



Original Article

Oral Microbiota Transmission Partially Mediates Depression and Anxiety in Newlywed Couples



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Abstract

Background and objectives: Oral microbiota dysbiosis and altered salivary cortisol levels have been linked to depression and anxiety. Given that bacterial transmission can occur between spouses, this study aimed to investigate whether the transmission of oral microbiota between newlywed couples mediates symptoms of depression and anxiety.

Methods: Validated Persian versions of the Pittsburgh Sleep Quality Index, Beck Depression Inventory-II, and Beck Anxiety Inventory were administered to 1,740 couples who had been married for six months. The researchers compared 268 healthy control spouses with 268 affected cases in a cross-sectional study. Data were analyzed using appropriate statistical methods.

Results: After six months, healthy spouses married to an insomniac with the depression-anxiety (DA) phenotype scored significantly higher on the Pittsburgh Sleep Quality Index, Beck Depression Inventory-II, and Beck Anxiety Inventory compared to their baseline scores. This indicates that their sleep quality, depression, and anxiety scores became more similar to those of their affected spouses. Additionally, the composition of their oral microbiota changed significantly, becoming increasingly similar to that of their spouses. Specifically, in couples where one partner had the DA phenotype, the oral microbiota of the healthy spouse mirrored that of the affected partner ($p < 0.001$). These microbial changes correlated with alterations in salivary cortisol levels as well as depression and anxiety scores. Linear discriminant analysis revealed that the relative abundances of *Clostridia*, *Veillonella*, *Bacillus*, and *Lachnospiraceae* were significantly higher in insomniacs with the DA phenotype compared to healthy controls ($p < 0.001$).

Conclusions: Oral microbiota transmission between individuals in close contact partially mediates symptoms of depression and anxiety.

Introduction

Oral microbiota dysbiosis is significantly correlated with various neuropsychiatric disorders, including autism spectrum disorder, dementia, Parkinson's disease, schizophrenia, anxiety, epilepsy, and depression.¹ Wingfield *et al.*² demonstrated that the composi-

tion of the oral microbiota is significantly associated with depression in young adults who met the criteria for depression outlined in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (hereinafter referred to as DSM). They found that 21 bacterial taxa exhibited significantly different levels of abundance in depressed young adults. Specifically, there was an increase in *Prevotella nigrescens* and *Neisseria* spp., while 19 taxa showed a decrease in abundance. Oral microbiota exhibits variability influenced by individual factors, including oral hygiene practices, substance use, and tobacco consumption.¹

Recently, Simpson *et al.*³ demonstrated that the composition of the oral microbiota significantly correlates with symptoms of anxiety and depression in adolescents. Similar patterns have been observed in other populations, including pregnant women,⁴ patients experiencing burning mouth syndrome with psychiatric

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symptoms,⁵ individuals with anxiety, mood disorders, trauma- and stress-related disorders,⁶ and those with irritable bowel syndrome,⁷ among others.

Salivary cortisol does not directly cause depression or anxiety but serves as an indicator of these disorders. The suggested mechanism of action is that the oral microbiota may directly compromise the blood-brain barrier or exert indirect effects through the oral microbiota-brain axis.¹

On the other hand, a diminished cortisol awakening response is linked to an increased likelihood of experiencing a negative and persistent progression of depression and/or anxiety disorders.^{8,9}

The first author has previously discussed how closeness may facilitate bacterial transmission and, consequently, impact microbiota-related research.¹⁰ In various statistical simulations, I demonstrated significant differences in the occurrence of microbiota phenotypes between two groups of otherwise healthy spouses of individuals with a specific disease condition. These differences can largely be attributed to varying levels of social closeness. It is important to note that social closeness refers to one's sense of belonging and physical and psychological bonds in personal relationships. More specifically, it is defined as the average distance from a given node (a small group of individuals) to all other nodes (a higher number of small groups or nodes). A network consists of a collection of nodes (representing variables or the same small group of individuals) and edges (representing connections between or among these small groups) that link the nodes. The width of an edge indicates the strength of the relationship between the connected nodes, with wider edges signifying stronger associations. To assess the significance of each node within the network, three centrality measures—strength, betweenness, and closeness—are utilized.¹¹

Prior research has identified various forms of physiological synchrony between couples, including synchrony in diurnal cortisol patterns,¹² cardiac synchrony,¹³ and sleep concordance.¹⁴ The bidirectional associations between sleep disturbances and ocular surface parameters,^{15,16} in conjunction with other physiological synchronies observed in couples,^{12–14} lead the authors to hypothesize that the transmission of oral microbiota partially mediates depression and anxiety. Furthermore, cortisol is recognized as a biomarker for anxiety and depressive states in couples and partners.^{8,9}

Based on these interconnected premises, we hypothesized that oral microbiota partially mediates psychometric parameters in newlywed couples through person-to-person contact. To assess this hypothesis, the researchers enrolled couples in which one spouse simultaneously experienced depression and insomnia (referred to as the depression-anxiety (DA) phenotype,¹⁷ see *Psychometric assessments* under Materials and methods). To facilitate contact, the spouses lived in the same household. We aimed to investigate whether oral bacterial transmission among newlywed couples partially mediates depression and anxiety.

