Article

Discordant Serum and Salivary Reelin Patterns in Major Depressive Disorder: Evidence From Transcranial Magnetic Stimulation

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Abstract

Background: This study aimed to investigate serum and salivary Reelin levels in patients with major depressive disorder (MDD) and to evaluate their changes following Transcranial Magnetic Stimulation (TMS).

Methods: This prospective study included 29 patients with MDD and 30 healthy controls recruited between 2024 and 2025. Depression and anxiety severity were assessed using the Beck Depression Inventory and Beck Anxiety Inventory. Serum and salivary Reelin levels were measured before and after treatment. Pre- and post-treatment comparisons were performed using paired samples *t*-test, and between-group comparisons were conducted.

Results: At baseline, serum Reelin levels were significantly higher in patients than in controls ($p = 0.009$), whereas salivary levels were significantly lower ($p = 0.036$). Following treatment, both depression and anxiety scores showed significant reductions ($p < 0.001$), indicating improvement in clinical symptom severity. Serum Reelin levels showed a non-significant increase, while salivary levels demonstrated a slight, non-significant rise and remained below control values.

Conclusions: Serum and salivary Reelin levels demonstrated compartment-specific differences in patients with MDD. Despite improvement in clinical symptom severity after treatment, peripheral Reelin levels showed no significant changes. Because concomitant psychotropic treatments were not systematically documented, the observed biomarker findings cannot be attributed specifically to TMS.

Keywords

depression; Reelin; TMS; biomarkers; saliva; serum

Introduction

Major depressive disorder (MDD) is a highly prevalent and debilitating psychiatric condition, representing one of the leading causes of disability worldwide. Despite advances in pharmacological and non-pharmacological treatments, a substantial proportion of patients remain treatment-resistant, highlighting the need for a deeper understanding of underlying biological mechanisms and the identification of reliable biomarkers. In this context, synaptic plasticity and neurobiological dysregulation have emerged as key components in the pathophysiology of depression [1,2].

Reelin, a large extracellular matrix glycoprotein, plays a critical role in neuronal migration, synaptic plasticity, and neurodevelopment, as well as in the regulation of adult brain function. It is predominantly expressed in the hippocampus and prefrontal cortex, regions strongly implicated in mood regulation. Increasing evidence suggests that alterations in Reelin signaling may contribute to the development of depressive disorders by affecting synaptic organization and neuroplasticity [3–5].

Previous studies have demonstrated that Reelin expression is reduced in the brains of individuals with mood disorders, including major depression, suggesting a poten-

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tial role as a vulnerability factor in the development of depressive phenotypes. Experimental and clinical data further indicate that stress-related mechanisms and glucocorticoid exposure may downregulate Reelin expression, thereby contributing to impaired neurogenesis and increased susceptibility to depression [6–8].

In addition to central nervous system alterations, peripheral biomarkers have gained increasing attention for their potential role in the diagnosis and monitoring of psychiatric disorders. Saliva has emerged as a promising non-invasive biological medium for exploratory biomarker research in psychiatric disorders. However, the biological origin and analytical characteristics of many salivary biomarkers, including Reelin, remain incompletely understood. Recent studies have reported alterations in salivary biomarker profiles, including cytokines and stress-related molecules, in patients with major depressive disorder [9,10].

Transcranial Magnetic Stimulation (TMS) has been established as an effective and well-tolerated treatment modality for MDD, particularly in treatment-resistant cases. Its therapeutic effects are believed to be mediated through modulation of cortical excitability and enhancement of neuroplasticity. Consensus guidelines and accumulating evidence support the clinical efficacy and safety of TMS, while ongoing research continues to explore its biological mechanisms of action and potential biomarkers of treatment response [2,11].

However, despite growing interest in Reelin as a biomarker, limited data exist regarding its behavior across different biological compartments and its response to neuromodulatory treatments such as TMS. In particular, the relationship between serum and salivary Reelin levels remains poorly understood, and whether these compartments reflect synchronized or distinct biological processes is still unclear.

Saliva has emerged as an attractive biological matrix for psychiatric biomarker research because it can be collected non-invasively and repeatedly with minimal patient burden. While several salivary biomarkers have been investigated in MDD, the potential role of salivary Reelin remains largely unexplored. Therefore, the aim of this study was to investigate serum and salivary Reelin levels in patients with MDD and to evaluate their changes following TMS treatment, with a particular focus on potential compartmental differences and their relationship to clinical improvement. Salivary Reelin was evaluated as an exploratory candidate biomarker rather than as an established diagnostic or treatment-response biomarker.

Materials and Methods

Study Design and Setting

This prospective observational biomarker study was conducted at Moodist Hospital between 2024 and 2025. The study population consisted of patients diagnosed with MDD by experienced psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria as part of routine clinical psychiatric assessment, who had a clinical indication for TMS. Detailed information regarding concomitant psychotropic medication classes, medication stability, dose modifications during treatment, and concurrent psychotherapy was not systematically collected as part of the study protocol, as the study was designed to evaluate biomarker dynamics under routine clinical practice conditions.

