RESEARCH ARTICLE

Folic Acid and Vitamin B12 Supplementation on Male Patients with Schizophrenia Predominant Negative Symptoms

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Abstract: Background: Schizophrenia is a chronic mental disorder that affects approximately 1% of the world’s population. Particularly, negative symptoms are frequently resistant and are the main contributors to the disability on schizophrenia patients. Folic acid and vitamin B12 supplementation is the safe and affordable approach, which can significantly improve the outcome on the patients with residual symptoms.

Objectives: We aimed to understand the difference of negative subscale of Positive and Negative Syndrome Scale (PANSS) Score on patients with schizophrenia who receive risperidone with the addition folic acid and Vitamin B12 and patients who receive only risperidone after 6 weeks.

Methods: This study is a quasi experimental pre-test and post-test designs that are divided into two groups, they are group receiving risperidone with the addition of folic acid and vitamin B12 and the group receiving only risperidone. Diagnosis of schizophrenia according to the International Classification of Diseases (ICD-10) criteria and negative symptoms assessed by using negative subscale of PANSS Scores are observed. Statistical analysis is conducted using Statistical Package for the Social Sciences (SPSS) software.

Results: There was a significant difference in the mean score of negative-scale PANSS scores at the end of week 6 between the group receiving folic acid and vitamin B12 supplementation and the group receiving only risperidone with p = 0.002; p <0.05.

Conclusion: Folic acid and vitamin B12 Supplementation provide benefits to patients schizophrenia.

Keywords: Folic acid, homocysteine, negative symptom, risperidone, schizophrenia, vitamin B12.

1. INTRODUCTION

Schizophrenia is a debilitating and usually chronic disease associated with social, cognitive, and emotional dysfunctions [1]. Schizophrenia affects about 1% of the world's population and the peak of schizophrenia disease usually occurs in late adolescence or early adulthood [2]. Negative symptom is a disorder that generally includes inability to experience pleasure, speech impairment, flat affects, and lack of motivation [3].

Negative symptom involves the significant decline or the reduction of the normal functions, usually includes affective experience (anhedonia), interest and motivation (volition or apathy), social drive or interest and willingness to affiliate (asocial), expressive or communicative behavior, (flat or blunt affect) [4-7], but it has to be obvious that all of the above are not caused by depression or neuroleptics medication [8].

Folic Acid is a natural vitamin B (vitamin B9) consisting of three primary structures, a hetero-bicyclic pteridine ring, Para Amino Benzoic Acid (PABA), and glutamic acid [9, 10]. The richest sources are yeast, liver, kidney, and green vegetables, typically, 5-20 mg of folate is stored in the liver and other tissues [11]. Folic acid is also involved in the synthesis of neurotransmitters and in other metabolic pathway [9].

Vitamin B12 is one of the elements of vitamin B which is a water-soluble vitamin and is also known as cobalamin or cyanocobalamin. Vitamin B12 deficiency causes the deficiency of the folic acid cofactors which is required for some biochemical reactions involving the change of one carbon unit [12].

Negative symptoms are often resistant to treatment and are the major contributor to the disability in schizophrenia. Folic acid supplementation and vitamin B12 is a safe and inexpensive approach that significantly improves outcome in patients with residual symptoms [4, 13]. The role of vitamin supplementation has been described in some psychiatry case studies. Vitamins and minerals are involved in one or more biochemical and/or physiological pathways that affect the...
functions of the human brain and play an important role in the number of vital functions, acting as co-factors or co-enzymes and catalyzing a number of reactions occurring in the body [14].

Although the pathophysiology of schizophrenia remains unknown, biological studies support that high level of homocysteine play an important role that can lead to neurodegeneration in schizophrenia [15]. Homocysteine is an endogenous amino acid, containing the free thiol group, which is involved in the synthesis of methionine and cysteine. Indirectly, it plays a role in the methyl, folate, and thiol cell metabolism, where homocysteine is converted into methionine which can reduce homocysteine levels [16].

