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Association of vitamin B12 mediated hyperhomocysteinemia with depression and anxiety disorder: A cross-sectional study among Bhil indigenous population of India

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SUMMARY

Background: Indigenous populations in India are amongst the poorest and most marginalized population groups experiencing severe health deprivation.
Aim: The present study is the first study that aims to understand the association of micronutrient deficiencies (vitamin B12 and folate) and MTHFR C677T gene polymorphism with depression and generalized anxiety disorder (GAD) among the Bhil indigenous population of India.
Methods: A total of 303 participants aged 25–65 years of both sexes and unrelated up to first cousins belonging to Bhil indigenous population were recruited for the present study. Depression and generalized anxiety disorder were assessed using Patient Health Questionnaire and Generalized Anxiety Disorder scale, respectively. Biochemical analysis, DNA extraction and MTHFR C677T gene polymorphism analysis were done using standard protocols.
Results: Although, vitamin B12 and folate status was not found to be directly associated with depression and GAD, but hyperhomocysteinemia was posing more than three folds and six folds significant increased risk for depression and GAD, respectively. Further, it seems hyperhomocysteinemia was mediated by vitamin B12 deficiency among depressed and anxious individuals. T allele of MTHFR C677T gene polymorphism was posing increased risk for depression and anxiety disorder though not significant.
Conclusion: The present study highlights the significance of micronutrient deficiencies in the causation of depression and anxiety disorder.

1. Introduction

The indigenous populations are the weakest sections of the country in view of socio-economic and socio-demographic factors like poverty, low literacy rate, lack of developmental and insufficient health services [1,2]. Most of the studies on indigenous populations in India have focused on issues like hypertension, diabetes, tuberculosis and undernutrition [1,3,4]. Mental health issues are the most neglected concerns in indigenous populations of India. Although the determinants of mental health are complex, the emerging studies suggest that nutritional deficiencies have been associated with various mental disorders [5,6]. However, there is paucity of data on association of nutrition with mental disorders among indigenous populations [7,8] Also, in light of mental disorders like depression and anxiety disorders, genetic predisposition due to mutations in various genes involved in various metabolic pathways have also been broadly studied [9]. Further, mutations in methylenetetrahydrofolate reductase (MTHFR) gene have been widely implicated in mental disorders like depression and anxiety disorders [10], where the T allele of MTHFR C677T gene polymorphism has also been reported to be associated with hyperhomocysteinemia [11]. In the current state, wherein indigenous populations of India are thought of consuming nutrient-poor food, as food availability and accessibility itself is a challenge, makes

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indigenous populations more susceptible to mental disorders. To best of our knowledge, the present study is the first study that aims to understand the association of micronutrient deficiencies (vitamin B12 and folate) and MTHFR C677T gene polymorphism with depression and generalized anxiety disorder among the Bhil indigenous population of India.

2. Materials and methods

2.1. Study population

Bhil is the third largest indigenous population of India [12]. The Bhil are economically very poor [12].

2.2. Data collection

The present cross-sectional epidemiological study was part of a major government-funded project entitled *"Psycho-social and biological determinants of cardiovascular disease*: *a study among Bhils and Minas of Rajasthan, India"*. The aim of the project was to understand, estimate and document the distribution of various cardiovascular diseases and associated risk factors (both traditional and nontraditional) and their interaction with genetic and immunological variables with respect to cardiovascular sub phenotypes among Mina and Bhil indigenous population of Rajasthan. Only individuals who were unrelated upto first cousin, aged 25–65 years (both males and females) and belonged to Bhil indigenous population were recruited after taking and informed written consent in the present study. The study was approved by the Ethical Committee, Department of Anthropology, University of Delhi, Delhi, India. Fieldwork, data collection and data analysis for this project was supported by the Delhi University- Department of Science and Technology (DU-DST), Ministry of Science and Technology, Government of India [Grant number RC/2014/7114].