Materials and methods

Participants

This is a cross-sectional longitudinal study. Data were collected prospectively from two private sleep clinics in Tehran, Iran, between February and October 2024. Our primary outcome measures included the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Pittsburgh Sleep Quality Index (PSQI), serum cortisol levels, and the composition of oral microbiota.

The sample size required for subgroup comparisons was deter-

mined by Placzek *et al.*¹⁸ Accordingly, 422 samples (211 in the case group and 211 in the control group) were deemed sufficient. However, we enrolled 268 participants as cases and 268 as controls, as we had sufficient budget and resources. This ensured even greater statistical power than initially calculated.

We sent invitations to all 1,740 couples at the two private clinics to attend briefing sessions about the study. Data were collected using paper-based self-administered questionnaires, which were distributed by a healthcare assistant. Both otherwise healthy controls and individuals with insomnia and/or hypersomnia were screened based on their scores on the BAI, BDI, and PSQI at baseline and at the six-month follow-up.

Screening for insomnia, depression, and anxiety

For the case group, inclusion criteria consisted of having insomnia and/or hypersomnia, as determined by the self-administered PSQI,¹⁹ and experiencing depressive or anxiety states based on the validated Persian versions of the BDI-II and the BAI.^{20,21} Exclusion criteria included the use of medications known to affect gut, oral, or ocular microbiota composition, pregnancy, divorce during the study, antibiotic use in the past month, ongoing active ocular infections (including conjunctivitis), and a lack of prior history with dry eye disease (DED). Otherwise healthy controls were included based on a comprehensive clinical examination. Participants were asked to specify the exact date of their official marriage and to indicate whether they were cohabiting in the same household.

Participants who had been married within the past six months and were in a cohabiting relationship were screened for the presence or absence of insomnia. Those selected were enrolled in the study along with their official spouses. Two hundred ninety-six couples were selected, comprising healthy spouses and insomniacs with a DA phenotype.

Three couples were excluded from the study because the women were either taking antibiotics known to affect the composition of oral microbiota or were pregnant. None of the female spouses were using medications known to interact with the hypothalamic-pituitary-adrenal axis. Seventeen participants were excluded due to low readings ($n = 10$) or missing data ($n = 7$). Additionally, one couple had divorced and had spent significant time living apart, while seven couples who had relocated to another city were also excluded.

The remaining 268 couples lived together in the same household. All spouses were instructed to maintain their baseline dietary habits, oral hygiene practices, and exercise routines. On Day 1 and Day 180, all couples participated in a study measuring oral microbiota composition and salivary cortisol levels. Data collected from the 268 couples were analyzed. A diagram illustrating the categorization and enrollment of participants is shown in Figure 1.

Psychometric assessments

The validated Persian versions of the BDI-II,¹⁹ BAI,²¹ and PSQI,²⁰ were utilized to assess depression, anxiety, and sleep quality, respectively. Participants were then categorized into two groups: (i) healthy spouses and (ii) insomniac spouses. Each group was further divided based on BDI-II and BAI scores into categories of “moderate depression” and “moderate anxiety”. A combined DA phenotype was defined as having a BAI score between 16 and 25 and a BDI-II score of 14 or higher.¹⁷

The BDI-II is a widely used 21-item self-report inventory designed to assess depressive symptoms experienced over the past two weeks. Higher scores indicate more severe depressive symp-