Haidemenos (2007) reported increased plasma levels of homocysteine amino acid that have been associated with schizophrenia, low levels of folic acid and vitamin B12 are involved in elevated levels of homocysteine [17]. Hill et al. (2011) reported that the addition of folic acid is a treatment strategy that can lower homocysteine levels in schizophrenia patients [18].

The deficiency of folic acid and vitamin B12 may contribute to the pathogenesis of neuropsychiatric disorders such as mental disorders, memory changes, cognitive impairment, mood disorders, violent behavior, fatigue, delirium and paranoid psychosis [19]. Interactions of environment and genes, where dietary intake that contains folic acid with genetic variant generally affect the metabolism of folic acid. This has potential implications on the pathogenesis and the treatment of schizophrenia [20].

Approximately 20-40% of individuals diagnosed with schizophrenia experience persistent symptoms and long-lasting negative symptoms [21]. Changes in the neurotransmitter system in both primary and secondary neurodevelopmental forms arise from the use of dopaminergic inhibitors may become the predisposition of one to develop a negative symptom in schizophrenia. [22].

The negative symptoms of schizophrenia including anhedonia, lack of motivation, and speech poorness, are caused by the reduced activation of D1 receptors in the prefrontal cortex and decreased activity of the caudatus nucleus [23, 24].

The abnormalities in the receptor function of N-methyl-D-aspartate (NMDA) seem to disrupt brain plasticity (the ability to form long-term synaptic connections), which can cause not only psychosis but also negative symptoms and cognitive impairment of schizophrenia [25]. Serotonin also plays a role in negative symptoms, cholinesterase inhibitors indicate a signal for the improvement of negative symptoms [26].

Fisekovic et al. (2013), reported that there was a significant difference in the levels of folic acid among schizophrenia patients (5.94 ± 2.30) in Clinical Centre University of Sarajevo with control group (15.11 ± 5.42) with p ≤0.001 [27]. Low folic acid levels in schizophrenia were also reported by Kim and Moon (2011) [28].

Misiak (2014) reported that serum levels of vitamin B12 and folic acid were significantly lower in patients with schizophrenia receiving antipsychotic treatment of olanzapine and risperidone (p <0.001), on the use of risperidone there was a difference in negative symptom at baseline and after 12 weeks of treatment measured with PANSS rating scale [29].

Based on these information, it can be concluded that the deficiency of folic acid and vitamin B12 are common in patients suffering from schizophrenia. Therefore, supplementation of folic acid and vitamin B12 as the coenzyme can be an alternative to the process of treatment of schizophrenia with negative symptom that is affordable and efficient.

2. MATERIALS AND METHOD

2.1. Data Resources and Subject

This study is a quasi-experimental study of pre-test and post-test that recruited as many as 60 Schizophrenia patients who were hospitalized at Prof. Muhammad Ildrem North Sumatera Hospital, from September to November 2017 and recruited using non probability sampling, which was consecutive sampling, divided into two groups, one is the group receiving risperidone with the addition of folic acid and vitamin B12 and another group receiving risperidone only.

The inclusion criteria were male patients with a diagnosis of schizophrenia according to the International Classification of Diseases (ICD-10) criteria [8], the presence of predominant negative symptoms, the negative subscale of PANSS scores with each item ≥ 4, on at least three items negative subscale of PANSS scores [25]; the subjects were treated with a typical antipsychotic (risperidone 4mg) [30]; subjects with 25-40 years of age; duration of illness of 5-10 years [31]; and normal Body Mass Index (BMI). The Exclusion criteria were comorbidity; organic brain damage; other medical conditions; alcohol and drug abuse; use of antiepileptic drugs [32]; and supplements containing folic acid and vitamin B12 for 3 months.