The present study was designed as a prospective observational biomarker study conducted under routine clinical practice. TMS was administered exclusively according to clinical indications determined by the treating psychiatrists, and no study-specific treatment allocation, randomization, or protocol-driven therapeutic intervention was performed by the investigators.

Participants

Patients were diagnosed with MDD by experienced psychiatrists according to the DSM-5 criteria [2], as part of routine clinical psychiatric assessment. Structured diagnostic interviews were not incorporated into the study protocol. A total of 30 patients initially met the inclusion criteria. However, one patient was excluded from the final analysis due to missing Reelin measurements, resulting in a final sample size of 29 patients in the depression group. The control group consisted of 30 healthy participants matched for basic demographic characteristics. Healthy controls were recruited from volunteers without a known history of psychiatric disorders based on medical history and self-report. Individuals reporting a previous psychiatric diagnosis or current psychotropic medication use were excluded. Formal structured psychiatric interviews were not performed.

Inclusion and Exclusion Criteria

Patients were included if they were diagnosed with MDD by experienced psychiatrists according to the DSM-5 criteria as part of routine clinical psychiatric assessment and were eligible for TMS treatment.

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Exclusion criteria included the presence of severe neurological disorders, active systemic diseases that could affect biomarker levels, or inability to complete clinical assessments.

Clinical Assessment

Depression and anxiety severity were assessed using the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI). Both instruments are self-report scales consisting of 21 items, with each item scored on a 4-point Likert scale ranging from 0 to 3. Total scores range from 0 to 63, with higher scores indicating greater symptom severity [5,6].

The BDI evaluates cognitive, emotional, and somatic symptoms of depression, while the BAI primarily assesses somatic and physiological symptoms of anxiety. Assessments were performed before and after TMS treatment.

Sociodemographic data, including age, sex, marital status, income, and education level, were recorded. Income status was determined based on participants' self-reported assessment in the study questionnaire and categorized as low, medium, or high. Clinical variables including disease duration, psychiatric comorbidities, family history, and suicidal ideation were evaluated. Additional clinical characteristics, including episode subtype, treatment resistance, psychotic features, detailed substance use history, and structured psychiatric interview findings, were not systematically collected.

Transcranial Magnetic Stimulation Protocol

TMS was administered in a real-world clinical setting according to standard therapeutic principles. Stimulation protocols were individualized based on clinical judgment, patient characteristics, comorbid psychiatric conditions, and treatment accessibility.

Different TMS modalities were applied across patients, including conventional repetitive TMS (rTMS), deep TMS, and intermittent theta burst stimulation (iTBS). Conventional rTMS and deep TMS sessions typically lasted 20–30 minutes, whereas theta burst sessions were considerably shorter, with a duration of approximately 10 minutes per session.

Stimulation targets were selected according to the dominant clinical presentation. Left dorsolateral prefrontal cortex (DLPFC) stimulation was primarily used for depressive symptoms, right-sided stimulation was applied in patients with prominent anxiety symptoms, and combined left DLPFC and orbitofrontal cortex stimulation was used in patients with obsessive symptoms.

Treatment frequency varied depending on the protocol. Patients receiving standard rTMS or deep TMS underwent either one or two sessions per day. Patients receiving intermittent theta burst stimulation followed an accelerated protocol consistent with the Stanford Accelerated Intelligent Neuromodulation Therapy (SAINT) approach, receiving up to 10 sessions per day.

The total treatment duration also varied according to the applied protocol. Theta burst stimulation protocols were completed within approximately 5 days, whereas patients receiving one session per day completed treatment within 20–30 days. Patients receiving two sessions per day completed treatment within 10–15 days. Treatments were administered daily, including weekends, resulting in a 7-day treatment schedule.

Stimulation intensity was generally set between 100–120% of the individual resting motor threshold. All procedures were conducted in an outpatient clinical setting under the supervision of trained medical personnel.

No serious adverse events were observed during the treatment period, and all procedures were well tolerated.

The choice of stimulation modality, cortical target, stimulation intensity, treatment frequency, and treatment duration was determined by the treating psychiatrist according to routine clinical practice and individual patient characteristics. Accordingly, the present study was designed to evaluate overall biomarker changes associated with clinically indicated TMS rather than to compare the biological effects of specific stimulation protocols. Owing to the limited sample size and the exploratory nature of the study, protocol-specific subgroup analyses and adjusted multivariable models were not performed.

The stimulation parameters used in the present study were selected according to established clinical recommendations and contemporary evidence for the application of repetitive transcranial magnetic stimulation, deep TMS, and intermittent theta burst stimulation in MDD. Treatment protocols were individualized according to routine clinical practice while remaining consistent with published clinical guidelines and evidence-based recommendations [4,12,13].