Data related to socio-demographic variables (age, gender, marital status, occupation, and lifestyle habits like smoking and alcohol consumption) was collected using pre-tested and modified interview schedules. Depression and generalized anxiety disorder were assessed using Patient Health Questionnaire (PHQ-9) [13] and Generalized Anxiety Disorder (GAD-7) Scale [14], respectively. PHQ-9 is a 9-item questionnaire that is used for screening purposes in both clinical and research settings [13]. Its total score ranges from 0 to 27, where each of the 9 items is scored from 0 ("not at all") to 3 ("nearly every day). The generalized anxiety disorder-7 (GAD-7) scale was used in this study to determine the presence of generalized anxiety disorder [14]. It consists of 7 items, with each item scored from 0 to 3, its total scores ranges from 0 to 21. Both PHQ-9 and GAD-7 are already translated and validated in Indian populations including indigenous populations [15]. Psychometric analysis of responses for PHQ-9 (Cronbach's $\alpha = 0.964$) and GAD-7 (Cronbach's $\alpha = 0.916$) in the present cohort indicated acceptable internal consistency. The score of 10 on PHQ-9 and GAD-7 was used for cut-off for depression and generalized anxiety disorder, respectively [15,16].

2.3. Sample size calculation

The sample size was calculated to be 271 individuals at 90% confidence interval and considering total population size of Bhil in Rajasthan (4,100,264 individuals) [12] following the standard formula given by Krejice and Morgan [17]. Considering almost 10% dropout rate, sample size was increased to 303 individuals for both depression and anxiety disorder respectively.

2.4. Biochemical and genetic analysis

An overnight fasting blood (5 ml) was drawn by standard venipuncture from all the recruited participants and collected into EDTA coated and without EDTA evacuated tubes. The blood samples were centrifuged at 15,000 rpm, plasma and serum were separated and stored at −80 °C until required for analysis. Plasma homocysteine, folate and vitamin B12 concentration analyses were done using Chemiluminescence Immunoassay technique (Immulite 1000). Quality control were run with each reaction to check inter assay and intra assay variations that were found to be <10%. Vitamin B12 deficiency was defined as concentration <220 pmol/L, folate deficiency as <3 ng/ml and hyperhomocysteinemia as >15 μmol/L [18]. DNA extraction was done using salting out method [19]. The MTHFR C677T gene mutation was detected by using *Hinf* I restriction digestion enzyme digestion [20]

2.5. Statistical analysis

Pearson's Chi-square test was employed for assessing the distribution of various categorical variables. Logistic regression analyses were used to estimate odds ratios (ORs) for being case comparing each category with the reference categories of metabolites and the MTHFR C677T polymorphism. The precision of odds ratios estimates was expressed with 95% confidence intervals (CIs). All the confounders (like age, gender, marital status, occupation, smoking status) have been statistically controlled for adjusted odds ratio analysis. In 2 × 2 tables whenever any cell frequency was observed to be lower than 5, necessary Yates' correction was made. Additionally, Spearman's correlation was used as measures of association for the continuous variables.

3. Results

Depression and generalized anxiety disorder were found to be present in 34.98% and 29.8% of the participants of the studied indigenous population (*communicated elsewhere*). In the present study, it was found that normal and morbid (depressed and anxious) individuals do not seem to differ with respect to the distribution of vitamin B12 and folate status. Also, odds ratio analysis reveals no direct association of vitamin B12 and folate with depression and generalized anxiety disorder. However, individuals with hyperhomocysteinemia were found to be significantly higher in the anxious group (p-0.04) as compared to normal individuals. After adjusting for all the confounders (like age, gender, marital status, occupation, smoking status) odds ratio analysis indicates that hyperhomocysteinemia was posing more than three folds and six folds significant increased risk for depression (suggestive p value-0.07) and generalized anxiety disorder (p value-0.05), respectively. Distribution of MTHFR C677T gene polymorphism seems to be similar in both normal and morbid (depressed and anxious) group. Also, odds ratio analysis found that T allele of MTHFR C677T gene polymorphism was posing increased risk for depression and anxiety disorder though not significant (Tables 1.1 and 1.2).