2.2. Covariates

The socio-demographic characteristics include age, marital status that is classified into two categories (Married or Single), education level that is categorized as primary education, secondary education and higher education, tribal groups that are categorized as Batak and Non Batak. Other medical conditions were assessed through interviews. Body mass index (BMI) was calculated using weight and height (kg)/height (m2) measurements, with normal values of 18.50-24.99 kg/m2 [33].

2.3. Negative Symptoms Assesment

The implementation of this study strives to follow the standardized patterns and ethics of scientific studies. The study was approved by The Research Ethics Committee of the Faculty of Medicine of University of Sumatera Utara. Male patients with Chronic schizophrenia predominant negative symptoms from Prof. Muhammad Ildrem North Sumatera Hospital Inpatient service recruited for study after signed an informed consent with the submission of information that the data or the confidentiality of the subjects would be guaranteed.

Schizophrenia diagnosed was according to International Classification of Diseases (ICD-10). Negative symptoms
assessed by using negative subscale of PANSS Scores as the baseline to be performed for ten minutes, the negative subscale of PANSS score with each item ≥ 4, on at least three items negative subscale of PANSS scores [25]. In PANSS assessment, suitability test was conducted between the researchers and the interraters. Equation or analysis test for each positive subscale, negative subscale and total PANSS scores were performed with comparative numerical suitability test (Bland Altman).

2.4. Statistical Analysis

Sixty Subjects completed the study allocated into two groups: first group is the intervention group, started the study with risperidone of 4 mg/day/oral divided into 2 doses with the addition of folic acid of 2 gr/day/oral and vitamin B12 400 mcg (30 subjects). The second group is the control group, started the study with risperidone of 4mg/day/oral divided into 2 doses (30 subjects). After 6 weeks, negative symptoms were re-assessed by using negative subscale of PANSS.

One patient did not comply with the treatment regimen and was excluded. One patient developed an extra pyramidal side effect and unresolved side effect after 2 weeks received the trihexyphenidyl with dose range of 5-15mg/day and was excluded [34]. Since this study was designed on the treatment analysis so that for each excluded subjects were replaced with new subjects for data analysis.

All statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) software. Normality test was performed on each group using Saphiro-Wilk test. The test was administered at baseline and at the end of 6 weeks. Analysis was performed by comparing scores PANSS negative subscale between the group of male patients with schizophrenia who received risperidone with addition of folic acid and vitamin B12 and group that received risperidone only. Analysis of changes in PANSS negative subscale scores from baseline to the end of 6 weeks in both group were conducted via Paired T test. While the analysis of PANSS negative subscale scores at the end 6 weeks between the two groups were performed with Unpaired T-test.

3. RESULT

The study included 30 patients with schizophrenia receiving risperidone of 2mg with the addition of 2 mg of folic acid and 400mcg of vitamin B12 (intervention group) and 30 patients receiving 2mg of risperidone only (control group). The demographic characteristics of each group are shown in Table 1. The average age in the intervention group is 33.73 years while in the control group is 33.33 years. Most tribes in both groups was Batak, which is 18 (60%) in the intervention group and 16 (53.3%) in the control group. The highest level of education in both groups was in secondary education with 13 in amount (50%). Most marital status in both groups is single; in the intervention group is 20 (66.7%) and in control group is 22 (73.3%). The average duration of