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Measurement of Serum and Salivary Reelin Levels

Previous postmortem studies have reported reduced hippocampal Reelin expression in MDD and other psychiatric disorders, providing a rationale for investigating peripheral Reelin levels in the present study [14,15].

Biological samples were collected from patients both before and after TMS treatment. Pre-treatment samples were obtained prior to the initiation of TMS sessions, while post-treatment samples were collected on the same day immediately after completion of the final TMS session.

To obtain serum, 5 mL of venous blood was drawn into gel-containing biochemistry tubes and allowed to clot at room temperature, followed by centrifugation at 3000 rpm for 20 minutes.

For saliva analysis, approximately 2 mL of unstimulated whole saliva was collected under standardized conditions using sterile Salivette tubes (Cat. No. 51.1534; SARSTEDT AG & Co. KG, Nümbrecht, Germany), avoiding masticatory stimulation. Collections were performed in the morning under fasting conditions. The saliva collection procedure was conducted in accordance with previously validated oral health sampling methodologies [15]. Saliva samples were centrifuged at 4000 rpm for 15 minutes to eliminate cellular debris and sediments, following established biochemical protocols [16]. Following separation, both serum and saliva samples were aliquoted and stored at −80 °C until analysis.

Cryopreserved serum and saliva samples were thawed under controlled conditions prior to analysis. Reelin (RELN) concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human RELN ELISA Kit, Lot No: KD0DAB60207616, Catalog No: RE2380H; Reed Biotech Ltd., Wuhan, China), according to the manufacturer's instructions.

All samples were analyzed in duplicate. Plate washing was performed using an automated microplate washer (Epoch; BioTek Instruments, Inc., Winooski, VT, USA), and optical density was measured at 450 nm using an Epoch® microplate spectrophotometer (BioTek Instruments, USA). Calibration curves were generated using standard solutions provided in the kit, and concentrations were calculated accordingly and expressed in ng/mL, taking dilution into account.

All biochemical analyses were performed in the Medical Biochemistry Research and Development Laboratory at Kafkas University Faculty of Medicine under standardized laboratory conditions by personnel blinded to clinical data.

Statistical Analysis

All statistical analyses were performed using Wistats v3.0 (WisdomEra Corp., Istanbul, Turkey), an integrated Python-based platform for statistical evaluation (https://wistats.wisdomera.io).

Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as number (percentage). Comparisons between the control and patient groups were performed using the independent samples *t*-test or Mann–Whitney U test, as appropriate. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate.

For within-group comparisons, changes between pre-TMS and post-TMS measurements were analyzed using the paired samples *t*-test. This analysis was applied to evaluate differences in BDI and BAI scores as well as serum and salivary Reelin levels before and after TMS treatment.

For paired pre- and post-treatment comparisons, mean differences with 95% confidence intervals and Cohen's d effect sizes with corresponding 95% confidence intervals were calculated.

All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

Continuous variables were assessed for normality using the Shapiro–Wilk test together with skewness and kurtosis. Variables satisfying parametric assumptions are presented as mean ± standard deviation and were analyzed using parametric statistical tests, whereas non-normally distributed variables were summarized using appropriate descriptive statistics and analyzed using corresponding nonparametric methods.

The primary analyses consisted of comparisons between patients and healthy controls for serum and salivary Reelin levels and paired pre- and post-treatment comparisons within the patient group. Because sociodemographic and clinical variables were unavailable for the healthy control group and the patient cohort was relatively small, multivariable adjustment was not performed.

The present study was designed as an exploratory observational investigation based on consecutively recruited eligible patients rather than a hypothesis-driven confirmatory trial. Consequently, a formal prospective sample size

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Table 1. Demographic characteristics of the control and patient groups.

Variable	control (n = 30), Mean ± SD, n (%)	patients (n = 29), Mean ± SD, n (%)	*p*-value
Sex			0.449
Female	16 (53.33%)	14 (48.28%)	
Male	14 (46.67%)	15 (51.72%)	
Age (years)	35.77 ± 12.47	35.00 ± 14.30	0.622
Serum Reelin before TMS (ng/mL)	8.65 ± 1.73	10.08 ± 2.32	0.009
Salivary Reelin before TMS (ng/mL)	0.73 ± 0.14	0.64 ± 0.17	0.036

TMS, Transcranial Magnetic Stimulation; SD, standard deviation.

calculation was not performed. The relatively limited sample size should therefore be considered when interpreting analyses of biomarker changes and precluded reliable protocol-specific subgroup analyses.

Results

Baseline Characteristics

At baseline, there was no significant difference between the control and patient groups in terms of sex distribution (female: 53.33% vs. 48.28%; male: 46.67% vs. 51.72%; $p = 0.449$) or age (35.77 ± 12.47 vs. 35.00 ± 14.30 years; $p = 0.622$). Serum Reelin levels were significantly higher in the patient group compared to controls (10.08 ± 2.32 ng/mL vs. 8.65 ± 1.73 ng/mL; $p = 0.009$). In contrast, salivary Reelin levels were significantly lower in patients than in controls (0.64 ± 0.17 ng/mL vs. 0.73 ± 0.14 ng/mL; $p = 0.036$) (Table 1). The overall study population and inclusion process are summarized in Fig. 1.