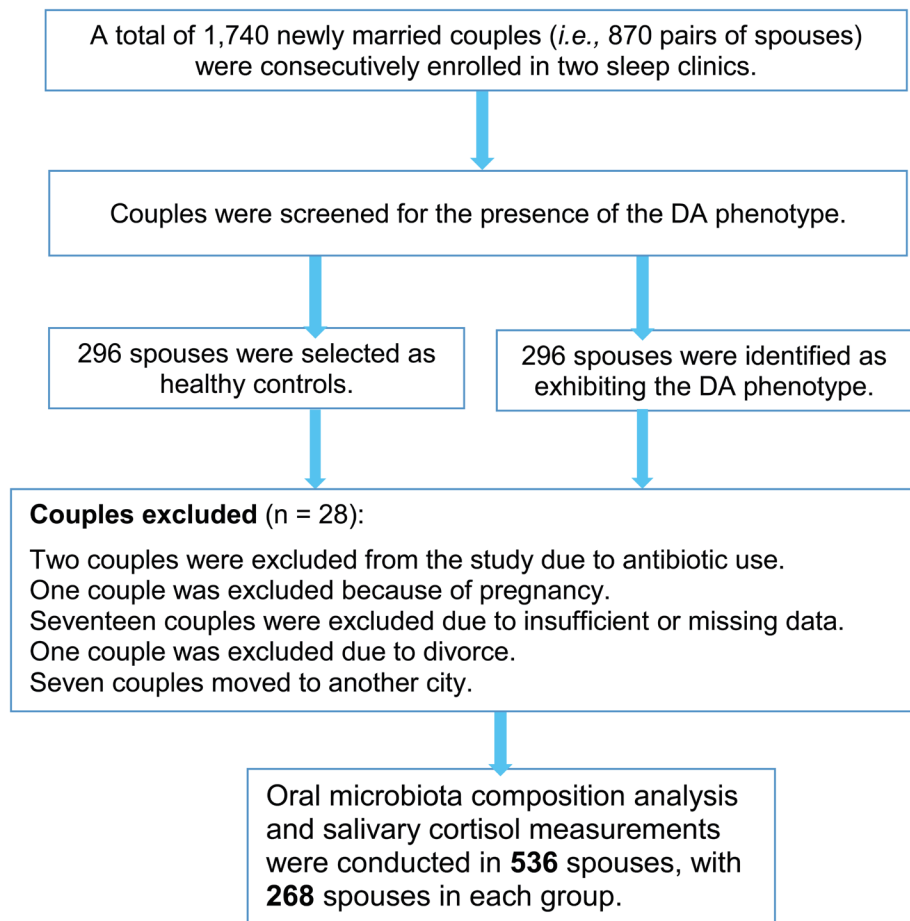


Fig. 1. Diagram showing the categorization of participants based on sleep disturbance status and depression-anxiety (DA) phenotype.

tomatology. In this study, depression was defined as a BDI-II score of 14 or higher.²²

The self-administered PSQI questionnaire assesses sleep quality over the past month. Scores range from 0 to 21, with lower scores indicating better sleep quality. A score higher than 5 is considered indicative of poor sleep quality. Insomnia, defined as the coexistence of difficulty resuming sleep and daytime dysfunction, is referenced in the literature.²³

The BAI questionnaire consists of 21 items, each scored on a scale from 0 to 3. A score of 0 indicates “not at all”, 1 indicates “mildly, but it did not bother me much”, 2 indicates “moderately, it was not pleasant at times”, and 3 indicates “severely, it bothered me a lot” for all the items. The total score can range from 0 to 63, reflecting varying levels of anxiety. Scores from 0 to 7 indicate a minimal range (no anxiety), scores of 8–15 indicate mild anxiety, scores of 16–25 indicate moderate anxiety, and scores ≥ 26 represent severe anxiety. Furthermore, in comparison to the category of no anxiety, the three categories of mild, moderate, and severe anxiety are collectively considered as “yes”.²⁴

We did not include any spouses with severe symptoms who would require additional support.

Oral parameters

Oral samples were collected during clinic visits from enrollment until five to six months after marriage, as briefly described below.

Oral microbiota

We adhered to the guidelines established by Brzychczy-Sroka *et al.*²⁵ Accordingly, oral samples were collected from the palatine tonsils at the end of the examination, prior to the collection of posterior pharyngeal swabs. Swab samples were obtained from both the palatine tonsils and the pharynx. The samples were preserved in saline, placed in ziplock bags, and stored in portable freezer bags at -20°C before being promptly delivered to the laboratory (within a maximum of 1–2 h). Samples were collected on three occasions: at baseline and again six months later.

The samples were then frozen at -80°C until analysis. One of the researchers (RR) secured the collected materials in MoBio buffer and placed them in a small ice-filled cooler for transport, without specifying a precise transport temperature, as recommended by Brzychczy-Sroka *et al.*²⁵ The final materials were delivered to the laboratory within 3–4 h.

Bacterial DNA was extracted from each clinical sample. The subsequent step involved amplifying the bacterial DNA using polymerase chain reaction, specifically targeting the V3–V4 region of the 16S ribosomal RNA subunit. The resulting amplicons were utilized to create a genomic library through several stages: purification of the polymerase chain reaction products, indexing of the samples, and re-purification. Following this, the samples were quantified using fluorometry, and the genomic library was combined for next-generation sequencing on the MiSeq platform.