<table>
<thead>
<tr>
<th>Sociodemographic Characteristics</th>
<th>Risperidone + Folic Acid and Vitamin B12 n = 30</th>
<th>Risperidone n = 30</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year), Mean</td>
<td>33.73 (3.99)</td>
<td>33.33 (4.00)</td>
<td>0.700*</td>
</tr>
<tr>
<td>Tribe, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batak</td>
<td>18 (60.00)</td>
<td>16 (53.30)</td>
<td>0.795**</td>
</tr>
<tr>
<td>Non Batak</td>
<td>12 (40.00)</td>
<td>14 (46.70)</td>
<td></td>
</tr>
<tr>
<td>Education level, n(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>12 (40.00)</td>
<td>10 (33.30)</td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>13 (43.30)</td>
<td>13 (43.30)</td>
<td>0.773**</td>
</tr>
<tr>
<td>Higher school</td>
<td>5 (16.70)</td>
<td>7 (23.30)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>10 (33.33)</td>
<td>8 (26.70)</td>
<td>0.799**</td>
</tr>
<tr>
<td>Single</td>
<td>20 (66.70)</td>
<td>22(73.30)</td>
<td></td>
</tr>
<tr>
<td>Duration of illness (year), Mean</td>
<td>7.63 (1.56)</td>
<td>7.53 (1.57)</td>
<td>0.806*</td>
</tr>
<tr>
<td>Body Mass Index, Mean</td>
<td>22.65 (1.43)</td>
<td>22.46 (1.19)</td>
<td>0.584*</td>
</tr>
<tr>
<td>PANSS Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive subscale, Mean</td>
<td>12.97 (1.92)</td>
<td>12.93 (1.93)</td>
<td>0.947*</td>
</tr>
<tr>
<td>Negative subscale, Mean</td>
<td>32.77 (2.65)</td>
<td>32.33 (2.44)</td>
<td>0.512*</td>
</tr>
<tr>
<td>Total Score, Mean</td>
<td>75.07 (1.98)</td>
<td>74.93 (2.35)</td>
<td>0.813*</td>
</tr>
</tbody>
</table>

*Unpaired T test; **Chi square test.

Table 1. Demographic data of the subjects.
Table 2. The difference of the mean of negative subscale PANSS score on male patients with schizophrenia receiving risperidone with addition of folic acid and vitamin B12.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Mean Difference</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0 (n=30)</td>
<td>32.77 (2.65)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 6 (n=30)</td>
<td>24.17 (3.64)</td>
<td>8.60 (2.08)</td>
<td>7.82 - 9.37</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Paired T Test; Difference of Week 0 and Week 6.

Table 3. The difference of the mean of negative subscale PANSS score on male patients with schizophrenia receiving risperidone only.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Mean Difference</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0 (n=30)</td>
<td>32.33 (2.44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 6 (n=30)</td>
<td>26.70 (1.97)</td>
<td>5.63 (1.85)</td>
<td>4.94 - 6.32</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Paired T Test; Difference between Week 0 and Week 6.

Table 4. The difference of the mean of negative subscale PANSS score between male patients with schizophrenia receiving risperidone with addition of folic acid and vitamin B12 and patients receiving risperidone only.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>p-value</th>
<th>Mean Difference (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risperidone + Folic Acid and Vitamin B12</td>
<td>30</td>
<td>24.17 (3.64)</td>
<td>0.002</td>
<td>2.53 (1.021 - 4.045)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>30</td>
<td>26.70 (1.96)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unpaired T Test.

illness in the intervention group is 7.63 and the control group is 7.53. The average body mass index in the intervention group is 22.65 kg/m² and in the control group is 22.46 kg/m². The average positive subscale PANSS scores in the intervention group are 12.97 and in the control group is 12.93. The mean of negative subscale PANSS scores in the intervention group is 7.53 and in the control group is 7.63. The average body mass index in the intervention group is 22.65 kg/m² and in the control group is 22.46 kg/m².

Based on the result of comparative test between characteristic demographic variables of the subject, there were no significant difference for average age, tribe, marital status, education level, duration of illness, body mass index and PANSS scores in both groups (p-value <0.05).

The change from baseline to end point in negative subscale PANSS scores on male patients with schizophrenia receiving risperidone with addition of folic acid and vitamin are shown in Table 2. The mean of negative subscale PANSS score in the group receiving risperidone with the addition of folic acid and vitamin B12 at week 0 is 32.77±2.65 and at the end of week 6 is 26.70±1.97. The result of the analysis using paired T-test resulted a significant difference from negative subscale PANSS score at week 0 and end of week 6 (p-value <0.001; p-value <0.05).

