany showed that one out of three children has PTSD46. Several other studies also reported high rates of PTSD and depression among Syrian refugee children15,46,47. Accordingly, our findings which gathered by a well known and widely used screening instrument7,42 revealed very high rates of peer (64.9%), emotional (45.5%), conduct (27.3%) and, hyperactivity (19.5%) problems among refugee kids which point out likelihood of having psychopathology. Very high rates of peer problems which are not commonly studied among child survivors of war is a prominent finding which point out that adverse psychological consequences of war-related experiences might not be limited to emotional problems. Similarly, we have found high rates of conduct problems which is not often studied among child survivors of war and violence. We also found out that hyperactivity scores which may be related to hyperarousal symptoms of PTSD may worsen social functionality by causing peer problems. We think that later studies which will be carried out with refugee children should also focus on behavioural as much as emotional problems.

Although several studies have reported that girls may be more adversely affected by displacement46, it has not been shown in all studies46. However, traumatic events may influence girls and boys in different ways such as boys are prone to emerge externalisation problems and girls are prone to develop internalisation problems after traumatic events46. Accordingly, emotional problems were higher among girls than boys and conduct problems were more prevalent among boys than girls in our study. Previous findings that indicate girls may be affected more adversely by war might be related to the methodology of these studies which investigate internalising problems (depression and anxiety disorders) while only a few studies examine externalising or behavioural problems which are more prevalent among boys46. We think all of the psychiatric disorders must be evaluated precisely to explore effects of war and displacement on the mental health of both, girls and boys.

Children’s reaction to stressful events may vary by age44. It has been reported that depression might be more common after traumatic exposure among older children44. A new study which was carried out with refugee children in Turkey also had reported higher psychiatric disorder rates among older children44. However several study findings have reported war-related adversities might have more adverse effects on younger children’s mental health4. It has been proposed that younger children are more commonly tend to externalise the causes and consequences of events compared to older children44. However, no difference was observed between child and adolescent age groups in any SDQ problem sub-scores in our study sample. We think that when the community is exposed to a single traumatic event children’s different resilience capacities may cause different reactions. But by the accumulation of long-lasting traumatic events such as living in a war-torn area, resilience capacities of every person may break down that make it hard to observe unique reactions and resilience capacities of different individuals.

Our findings which revealed higher prosocial scores among younger children deserves attention. Traumatic events may trigger fight or flight response that may undermine children’s basic trust to others and decrease their social attendance. The results might be interpreted that older children might be more vulnerable to a breakdown in social interactions in the face of war-related adversities. This finding might also be due to increased exposure to traumatic events among older children.

Traumatic events may have a cumulative adverse effect on children’s mental health42,45. Similarly, in both, 7–12 and 13–17 age groups, exposing traumatic events strongly associated with increased emotional and behavioural problems. The number of traumatic events was also predicting higher TDS. These findings may conceptualise as more traumatic events worst mental wellbeing for refugee children. The results also point out the importance of protecting children from any more traumatic incidents as soon as possible to prevent them emerging from further psychological problems.
Prosocial skill is a protective factor against the development of psychosocial problems. Similarly, our findings showed negative correlations between prosocial behaviour and emotional, conduct and peer problems. Children's hyperactivity and conduct scores correlated to peer problems. Neurodevelopmental disorders such as hyperactivity may be a risk factor for the development of other psychiatric disorders by deteriorating social attendance. It is also important to point out that high rates of hyperactivity problems which were observed in our study may be related to PTSD's hyperarousal symptomatology which may lead to irritability, anger, concentration problems and easily startling.

Although several factors found to be associated with higher TDS, parent's history of maltreatment/torture was the only factor predicting children's TDS in logistic regression. The finding points out how parent's previous stressful experiences may have adverse effects on children's mental health. Having a less educated father was also predicting more emotional problems in children. In a new study, similar findings were also reported which pointed out the importance of psychosocial well-being of parents on the mental well-being of their siblings. Fathers with a higher education background may have better-coping strategies to protect their children or may be more successful in maintaining a supportive milieu that might be protective for refugee children.