Table 1. Baseline characteristics of participant spouses

	Healthy spouses (n = 268)	Insomniacs with depressive-anxiety phenotype* (n = 268)	p-value
No (%)	268 (50)	268 (50)	-
Age (years)	34.02 ± 8.1	34.70 ± 8.80	NS
Males	37.41 ± 7.96	38.02 ± 8.02	NS
Females	31.08 ± 8.5	32.11 ± 9.01	NS
Body mass index (kg/m ²)	23.33 ± 3.82	23.25 ± 3.60	NS
Males	24.98 ± 4.03	23.80 ± 4.24	NS
Females	23.10 ± 2.90	23.90 ± 3.51	NS
Beck depression inventory II	6.60 ± 2.20	15.60 ± 9.10	0.004**
Beck anxiety inventory	12.10 ± 4.90	24.80 ± 3.40	0.0001**
Global PSQI score	5.40 ± 2.80	8.10 ± 3.20	0.0001**
Salivary cortisol (ng/mL)	10.40 ± 13.20	39.10 ± 11.65	0.0001**
Socioeconomic status			
High	8 (2.70)	10 (3.37)	NS
Medium-high	291 (97.30)	282 (96.63)	NS

*A combined DA phenotype in this study was defined as the simultaneous presence of a Beck Anxiety Inventory score of 16–25 and a Beck Depression Inventory-II score of 14 or higher. Insomnia (sleeplessness) was defined as the coexistence of both difficulty resuming sleep and daytime dysfunction. **The Student's t-test revealed that insomniacs exhibiting a depressive-anxiety phenotype scored significantly higher on measures of depression (test statistic $T = -15.7374$), anxiety (test statistic $T = -34.8601$), and insomnia (test statistic $T = -10.3952$), and had notably elevated salivary cortisol levels (test statistic $T = -26.6867$) compared to their healthy spouses. DA, depression-anxiety; NS, non-significant; PSQI, Pittsburgh Sleep Quality Index.

(Illumina, San Diego, California, United States). The process of developing the genomic library for sequencing has been detailed in previous work.²⁶

Salivary cortisol

Salivary samples were collected from participants using Oragene OG-500 kits (DNA Genotek, Ontario, Canada), which facilitate self-collection and stabilization of DNA at room temperature. Participants were instructed to refrain from consuming any food or drink, except water, for at least 30 min prior to sample collection. Salivary cortisol was measured using liquid chromatography-tandem mass spectrometry as previously described.²⁷

All saliva specimens were stored at -80°C for subsequent analysis. We utilized samples collected immediately upon awakening, prior to drinking, eating, or performing oral hygiene.

Covariates

Gender, age, body mass index (BMI), alcohol consumption and smoking status, total dietary sugar intake, and the presence of chronic kidney disease or hypertension were considered as covariates. Hypertension was defined as an average systolic blood pressure of ≥ 140 mmHg and an average diastolic blood pressure of ≥ 90 mmHg, or the use of antihypertensive medications, or a physician's diagnosis. Chronic kidney disease was defined as an estimated glomerular filtration rate of < 60 mL/min/1.73 m².

Statistical analysis

Statistical analyses were conducted using SPSS software (version 17.0; SPSS, Chicago, IL). Differences between groups and subgroups were analyzed using Student's t-test for continuous parameters and the χ^2 test for categorical parameters. Where appropriate, the Bonferroni correction was applied to adjust for multiple testing. Intragroup changes were compared using a paired t-test. Pri-

mary and secondary endpoints were analyzed using analysis of covariance, with groups as fixed factors and baseline measurements as covariates. Furthermore, Pearson's and Spearman's correlation tests were employed to explore correlations between oral microbiota, anxiety, depression, and insomnia. Oral microbiota analysis was conducted using QIIME 2 version 2019.07.²⁸ Processed data were imported into phyloseq version 1.28.024 for further analysis.²⁹ Beta diversity was evaluated using Shannon's diversity index and Bray-Curtis dissimilarity. For a detailed description of the methodology used for bioinformatic processing, please refer to Wingfield B. *et al.*'s study.² To determine which taxa may be correlated with the DA phenotype, we performed L2-regularized logistic regression using the mikropml package in R,³⁰ a commonly applied methodology for conducting differential abundance analysis of microbiota data.³¹

Socioeconomic status was evaluated utilizing data on family size, parental educational qualifications, possession of a vehicle and particular household appliances, the square footage of the family's dwelling, and the average monthly income of the household.³²

Results

Table 1 presents the baseline characteristics of the participants. Demographic factors, including gender, age, BMI, and socioeconomic status, were comparable between the healthy control group and the DA phenotype group. The mean $\pm$ standard deviation for age among male and female spouses was 37.20 ± 8.01 years and 31.02 ± 9.30 years, respectively. Couples had been married and cohabiting for an average of 5.91 ± 2.03 months. As expected, there were significant differences in salivary cortisol levels, global PSQI, BDI-II, and BAI scores between the healthy controls and the insomniacs in the DA phenotype group at baseline.