The difference of the mean of negative subscale PANSS score on group receiving risperidone only are shown in Table 3. The mean of negative subscale PANSS score in the group receiving risperidone only at week 0 is 32.33±2.44 and at the end of week 6 is 26.70±1.97. The result of the analysis using paired T-test resulted a significant difference from negative subscale PANSS score at week 0 and end of week 6 (p-value <0.001; p-value <0.05).

Table 4 reveals the difference of the mean of negative subscale PANSS scores between male patients with schizophrenia receiving risperidone with addition of folic acid and vitamin B12 and patients receiving risperidone only. Using Unpaired T-test, the mean of negative subscale PANSS scores on the group receiving risperidone with the addition folic acid and vitamin B12 is shown to be 24.17±3.64, compared to the group who receiving risperidone only with 26.70±1.97. Finally, a significant difference between the treatment effect in improvement in negative subscale PANSS scores is confirmed (p-value=0.002; p <0.05), with the mean difference of 2.53.

4. DISCUSSION

This study reported that 6 weeks of folate and vitamin B12 as supplementation to treat male patients with schizophrenia predominant negative symptoms has demonstrated a clinical benefit. There were significant difference between the mean of negative subscale PANSS scores in group receiving risperidone and addition of folic acid and vitamin B12 and group receiving risperidone only, based on the negative subscale PANSS score. PANSS is the
instrument has demonstrated good validity and reliability for assessing the negative symptoms. The risperidone dose used was 4 mg in divided doses of 2 mg, the dose of folic acid was 2 g/day/oral and vitamin B12 was 400 mcg/day/oral.

In this study, the gender included was male because of the physiological differences such as weight, height, body surface area, total body water, and body fat between male and female affecting the body’s response to drugs. Folic acid intake is usually higher in male patients than in females, male patients will get a lower response biologically because male patients have larger body sizes so the dose distributed should also be greater [35].

Based on the result of the comparative test between characteristic variables of the subject, we have concluded that there were no significant differences between mean age, tribe, marital status, education level, duration of illness, body mass index and PANSS score.

The mean of negative subscale PANSS score in the group receiving risperidone treatment with addition of folic acid and vitamin B12 in week 0 was 32.77±2.65 and in the end of week 6 was 24.17±3.64. Using paired T-test we have concluded that significant difference in the negative-scale PANSS scores in week 0 and in the end of week 6 with the p-value <0.001. Our result are compared with the results Roffman et al. (2013) on 140 subjects who received risperidone and randomly added 2 g of folic acid and vitamin B12 400 mcg or placebo orally, have shown that patients receiving folic acid and vitamin B12 showed a significant decrease in negative symptoms (r = -0.19 SANS changes per week, 95% CI (-0.35 to -0.03), p = 0.02) and subjects given placebo showed no change (0.02 per week ; 95% CI -0.21 to 0.24; p = 88). These results suggest that the administration of antipsychotic drugs with folic acid supplementation and B12 improves the negative symptoms of schizophrenia [12].

The mean score of negative subscale PANSS in groups receiving risperidone only at week 0 was 32.33±2.44, and the end of week 6 was 26.70±1.97. Using paired T-test, it can be concluded that negative subscale of PANSS score on week 0 and end of week 6 were significantly different (p<0.001; p<0.05). This is consistent with studies conducted by Misia et al. (2014), which reported that serum levels of vitamin B12 and folic acid were significantly lower in patients with schizophrenia receiving antipsychotic treatment of olanzapine and risperidone (p<0.001), on the use of risperidone there was a negative symptom difference in baseline and after 12 weeks of treatment as measured by PANSS (p<0.001) [29].