It has been criticised that treatment of contagious diseases which threat host countries are prioritised while mental health disorders are not attracting attention. Our findings showed high rates of emotional and behavioural problems which might have an everlasting effect on the psychosocial and academic development of children and adolescents. Refugee mental health must be one of the leading components of the support program for refugee children and adolescents that requires strong collaboration with the global community. Despite the substantial evidence which shows psychiatric disorders are prevalent among refugee children, there is only one specialised child psychiatry unit to take care of refugee children which hire a culturally oriented translator in Turkey, the leading refugee-hosting country. We hope that our findings will encourage clinicians, health care workers, and policymakers to provide psychosocial support in the care programs with the collaboration of schools, health care services, and specialised child psychiatry units to help refugee children and adolescents. We also recommend focusing on comparison of mental wellbeing of refugee children who attend to transient education centres and public schools in further studies.

Limitations

It is required to acknowledge several limitations of our study. Psychological assessment of children requires multiple informants and careful observation of the child. Gathering information by questionnaire and using parents as the only source of information are limitations of the present study. Moreover, lack of control sample is another limitation of our study.

In conclusion, the study pointed out high prevalence rates of emotional and behavioural problems among Syrian kids in a school sample, years after resettlement in Turkey. Results also showed high exposure rates to severe traumatic events during the war. Despite high rates of psychological problems that may diminish their social and academic functioning, none of the children was able to reach mental health care services due to several barriers. We think that mental health of refugee children is a public health crisis that requires the collaboration of international community and policymakers to support mental health providers in underdeveloped countries which host the most of refugee children to protect child survivors against the development of future psychiatric disorders and functionality loss that may cause a lost generation.

Conflict of interest

The authors disclosure no conflict of interests.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all the parents and children participated in the study.

References


Dear Editor,

Cognitive disorders and depression are intensively correlated, not only by sharing risk factors, but also by frequently showing similar presentations. This might lead to challenging cases among the elderly population, since, in old age, depression commonly manifests with prominent neurovegetative and cognitive symptoms. Here we report about a case of a patient who had been treated, but after clinical evaluation demonstrated to be a case of sporadic Creutzfeldt-Jakob disease (sCJD), a rare and fatal neurodegenerative condition.

A 77 year-old female, without relevant psychiatric or medical history. Six months before admission, she presented with increasingly irritability and sadness. Within days, clinical condition declined, progressing to mutism, lack of interaction, anorexia, immobility and was treated with different combinations of antidepressants and antipsychotics, with the hypothesis of major depression. She was then referred to the Psychiatry inpatient unit of the local University Hospital for diagnostic investigation and clinical support. Mental status examination at admission was compatible with stupor, but three days later she begin to show spontaneous high amplitude myoclonus and intermittent inhibitory paratonia in inferior limbs, leading us to request Neurology consultation. Previous electroencephalography showed no relevant findings, but new analysis of a previous MRI demonstrated hyperintensity in cortex at DWI, suggestive of sCJD (Figure 1). Cerebrospinal fluid was sent to specific analyses which showed no relevant findings, but new analysis of a previous MRI demonstrated hyperintensity in cortex and caudate at DWI, suggestive of sCJD. Cerebrospinal fluid was sent to specific analyses which showed no relevant findings, but new analysis of a previous MRI demonstrated hyperintensity in cortex and caudate at DWI, suggestive of sCJD (Figure 1).

Figure 1. MRI axial cuts showing hyperintensity in areas of the cortex (cortical ribboning) and in caudate nucleus at the DWI sequence.

Definite diagnosis of sCJD can only be made through direct analysis of brain tissue. A probable diagnosis is established in the presence of rapidly progressive dementia and two of the following: akinetic mutism, myoclonus, visual/cerebellar signs, pyramidal/extrapyramidal signs; plus one of the tests: EEG with periodic sharp-wave complexes, positive 14-3-3, or MRI with high signal abnormalities in caudate/putamen on DWI or FLAIR; and with presence of rapidly progressive dementia and two of the following: akinetic mutism, myoclonus, visual/cerebellar signs, pyramidal/extrapyramidal signs; plus one of the tests: EEG with periodic sharp-wave complexes, positive 14-3-3, or MRI with high signal abnormalities in caudate/putamen on DWI or FLAIR; and with stringent diagnostic criteria. Even when a diagnosis of CJD seems probable, the lack of definite diagnosis and the irreversibility of the condition, frequently leads clinicians to attempt empirical treatment for other possible etiologies.

This case demonstrates that sCJD is a possible underdiagnosed disease that can be clinically presented masquerading as major depressive disorder. We encourage close collaboration between Neurology and Psychiatry when faced with a patient presenting with a rapid and progressive cognitive deterioration.

References