Table 2. The healthy spouses who married an insomniac with a depressive-anxiety phenotype had higher psychopathology and salivary cortisol compared to their own baseline

	Healthy spouses	Same healthy spouses married to an insomniac with depressive-anxiety phenotype*	p-value
	Baseline values (n = 268)	Six-months values (n = 268)	
Depression (BDI-II)	6.60 ± 2.20	9.40 ± 8.20	
Males	6.50 ± 2.40	9.00 ± 7.70	0.0001 ^a
Females	8.20 ± 4.70	10.80 ± 1.20	0.0001 ^a
Anxiety (BAI)	12.10 ± 4.90	17.60 ± 4.90	
Males	11.90 ± 5.30	15.10 ± 1.70	0.0001 ^a
Females	14.20 ± 6.90	19.40 ± 5.30	0.0001 ^a
Global PSQI score	5.40 ± 2.80	7.80 ± 5.70	
Males	5.20 ± 1.90	7.50 ± 5.30	0.0001 ^a
Females	6.10 ± 3.20	8.10 ± 4.80	0.0001 ^a
Salivary cortisol (ng/mL)	10.40 ± 13.20	17.50 ± 18.30	
Males	9.60 ± 14.10	12.10 ± 16.20	0.05 ^a
Females	11.30 ± 8.40	20.80 ± 13.50	0.0001 ^a

*A combined depression-anxiety phenotype in this study was defined as the simultaneous presence of a Beck Anxiety Inventory (BAI) score between 16 and 25 and a Beck Depression Inventory-II (BDI-II) score of 14 or higher. Moderate depression was characterized by a BDI-II score of 14 or higher, while moderate anxiety was indicated by a BAI score between 16 and 25. ^aThe paired t-test for intra-group comparisons indicated that six months after marrying an insomniac with a DA-phenotype, healthy spouses exhibited significantly higher scores for depression, anxiety, and insomnia compared to their own baseline measurements. These differences were more pronounced in females than in males. DA, depression-anxiety; PSQI, Pittsburgh Sleep Quality Index.

Psychometric parameters

Table 2 shows that after six months, healthy spouses married to an insomniac with the DA phenotype scored significantly higher on PSQI, BDI-II, and BAI compared to their own baseline values. This indicates that sleep quality, depression, and anxiety scores changed and became more similar to those of their insomniac spouses, although they remained significantly lower than those of the insomniac group (all p -values <0.001). After six months, insomniacs with the DA phenotype exhibited a trend of increased salivary cortisol levels, as well as higher global PSQI, BDI-II, and BAI scores; however, these values did not reach statistical significance (non-significant differences are not shown).

In the gender subgroup analysis, the worsening of insomnia severity, an increase in salivary cortisol levels, and heightened depression and anxiety scores were more pronounced among female spouses after six months (Table 2).

In the crude model, insomnia severity was positively associated with anxiety and depression scores (odds ratio (OR): 1.62, 95% confidence interval (CI): 1.32–2.19, p < 0.001 for anxiety; OR: 2.30, 95% CI: 2.10–2.75, p < 0.001 for depression). After adjusting for age, gender, BMI, and education, insomnia continued to show a positive correlation with depression and anxiety scores, indicating a 1.8-fold and 2.1-fold increase, respectively, in odds compared to healthy spouses with normal sleep (OR: 1.8, 95% CI: 1.40–2.52, p < 0.001 for anxiety; OR: 2.1, 95% CI: 2.01–2.73, p < 0.001 for depression).

Salivary cortisol differences

Table 2 presents the results of a t-test analysis of salivary cortisol levels. In both male and female spouses with the DA phenotype, salivary cortisol was significantly higher in insomniac spouses at baseline compared to healthy control spouses (p < 0.0001). After six months, the salivary cortisol levels in spouses married to

an insomniac with the DA phenotype were significantly elevated compared to baseline values (p < 0.001). This finding suggests that healthy spouses were likely to resemble their insomniac partners with the DA phenotype. Gender subgroup analysis indicated that the increase in salivary cortisol levels was more pronounced in female spouses than in male spouses.

Oral microbiota characteristics

From the perspective of taxa composition, a total of 33 bacterial phyla were identified. Linear discriminant analysis (LDA) of oral microbiota composition showed that the relative abundances of *Clostridia*, *Veillonella*, *Bacillus*, and *Lachnospiraceae* were significantly higher in the DA phenotype spouses than in healthy controls (p < 0.001, LDA scores >2, alpha error = 0.01).

A high-level analysis of phyla differences revealed that the composition of oral microbiota in healthy spouses with normal sleep patterns was significantly altered, becoming similar to that of their partner. Specifically, if one spouse exhibited a DA phenotype, the oral microbiota composition of the other spouse mirrored that of the DA phenotype partner (p < 0.001, LDA scores >2, alpha error = 0.01) (Table 3).