A study conducted by Goff et al. (2004) reported the average level of folic acid in the group of people with schizophrenia was 5.74 ng/ml significantly lower than folic acid levels in the population of 10.00 ng/mL (p<0.001). The concentration of folic acid was significantly correlated with the severity of the negative symptom assessed according to the total Scale for Assessment of Negative Symptoms (r = -0.31, N = 91, p < 0.01) [36].

Our study reported that the mean of negative subscale PANSS scores after 6 weeks in the group receiving folic acid and vitamin B12 was 24.17±3.64 and group receiving risperidone only 26.70±1.97. According unpaired T-test we have concluded that mean of negative subscale PANSS scores at the end of week 6 between group receiving risperidone and addition of folic acid and vitamin B12 with group receiving risperidone only was significant difference for p = 0.002; p<0.05.

This is in accordance with a study reported by Levine et al. (2006) on the effectivity of addition of folic acid and vitamin B12 in the improvement of negative symptoms. In their study they performed the addition of folic acid 2 g and vitamin B12 400mcg, double blind, trial crossover with one tablet daily. After 3 months, it was found that in the intervention group the mean of negative symptom at baseline was 22.4±1.1 and post-test 2.9±0.5, the mean of negative symptom in baseline control group was 20.4±1.1 and posttest 0.9±0.5, statistically there were a significant difference in mean of negative symptom at control and intervention (p<0.001). They concluded that the addition of folic acid and vitamin B12 improved the negative symptoms in schizophrenia [37].

Although, in contrast to the result discovered by Hills et al. (2011) who assessed the effectiveness of supplementation therapies of folic acid and vitamin B12 in improving scores of PANSS chronic schizophrenia, reported that folate supplementation did not significantly affect the negative symptoms compared to placebo. However, there was a significant genotype versus the treatment effect on negative symptoms (F=7.13, df=1,39, P=0.01). In addition, Methyltetrahydrofolate Reductase (MTHFR) status significantly moderated the relationship between the change in folate serum folate and in negative symptoms (P=0.03) [18].

The increased levels of homocysteine in general population are caused by low levels of folate and vitamin B12 that function in one-carbon metabolism [38]. Therefore, a high folic acid consumption in schizophrenia patients provides an advantage by lowering the homocysteine levels, since folic acid is acting as a cofactor for the synthesis of methionine by transferring the methyl group to homocysteine [39]. Homocysteine acts as a methyl donor when converted to Sadenosyl-methionine, and shows a significant association between the total homocysteine and DNA methylation in schizophrenia, suggesting that homocysteine plays a role in the pathogenesis of schizophrenia through alterations in DNA methylation [40, 41].

The metabolism of folic acid, as well as its utilization, becomes increasingly important in schizophrenia. Folic acid is a water-soluble vitamin B that is involved in the synthesis, repair and methylation of Deoxyribonucleic Acid (DNA), whose use is effective depending on adequate daily intake, and genetically altered metabolism [3]. Studies conducted by Ramaekers and colleagues (2014) reported that 15 of 18 schizophrenia subjects (83.3%) had serum folate receptor alpha (FRa) positive antibodies compared with controls of only 1 of 30 subjects (3.3%) (p<0.0001). The administration of folic acid (0.3-1 mg/kg/day) in patients for at least 6 months resulted in clinical improvement [42].

CONCLUSION

Our study finding support that supplementation of folic acid 2 g and vitamin B12 400 mcg contribute to improve
outcomes in patients with negative symptoms. Supplementation of folic acid and vitamin B12 is safe and affordable approach for schizophrenia treatment.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study has obtained approval from the research ethics committee of Faculty of Medicine, University of Sumatra Utara, Indonesia (Approval number: 525/10/10/2019/KEPK FK USU-RUSP HAM/2017).

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. All reported human were experimented in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

CONSENT FOR PUBLICATION

Informed consent was obtain from the participants.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The author would like to extend his gratitude for the help and encouragement from all of the supervisors, residents, technical staffs, hospital and family who have provided moral and material support so that this research can be completed.

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