Clinical and Sociodemographic Characteristics of Patients

The mean age of patients was 35.00 ± 14.30 years, and 48.28% were female. The majority of patients were single (62.07%), followed by married (34.48%) and widowed (3.45%). Most participants had a medium income level (82.76%) and a bachelor's degree (62.07%). Family history was present in 44.83% of patients. The mean disease duration was 5.69 ± 6.65 years. Comorbidities were absent in 65.52% of patients, while individual conditions such as hypothyroidism (6.90%) and other disorders were less frequent. Suicidal ideation was reported in 20.69% of cases (Table 2). The study design and timing of clinical and biochemical assessments are illustrated in Fig. 2.

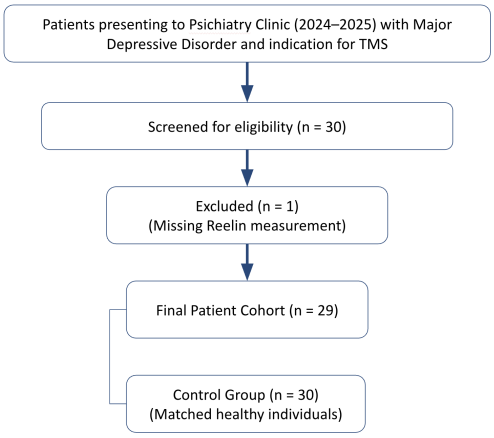


Fig. 1. Study flow diagram. Flow diagram of participant selection and study inclusion. Patients presenting to Moodist Hospital between 2024 and 2025 with a diagnosis of major depressive disorder and a clinical indication for Transcranial Magnetic Stimulation (TMS) were screened for eligibility. A total of 30 patients met the inclusion criteria; however, one patient was excluded due to missing Reelin measurements, resulting in a final patient cohort of 29 individuals. The control group consisted of 30 healthy participants matched for basic demographic characteristics.

Pre- and Post-TMS Comparisons

Baseline BDI and BAI scores were 31.62 ± 9.61 and 21.62 ± 11.22, respectively. After TMS treatment, BDI scores decreased to 16.59 ± 10.50, and BAI scores decreased to 14.59 ± 10.24. A statistically significant reduction was observed in both depression and anxiety scores following TMS treatment. BDI scores decreased from 31.62 (13–54) to 16.59 (0–45) ($p < 0.001$). Similarly, BAI scores decreased from 21.62 (0–47) to 14.59 (0–35) ($p < 0.001$) (Table 3). These clinical improvements are visually presented in Fig. 3.

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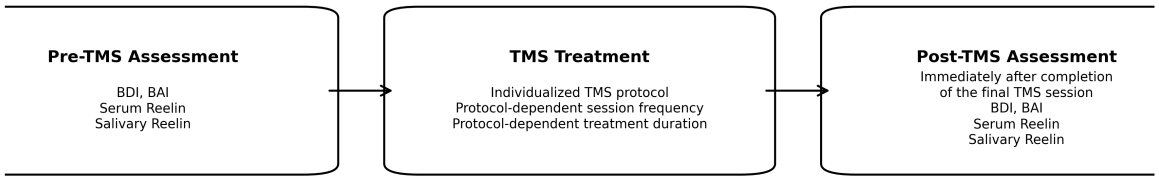


Fig. 2. Study design and sampling timeline. This schematic illustrates the prospective study design. Clinical assessments, including Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) scores, together with serum and salivary Reelin measurements, were obtained before initiation of Transcranial Magnetic Stimulation (TMS). Patients subsequently underwent individualized TMS treatment according to the applied protocol, with treatment duration varying depending on the stimulation modality and treatment schedule. Post-treatment clinical and biochemical assessments were performed immediately after completion of the final TMS session.

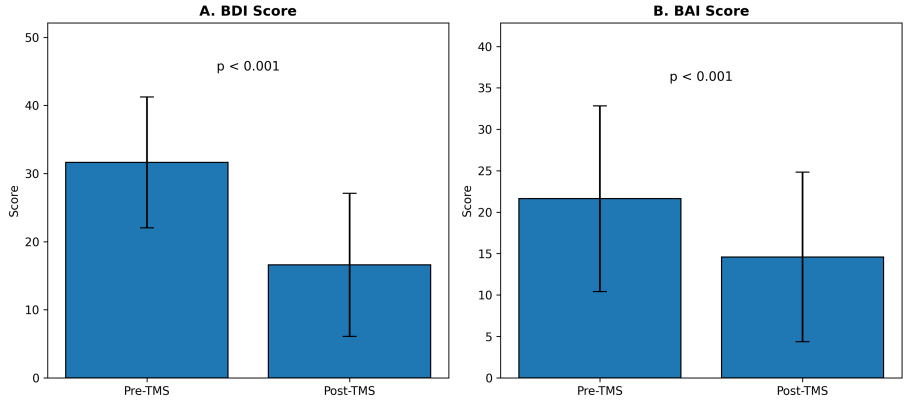


Fig. 3. Clinical outcomes after TMS. (A) Beck Depression Inventory (BDI) scores before and after TMS treatment. (B) Beck Anxiety Inventory (BAI) scores before and after TMS treatment. Both depression and anxiety scores showed a statistically significant reduction following TMS ($p < 0.001$ for both comparisons, paired samples *t*-test). Bars represent mean values, and error bars indicate standard deviation. These findings demonstrate a significant clinical improvement after TMS treatment.