The relative abundance of *Fusobacteria* (r = +0.49–0.57), *Patescibacteria* (r = +0.38–0.42), *Campylobacterota* (r = +0.32–0.36), *Spirochaetota* (r = +0.42–0.52), and *Gracilibacteria* (r = +0.29–0.37) in the oral microbiota was positively correlated with the severity of insomnia in individuals with the DA phenotype (all p -values <0.01).

We applied an L2-regularized logistic regression model to next-generation sequencing data to investigate potential associations between the oral microbiome and the DA phenotype. This model indicated that *Fusobacteria*, *Patescibacteria*, *Campylobacterota*, *Spirochaetota*, and *Gracilibacteria* may be associated with the DA phenotype.

Table 3. Baseline and six-month comparison of oral microbiota in healthy spouses and spouses married to individuals with depression-anxiety phenotype*

Oral microbiota in healthy spouses at baseline		Oral microbiota in healthy spouses six months after marriage			<i>p</i> -value
Phyla	% of abundance	Phyla	% of abundance	The absolute percent difference between the two groups	
Order of rank abundance					
<i>Firmicutes</i>	36.10	<i>Firmicutes</i>	31.42	4.68	0.005 ^{a,b} NS ^{c,d}
<i>Bacteroidetes</i>	17.88	<i>Bacteroidetes</i>	27.81	9.93	0.001 ^{a,b} NS ^{c,d}
<i>Proteobacteria</i>	17.42	<i>Proteobacteria</i>	20.60	3.18	0.01 ^{a,b} NS ^{c,d}
<i>Actinomycetota</i>	11.33	<i>Fusobacteria</i>	8.03	-e	-e
<i>Spirochaetes</i>	7.67	<i>Actinomycetota</i>	5.30	-e	-e
<i>Fusobacteria</i>	5.40	<i>Patescibacteria</i>	3.47	-e	-e
Six above-mentioned phyla contain ~96% of the taxa. The rest of phyla contain approximately 4% of the taxa					
TM7 12	1.88	Campilobacterota	1.40	-e	-e
Synergistetes	1.62	Spirochaetota	1.02	-e	-e
Chlamydiae	0.20	Gracilibacteria	0.03	-e	-e
Other rare phyla	0.50	Other rare phyla	<0.92	0.42	0.01 ^{a,b,c} 0.001 ^d

Oral microbiota in healthy spouses six months after marriage		Oral microbiota in insomniacs six months after marriage			<i>p</i> -value
Phyla	% of abundance	Phyla	% of abundance	The absolute percent difference between the two groups	
Order of rank abundance					
<i>Firmicutes</i>	30.20	<i>Firmicutes</i>	31.50	1.3	0.005 ^{a,b} NS ^{c,d}
<i>Bacteroidetes</i>	28.93	<i>Bacteroidetes</i>	27.51	1.42	0.001 ^{a,b} , NS ^{c,d}
<i>Proteobacteria</i>	20.60	<i>Proteobacteria</i>	20.89	0.29	0.01 ^{a,b} , NS ^{c,d}
<i>Fusobacteria</i>	7.98	<i>Fusobacteria</i>	8.14	0.16	NS ^{a,c,d} , 0.01 ^b
<i>Actinomycetota</i>	5.69	<i>Actinomycetota</i>	5.12	0.57	0.0001 ^{a,b} , NS ^{c,d}
<i>Patescibacteria</i>	3.40	<i>Patescibacteria</i>	3.23	0.17	0.0005 ^{a,b} , NS ^{c,d}
Six above-mentioned phyla contain ~96% of the taxa. The rest of phyla contain approximately 4% of the taxa					
TM7 12	1.40	Campilobacterota	2.38	-e	-e
Spirochaetota	1.02	Spirochaetota	1.04	0.02	0.0001 ^{a, b} , NS ^{c,d}
Gracilibacteria	0.03	Gracilibacteria	0.05	0.02	0.01 ^{a,b,c,d}
Other rare phyla	<0.75	Other rare phyla	<0.14	0.61	0.01 ^{a,b} , 0.001 ^c , 0.0005 ^d