Further, correlation analysis was performed to investigate the relationship of homocysteine with vitamin B12 and folate in both normal and morbid (depressed and anxious) individuals. It was found that vitamin B12 is significantly and negatively correlated with homocysteine in depressed (p value-0.01) and anxious individuals (p value −0.003). On the other hand, there was no significant correlation was found between folate and homocysteine in both normal and morbid (depressed and anxious) individuals (Table 2). It seems that hyperhomocysteinemia is mediated by

Table 1.1
Distribution of Biochemical variables and MTHFR C677T gene polymorphism among control and cases for depression of Bhil indigenous population of India.

	Depression				
Biochemical variables	Normal N (%)	Depressed N (%)	χ2 p value	Crude Odds Ratio OR (95% CI)	Adjusted Odds Ratio OR (95% CI)
B12 status					
Normal	69 (32.9)	31 (33.3)	0.93	0.97 (0.50–1.64)	1.36 (0.74–2.51)
Deficient	141 (67.1)	62 (66.7)			
Folate status					
Normal	134 (63.8)	64 (68.8)	0.39	0.79 (0.47–1.34)	0.94 (0.52–1.71)
Deficient	76 (36.2)	29 (31.2)			
Homocysteine status					
Normal	18 (8.6)	3 (3.2)	0.09$	2.81 (0.80–9.79)	3.89 (0.86–17.54)$
High	192 (91.4)	90 (96.8)			
MTHFR C667T polymorphism				**Genotypic models**	
				CC vs CT	1.35 (0.58–3.15)
CC	183 (88.8)	78 (85.7)	0.68		
CT	22 (10.7)	12 (13.2)			
TT	1 (0.5)	1 (1.1)		**CC vs CT + TT**	1.28 (0.54–3.03)

N = number of individuals; % = percentage; χ2 = chi-square; CI = confidence interval.
*p value significant level at ≤0.05; $ = suggestive p value significant level at ≤0.09.
OR=Odds Ratio adjusted for confounders (age, education, marital status, occupation, smoking).

Table 1.2
Distribution of Biochemical variables and MTHFR C677T gene polymorphism among control and cases for generalized anxiety disorder of Bhil indigenous population of India.

	Generalized anxiety disorder				
Biochemical variables	Normal N (%)	Depressed N (%)	χ2 p value	Crude Odds Ratio OR (95% CI)	Adjusted Odds Ratio OR (95% CI)
B12 status					
Normal	68 (31.5)	32 (36.8)	0.37	0.79 (0.46–1.33)	1.05 (0.57–1.93)
Deficient	148 (68.5)	55 (63.2)			
Folate status					
Normal	137 (63.4)	61 (70.1)	0.26	0.739 (0.43–1.26)	0.87 (0.47–1.60)
Deficient	79 (36.6)	26 (29.9)			
Homocysteine status					
Normal	19 (8.8)	2 (2.3)	0.04*	4.09 (0.93–17.99)$	6.71 (0.97–46.16)*
High	197 (91.2)	85 (97.7)			
MTHFR C667T polymorphism				**Genotypic models**	
				CC vs CT	1.14 (0.48–2.71)
CC	187 (88.2)	74 (87.1)	0.79		
CT	24 (11.3)	10 (11.8)			
TT	1 (0.5)	1 (1.2)		CC vs CT + TT	1.11 (0.46–2.73)

N = number of individuals; % = percentage; χ2 = chi-square; CI = confidence interval.
*p value significant level at ≤0.05; $ = suggestive p value significant level at ≤0.09.
OR=Odds Ratio adjusted for confounders (age, marital status, occupation, smoking).