Reelin Dynamics Before and After TMS

Serum Reelin levels increased from 10.08 ± 2.32 ng/mL before TMS to 10.69 ± 2.07 ng/mL after TMS. The mean change was 0.611 ng/mL (95% CI: −0.679 to 1.902), corresponding to a small effect size (Cohen's d = 0.180), and the change was not statistically significant ($p = 0.340$). Salivary Reelin levels increased from 0.64 ± 0.17 ng/mL to 0.66 ± 0.13 ng/mL. The mean change was 0.016 ng/mL (95% CI: −0.053 to 0.085), with a small effect size (Cohen's d = 0.090), and was also not statistically significant ($p = 0.633$) (Table 3). Comparative serum and salivary Reelin profiles across control, pre-TMS, and post-TMS conditions are presented in Fig. 4.

Integrated Evaluation of Serum and Salivary Reelin

At baseline, patients exhibited a distinct biochemical pattern characterized by elevated serum Reelin levels alongside reduced salivary Reelin levels compared to controls. Following TMS treatment, serum Reelin levels demonstrated a further numerical increase, whereas salivary levels showed only a limited increase and remained below control levels. The overall pattern and its proposed biological interpretation are illustrated in Fig. 5.

Discussion

In this prospective study, we investigated serum and salivary Reelin levels in patients with MDD and evaluated their changes following TMS treatment. The most important findings of our study were: (i) a significant dissociation between serum and salivary Reelin levels at baseline, char-

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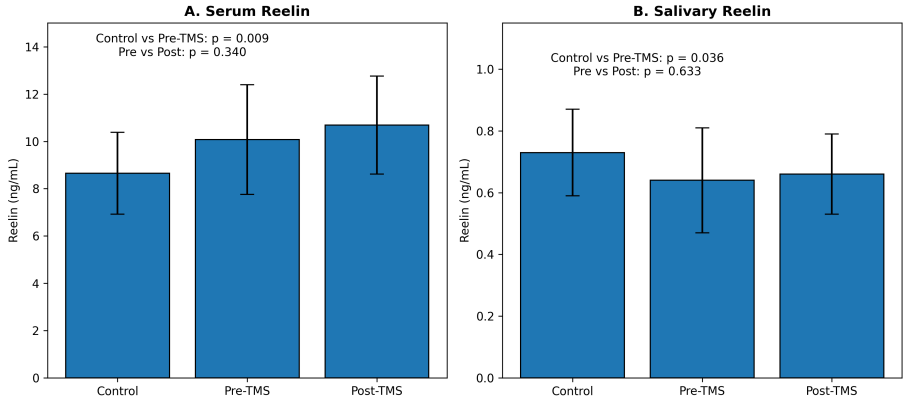


Fig. 4. Serum and salivary Reelin dynamics. (A) Serum Reelin levels in control subjects and patients before and after TMS treatment. Serum Reelin levels were significantly higher in patients compared to controls at baseline ($p = 0.009$), while no significant change was observed following TMS ($p = 0.340$). (B) Salivary Reelin levels in control subjects and patients before and after TMS treatment. Salivary Reelin levels were significantly lower in patients compared to controls at baseline ($p = 0.036$), with no statistically significant change after TMS ($p = 0.633$). Bars represent mean values, and error bars indicate standard deviation.