*The DA phenotype is defined as the simultaneous presence of a Beck Anxiety Inventory score between 16 and 25 and a Beck Depression Inventory-II score of 14 or higher. ^aComparison of the healthy group versus insomniacs at baseline. ^bComparison of the healthy group versus itself after six months. ^cComparison of healthy individuals versus insomniacs after six months. ^dComparison of insomniacs versus insomniacs after six months. ^eThe columns are left blank, indicating that the corresponding rankings for the phylum do not match. The composition of oral microbiota in the group of healthy spouses with normal sleep patterns was significantly altered, becoming similar to that of their respective partners. Specifically, if a spouse exhibited a DA phenotype, the oral microbiota composition of the other spouse also resembled that of the DA phenotype partner (all *p*-values <0.0001). At baseline, the three most abundant phyla in both healthy and insomniac spouses were *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, followed by *Actinomycetota*, *Spirochaetes*, and *Fusobacteria* in healthy spouses, and *Fusobacteria*, *Actinomycetota*, and *Patescibacteria* in insomniac spouses. Six months after marriage, the ranking of phyla changed; however, the top three phyla maintained their positions, albeit with some variations in their relative abundances. In healthy spouses, the phyla *Fusobacteria* and *Patescibacteria* were competitively displaced by *Actinomycetota* and *Spirochaetes*, respectively, after six months. The remaining ten phyla (i.e., TM7, *Synergistetes*, *Chlamydiae*, and other rare phyla) typically remained unchanged between the two groups of healthy spouses and insomniacs after six months. In terms of ranking, after six months of marriage to an insomniac, the most significant microbial shift was observed in the phyla *Fusobacteria*, *Patescibacteria*, and *Spirochaetes* among healthy spouses (*p*-value <0.01). In terms of percentage change, the most significant alterations were observed in the phyla *Bacteroidetes* (11.05% increase), *Proteobacteria* (3.18% increase), *Spirochaetes* (6.65% decrease), *Firmicutes* (5.9% decrease), *Actinomycetota* (5.64% decrease), and *Fusobacteria* (2.58% increase). In individuals with insomnia, after six months, both the ranking and percentage of the top ten phyla remained nearly unchanged (not significant). DA, depression-anxiety; NS, non-significant.

Gender differences

LDA and multivariable association analysis revealed multiple distinct bacterial genera that were significantly different in abundance between female and male spouses with the DA phenotype ($p < 0.001$, LDA scores >2 , alpha error = 0.05). The phylum *Proteobacteria* was significantly more abundant in female spouses with the DA phenotype compared to their male counterparts. Additionally, certain members of the phyla *Firmicutes* and *Bacteroidetes* were also significantly more abundant in these female spouses. Interestingly, the genus *Dialister* (family *Firmicutes*) was found to be significantly more abundant in female spouses with the DA phenotype than in male spouses (all p -values <0.001).

Mediation analysis

To better understand the role of oral microbiota in salivary cortisol levels and DA phenotype status, we conducted an assessment of interaction effects and mediation analyses. We hypothesized that there are interaction effects among these three variables, with oral microbiota status serving as the potential mediator in the relationship between salivary cortisol and DA phenotype status. Indeed, the oral microbiota pattern is a predictor of DA phenotype status ($\beta = 0.37$, $p < 0.001$), which, in turn, is also a relevant predictor of salivary cortisol levels ($\beta = 0.15$, $p < 0.001$). The mediation analysis accounted for 35% of the variability in the data ($R^2 = 0.35$).

Discussion

Oral microbiota transfer between individuals in close contact, such as couples in the present study, may mediate depression and anxiety. Although there are no directly comparable human studies, there is substantial evidence of bacterial exchange between humans and dogs and livestock.^{33,34} It is important to note that the results of studies investigating bacterial transmission from animals to humans may not be directly applicable to human studies. Nevertheless, there are reports of penile and genital microbiota exchange between partners.³⁵ These findings underscore the significance of bacterial exchange as a potential mediating mechanism for mood synchrony between spouses and partners. Various forms of physiological synchrony between couples have been documented, including cardiac synchrony,¹³ diurnal cortisol pattern synchrony,¹² and sleep concordance.^{4,14,36}

We found that changes in oral microbiota composition are associated with changes in the severity of insomnia, salivary cortisol levels, and depression and anxiety scores. Our findings align with previous studies on salivary cortisol levels as well as depression and anxiety scores.^{2-7,12,37-41}

Recently, in a large cohort of couples who had been married and living together for an average of 5.91 months, we demonstrated that sleep disturbances can be partially attributed to changes in the gut microbiota.¹⁵ Additionally, in another study, we identified a significant association between ocular microbiota and DED in individuals with insomnia, which may have been mediated through person-to-person contact.¹⁶ In that study, we found that six months after marriage, spouses married to an insomniac exhibiting the DED phenotype were significantly more likely to develop DED during the six-month follow-up. Supporting our initial hypothesis, these changes occurred in parallel with alterations in ocular microbiota composition.¹⁶

The oral and gut microbiomes are interconnected,⁴² exhibiting both distinct similarities and differences.⁴³ Additionally, established connections exist between oral microbiota and ocular mi-

crobiota.⁴⁴ Overall, these findings suggest that these networks are interrelated. Current literature supports this conclusion, indicating that the frequency and prevalence of DED in individuals with depression or anxiety are significantly higher than in healthy individuals, and vice versa.⁴⁵ Therefore, our findings have important implications for holistic medicine, family medicine, and personalized medicine. The practical and theoretical implications of this study encompass a wide range of areas, including sleep therapy and psychological states.