Table 2
Spearman's correlation analysis between Vitamin B12, folate and homocysteine among depressed and anxious individuals.

Variables	Homocysteine			
	Normal (r)	Depressed(r)	Normal (r)	Anxious (r)
Vitamin B12	−0.18*	−0.396*	−0.113	−0.494*
Folate	0.80	−0.008	0.57	−0.40

r = correlation coefficient;*p value significant level at ≤0.05.

vitamin B12 deficiency was posing the significant risk for depression and anxiety disorder in the presently studied population.

4. Discussion

It is interesting that India is the land of indigenous populations, it constitute 8.61% of the total population, which is about 104.28 million [12] but unfortunately, they are amongst the poorest and most marginalized population groups [12]. There is dearth of studies on mental health of indigenous populations of India. The present study clearly reveals that mental disorders are not exclusive to modernity, but is a serious issue among the simple and indigenous populations also who are still away from urban life in spirit and practice. The importance of nutrients in the causation of depression and anxiety disorder is suggested from epidemiologic studies [21,22], but evidence from indigenous populations are lacking. Along with social factors like poverty, illiteracy, lack of developmental facilities, discrimination and social stigma, genetics and environmental factors like poor nutrition seems to pose extra burden on the mental health of these indigenous population groups. As the presently studied population has very low socio-economic status, they cannot afford basic necessities of life and it is further leads to undernutrition in terms of vitamin B12 and folate deficiency [23]. Although, vitamin B12 and folate deficiency were not directly associated with depression and generalized anxiety disorder but hyperhomocysteinemia was

found to pose more than three folds and six folds significant increased risk for depression and generalized anxiety disorder, respectively (Tables 1.1 and 1.2). Hyperhomocysteinemia is the by-product of one-carbon metabolism which can be caused by various reasons like vitamin B12 or folate deficiency, mutation in MTHFR C677T gene, renal dysfunction, gastric atrophy and pollution etc. [24]. Further, vitamin B12 was found to be significantly and negatively correlated with homocysteine in both depressed and anxious individuals (Table 2). It seems vitamin B12 mediated hyperhomocysteinemia that was posing an increased risk for depression and anxiety disorder in the presently studied population. Further, non intrinsic biological factor in terms of T allele of MTHFR C677T gene polymorphism was posing increased risk for depression and anxiety disorder though not significant, these results are similar with previous studies reported [10,25–27].

Thus, the present study highlights the significance of micronutrient deficiencies in the causation of depression and anxiety disorder. There are similar studies which suggests the link between supplementation of micronutrients in terms of vitamin B12 and folate and a decrease in depression status [28–30]. But due to small sample size it could not be revealed in much detail. So, more such studies in these vulnerable population groups need to be replicated with a larger sample size. Another limitation associated with this investigation is that the increase in family-wise error rate across the reported statistical analyses was not controlled.

5. Conclusion

In conclusion, it is seen that depression and generalized anxiety disorder is majorly unexplored and neglected area especially among indigenous populations of India. There is dearth of knowledge regarding the factors (social as well as biological) associated with depression and anxiety disorder. In the present study hyperhomocysteinemia was posing significant increased risk for depression and anxiety disorder in the studied population. Thus, lowering of homocysteine levels by promoting vitamin B12 and folate rich food, in general, and supplementation with vitamin B12, in particular, may help in reduction of depression and anxiety disorder in the studied population. Moreover, promoting awareness about various mental disorders may reduce the risk in such indigenous populations.

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Conflict of interest

The authors declares that there is no conflict of interest.

CRediT authorship contribution statement

Kallur Nava Saraswathy: Conceptualization, Writing - original draft. **Shagufta Naaz Ansari:** Formal analysis, Writing - original draft. **Gurjinder Kaur:** Formal analysis. **Pooran Chand Joshi:** Writing - review & editing, Project administration. **Shivani Chandel:** Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2019.01.009.

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