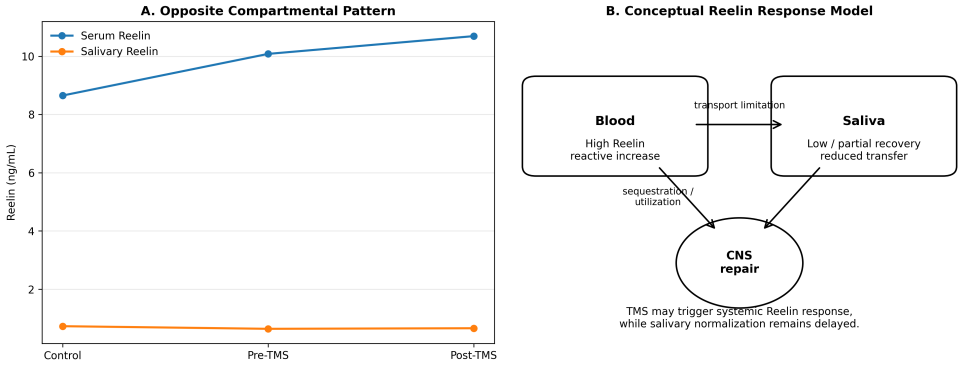


Fig. 5. Integrated compartmental model of Reelin response to TMS. (A) Graphical representation of serum and salivary Reelin levels across control, pre-TMS, and post-TMS conditions. An opposite pattern is observed between compartments, with higher serum but lower salivary Reelin levels in patients compared to controls. (B) Conceptual illustration of the observed serum and salivary Reelin patterns. The schematic summarizes the compartment-specific differences observed in the present study and is intended to generate hypotheses for future mechanistic investigations rather than to represent experimentally validated biological mechanisms. CNS, central nervous system; TMS, Transcranial Magnetic Stimulation.

acterized by higher serum (10.08 ± 2.32 ng/mL vs. 8.65 ± 1.73 ng/mL; $p = 0.009$) and lower salivary levels (0.64 ± 0.17 ng/mL vs. 0.73 ± 0.14 ng/mL; $p = 0.036$) in patients compared to controls, (ii) a significant clinical improvement after TMS, reflected by a marked reduction in BDI (31.6 to 16.6; $p < 0.001$) and BAI scores (21.6 to 14.6; $p < 0.001$), and (iii) a non-significant numerical increase in serum Reelin levels (10.1 ng/mL to 10.7 ng/mL; $p = 0.340$) with only a minimal and non-significant increase in salivary Reelin (0.64 ng/mL to 0.66 ng/mL; $p = 0.633$) following treatment. Together, these findings suggest compartment-specific differences in Reelin dynamics rather than a uniform systemic response.

Reelin has been increasingly recognized as a key regulator of synaptic plasticity and neuronal function, particularly in brain regions implicated in depression such as the hippocampus and prefrontal cortex. Experimental and clinical studies have demonstrated that decreased Reelin expression is associated with depressive phenotypes and impaired neuroplasticity, while restoration of Reelin signaling may reverse these alterations [1,6]. Importantly, landmark postmortem studies have consistently demonstrated reduced Reelin expression across multiple psychiatric disorders, particularly schizophrenia and bipolar disorder. For instance, Guidotti *et al.* [15] reported a 30–50% reduction in Reelin expression in the prefrontal cortex of patients with schizophrenia and bipolar disorder with psychosis, sug-

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Table 2. Demographic and clinical characteristics of patients with depression.

Variable	Patients (n = 29), Mean ± SD, n (%)
Sex	
Female	14 (48.28%)
Male	15 (51.72%)
Age (years)	35.00 ± 14.30
Marital status	
Married	10 (34.48%)
Single	18 (62.07%)
Widowed	1 (3.45%)
Income	
High	2 (6.90%)
Low	3 (10.34%)
Medium	24 (82.76%)
Education level	
Bachelor	18 (62.07%)
High school	5 (17.24%)
Master	2 (6.90%)
Other	4 (13.79%)
Family history	
No	16 (55.17%)
Yes	13 (44.83%)
Disease duration (years)	5.69 ± 6.65
Comorbidities	
None	19 (65.52%)
Brain surgery	1 (3.45%)
Breast cancer	1 (3.45%)
Diabetes mellitus	1 (3.45%)
Hashimoto thyroiditis	1 (3.45%)
Hypertension	1 (3.45%)
Hypothyroidism	2 (6.90%)
Other	3 (10.34%)
Suicidal ideation	
No	23 (79.31%)
Yes	6 (20.69%)

TMS, Transcranial Magnetic Stimulation; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; SD, standard deviation.

gesting a potential vulnerability marker for psychiatric illness. Similarly, Fatemi *et al.* [14] demonstrated decreased Reelin immunoreactivity in the hippocampus of patients with schizophrenia, bipolar disorder, and, to a lesser extent, major depression, supporting a shared neurobiological pathway across psychiatric conditions. In contrast to these findings, our study demonstrated elevated serum Reelin levels in patients with depression. This apparent discrepancy may reflect a compensatory or reactive peripheral response rather than a direct reflection of central nervous system levels.

Only a limited number of studies have evaluated peripheral Reelin concentrations in living patients with MDD. Jin *et al.* [17] reported reduced plasma Reelin levels in first-episode drug-naïve patients that increased following antidepressant treatment, whereas our study demonstrated higher baseline serum Reelin levels without significant post-treatment changes. Earlier work by Fatemi *et al.* [14] also demonstrated heterogeneous alterations in circulating Reelin isoforms in patients with mood disorders rather than a uniform directional change. These differences across studies may reflect variation in biological specimens (serum versus plasma), patient populations, medication exposure, disease stage, analytical methods, and treatment approaches.