This research was a non-invasive, prospective observational study; however, there is compelling preclinical evidence supporting our hypothesis that transplantation of fecal microbiota from patients with depression to microbiota-depleted rats can induce physiological and behavioral characteristics typical of depression in the recipient animals.⁴⁶ Furthermore, Lee *et al.*⁴⁷ demonstrated that fecal microbiota-induced insomnia, immobilization stress, and depression-like behaviors in a mouse model can be alleviated by microbiota-modulating probiotics. These two reports strongly suggest a causal association between changes in microbiota and psychometric parameters such as depressive states and anxiety.^{46,47} However, caution is necessary when translating findings from animal models to human studies. Taken together, our preliminary findings provide evidence that the association between changes in oral microbiota and mood alterations, or their synchrony in humans, is causally related.⁴⁷

Future research should further evaluate this hypothesis.

Strength

Firstly, this study employed a robust methodology, utilizing a large sample size to ensure an effective follow-up program. Secondly, to our knowledge, this is the first study of its kind to investigate a wide range of variables simultaneously. Lastly, while the participants were not homogeneous, the focused choice of this specific group allowed us to examine the short- to medium-term impacts of bacterial transmission on depression and anxiety in a real-life setting.

Limitations

After six months, the worsening of insomnia severity, depression, and anxiety scores was more pronounced in female spouses. It is possible that we underestimated or underreported the frequency of insomnia, depression, and anxiety in male spouses, as we used PSQI, BDI-II, and BAI to estimate insomnia, depressive, and anxiety symptoms, respectively. It is important to acknowledge that our participant couples were aware of the study's purpose due to ethical constraints. Previous well-controlled and well-conducted research has identified a main effect of condition, indicating that both males and females reported significantly higher levels of insomnia severity,⁴⁸ depression,²² and anxiety symptoms in the covert condition.⁴⁹ This suggests that we may have underestimated or underreported the frequency of insomnia, depression, and anxiety in male spouses, while potentially overestimating or overreporting these parameters in female spouses. Despite controlling for the most significant covariates, additional residual confounding may still exist. For instance, dietary intake is an important factor influencing the composition of the gut microbiome. Since couples were not randomly selected, the results may not be fully generalizable to all circumstances. We only measured morning salivary cortisol, and measuring salivary cortisol over three consecutive days could improve accuracy. While the primary sampling sites within the oral cavity include saliva, the supragingival region, the subgingival/submucosal area, infected root canals, and mucosal surfaces, our

assessment was limited to the palatine tonsils and pharynx due to financial constraints.

It can be argued that deriving a conclusive understanding from our preliminary findings is challenging without controlling for confounders (e.g., shared diet, stress exposure, and frequency of intimacy). Additionally, it is possible that the spouses may have underlying health issues that have not been verified through evaluations, which could affect the results. We acknowledge that the BDI-II has been criticized for its limitations in the absence of psychiatric assessments. However, the BDI-II is widely employed in non-clinical samples,⁵⁰ and in the present study, we did not include any spouses with severe symptoms requiring psychological care. Finally, we only employed the BDI-II and BAI, whereas clinical diagnoses of anxiety and depression based on the DSM would be a more precise method. We suggest that these important variables should be fully considered in future studies.

Future directions

Future research may involve the recruitment of clinical samples. Furthermore, since the body microbiota is implicated in many other diseases, it will be necessary to investigate the possibility of bacterial involvement in other seemingly non-communicable psychological and neurological conditions, as well as in other non-neurological conditions. Additionally, animal models will be instrumental in determining whether such relationships are causal. We also suggest that future research account for the possibility of bias in the study design due to the potential confounding effects created by bacterial transmission among individuals.

Conclusions

The transmission of oral microbiota plays a partial role in mediating depression and anxiety among couples. Since this study is associational, further research is needed to establish whether this association is causal. If it is determined that this association is indeed causal, it could have significant implications for contemporary research. We propose that, within the framework of diagnostic, predictive, preventive, and personalized medicine, the practical and theoretical implications of this study may enhance our understanding of various aspects of microbiota-host interactions.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Study design (RR, CGI, ND), manuscript conception (BV, AS), coordination of research implementation, provision of medical data (RR, AS), writing of the manuscript (RR, BV), research analysis (RR, CGI). All authors have read and approved the final manuscript. RR is responsible for the overall content (as guarantor).

Ethical statement

The study was approved by the ethics committee of the Iran National Science Foundation (Research Ethical Code INSF: 98R026323-2024) and adhered to the tenets of the Declaration of Helsinki. Participants gave informed consent to participate in the study before taking part.

Data sharing statement

Data are available on reasonable request.

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