Previous studies have demonstrated that Reelin is present in both the central nervous system and peripheral circulation [1,18]. However, the biological relationship between central and peripheral Reelin concentrations remains incompletely understood. Therefore, the elevated serum Reelin levels observed in our cohort should not be interpreted as directly reflecting central nervous system Reelin activity, and the underlying biological mechanisms remain to be elucidated.

One of the most striking findings of our study was the opposite pattern observed between serum and salivary Reelin levels. While serum levels were increased, salivary levels were significantly decreased in patients at baseline. Saliva has been proposed as a non-invasive medium for exploratory biomarker research in psychiatric disorders; however, variability in biomarker transport, secretion, and local regulation may limit its direct comparability with serum measurements [19]. Furthermore, the biological origin of salivary Reelin remains incompletely understood, and the present study was not designed to determine whether salivary Reelin primarily reflects local secretion, transudation, plasma ultrafiltration, or other biological processes. Therefore, the salivary findings should be interpreted as exploratory.

During the course of individualized TMS treatment, significant reductions in both depressive and anxiety symptoms were observed. However, because of the single-arm pre-post observational design, these improvements cannot be attributed specifically to TMS and should be interpreted descriptively. Consensus guidelines and accumulating evidence support the clinical efficacy and safety of TMS, while ongoing research continues to explore its biological mechanisms of action and potential biomarkers of treatment response [2,11]. However, despite the observed improvement in clinical symptom severity, changes in Reelin levels did not reach statistical significance. Serum Reelin showed a numerical increase, while salivary Reelin demonstrated

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Table 3. Comparison of BDI and BAI scores and serum and salivary Reelin levels before and after TMS.

Variable	Before TMS Mean ± SD	After TMS Mean ± SD	Mean difference (95% CI)	Cohen's d	*p*-value
BDI score	31.62 ± 9.61	16.59 ± 10.50			<0.001
BAI score	21.62 ± 11.22	14.59 ± 10.24			<0.001
TMS serum Reelin (ng/mL)	10.08 ± 2.32	10.69 ± 2.07	0.611 (−0.679 to 1.902)	0.18	0.34
TMS salivary Reelin (ng/mL)	0.64 ± 0.17	0.66 ± 0.13	0.016 (−0.053 to 0.085)	0.09	0.633

TMS, Transcranial Magnetic Stimulation; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; CI, confidence interval; SD, standard deviation. Mean differences were calculated as post-TMS minus pre-TMS values.

only a minimal rise and remained below control levels. Because neither serum nor salivary Reelin levels changed significantly following treatment and the corresponding effect sizes were small, the present findings do not demonstrate a measurable short-term effect of heterogeneous real-world TMS treatment on peripheral Reelin concentrations. Although delayed biological responses remain possible, this interpretation should be considered speculative and requires confirmation in larger prospective studies. Furthermore, because concomitant psychotropic medications, medication stability, and dose adjustments were not systematically documented, the observed peripheral Reelin findings cannot be attributed specifically to TMS.

Previous studies have demonstrated that deep TMS is an effective treatment for MDD, producing significant clinical improvement [4,20]. However, experimental studies investigating Reelin have suggested that this glycoprotein may contribute to vulnerability to depression rather than directly evaluating biomarker responses following TMS [21]. In this context, our findings support the notion that clinical efficacy and biomarker dynamics may not always be directly correlated. Additionally, the heterogeneity of TMS protocols applied in our study reflects real-world clinical practice and aligns with evidence emphasizing the importance of personalized neuromodulation approaches in depression treatment [22].

The heterogeneity of stimulation protocols should therefore be interpreted within the context of the study design. Since treatment parameters were individualized according to routine clinical decision-making, the observed biomarker responses represent the aggregate biological effects of real-world TMS practice rather than protocol-specific effects. Consequently, differences related to stimulation modality, cortical target, cumulative stimulation dose, or treatment duration cannot be distinguished in the present cohort.

From a mechanistic perspective, the observed compartment-specific serum and salivary Reelin patterns should be regarded as hypothesis-generating observations rather than evidence of a defined biological mechanism. However, this interpretation should be regarded as hypothesis-generating because the present study was not designed to formally evaluate inter-compartment relationships. Experimental studies have shown that Reelin exerts rapid antidepressant-like effects and plays a role in synaptic remodeling and neuroplasticity [23–25]. Although the observed peripheral Reelin patterns may reflect differences in biological regulation between serum and saliva, the present study was not designed to investigate the underlying mechanisms. Consequently, no conclusions can be drawn regarding the biological basis of these observations, and alternative explanations should be explored in future mechanistic studies. Although Reelin has been shown to regulate pre- and postsynaptic structural organization and synaptic density in the hippocampus [26], the present study was not designed to investigate the biological mechanisms underlying the observed peripheral Reelin patterns.

The present findings should be interpreted within the exploratory nature of the study. Although salivary Reelin demonstrated significant baseline differences between patients and healthy controls, these observations do not establish its clinical validity as a biomarker for MDD. Additional prospective studies evaluating diagnostic performance, longitudinal reproducibility, and relationships with clinical outcomes will be required before salivary Reelin can be considered a validated clinical biomarker.

Our study has several limitations. First, the single-arm pre-post observational design and the absence of a sham-TMS group, treatment-as-usual comparator, or repeated longitudinal assessment of healthy controls preclude causal attribution of the observed symptom reductions or peripheral Reelin findings specifically to TMS. Regression to the mean, expectancy effects, concurrent treatments, natural symptom fluctuation, and differences in treatment duration may all have contributed to the observed changes. Second, TMS was delivered according to routine clinical practice using individualized treatment protocols that differed in stimulation modality, cortical target, treatment schedule, stimulation intensity, and treatment duration. Although

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this design reflects real-world clinical care, it may have contributed to biological variability in peripheral Reelin responses. Because of the relatively small sample size, protocol-specific subgroup analyses and multivariable adjustment were not considered statistically reliable.

Another important limitation is the absence of systematically collected data regarding concomitant psychotropic medications, medication stability, dose adjustments during treatment, psychotherapy, and several other potential clinical confounders. Consequently, their potential influence on both symptom improvement and peripheral Reelin dynamics cannot be excluded, and the observed biomarker findings should be interpreted as reflecting routine clinical care rather than effects attributable exclusively to TMS. In addition, sociodemographic and clinical variables were unavailable for the healthy control group, preventing adjustment for potential confounders in patient–control comparisons. In addition, because depression and anxiety severity were assessed using self-reported questionnaires (BDI and BAI) in an unblinded study setting, expectancy effects and response bias may also have contributed to the observed improvements in symptom scores.

Furthermore, the present study was not designed to investigate the relationship between changes in Reelin levels and clinical improvement, nor were correlation or interaction analyses performed between serum and salivary Reelin concentrations. Therefore, no conclusions can be drawn regarding the association between peripheral Reelin dynamics and treatment response or whether the observed compartment-specific differences represent true biological compartmentalization. Finally, the study did not include long-term follow-up or direct central nervous system measurements, limiting interpretation of delayed biomarker responses and the relationship between peripheral and central Reelin dynamics. The findings should therefore be regarded as exploratory and require confirmation in larger prospective studies using standardized treatment protocols, comprehensive clinical characterization, multivariable statistical modeling, and longitudinal assessment of peripheral and central biomarkers.

In addition, several potentially relevant clinical and biological confounding variables were not systematically documented, including structured diagnostic interview findings, episode characteristics, treatment resistance, psychotic features, smoking status, caffeine consumption, oral health status, recent infections, inflammatory conditions, and medication-related factors. Although saliva samples were collected under standardized fasting morning conditions, the absence of these variables limits our ability to evaluate their potential influence on peripheral Reelin concentrations. Furthermore, the present study was not designed to evaluate the biological origin of salivary Reelin or the analytical performance of the ELISA assay in the salivary matrix, including matrix effects, recovery, or assay precision. Consequently, salivary Reelin measurements should be regarded as exploratory findings requiring further biological and analytical validation. Future prospective investigations should incorporate comprehensive psychiatric phenotyping together with standardized assessment of clinical and biological confounders.

Conclusions

In conclusion, patients with MDD exhibited opposite baseline serum and salivary Reelin patterns compared with healthy controls. Although significant clinical improvement was observed following heterogeneous real-world TMS treatment, neither serum nor salivary Reelin levels changed significantly. These findings support the presence of compartment-specific baseline differences in peripheral Reelin concentrations but do not demonstrate a measurable short-term biomarker response to TMS. Larger prospective studies using standardized treatment protocols are needed to clarify the biological significance of these observations.

Presentation at a Scientific Meeting

A preliminary version of this study was presented as an oral presentation at the 22nd International Hippocrates Congress on Medical and Health Sciences (online), 26–27 June 2026. The conference presentation consisted of the study abstract only and has not been published as a full-length peer-reviewed article.

Availability of Data and Materials

The data produced and examined in the present study are available through the Istinye University Dataset Sharing Platform. De-identified clinical datasets may be accessed via the following link: https://dataset.istinye.edu.tr/dataset?did=96. All records were anonymized in full compliance with applicable ethical standards. Data access is granted exclusively for research use within a controlled-access framework, in accordance with the platform's established data-sharing and licensing policies. The shared dataset contains de-identified, processed variables suitable for analysis rather than raw clinical records to ensure compliance with ethical and data-protection standards.

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Author Contributions

MSG: conceptualization, methodology, investigation, data curation, writing—original draft. ÖG: conceptualization, investigation, resources, supervision, writing—review and editing. HFG: methodology, formal analysis, visualization, writing—review and editing. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work..

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Istinye University (protocol code: 24-235, date of approval: 2024-11-22). Written informed consent was obtained from all participants prior to inclusion in the study. Institutional approval for the research was obtained from the management of Moodist Hospital on 05/09/2024.

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Conflict of Interest

None of the authors has any potential financial conflict of interest related to this manuscript.

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