

REVIEWER'S REPORT

Manuscript No.: JNHM- 172

Title: Association between kisspeptin and neurokinin B with menopausal symptoms in women at the premenopausal stage.

Recommendation:

Accept after minor revision

Rating	Excel.	Good	Fair	Poor
Originality			✓	
Techn. Quality			✓	
Clarity		✓		
Significance	✓			

Reviewer's Name:-Bilqees Hamza

Detailed Reviewer's Report

The submitted manuscript investigates the relationship between circulating concentrations of serum kisspeptin and neurokinin B (NKB) and the presence of menopausal transition-related symptoms in premenopausal women. The study recruited 190 participants (90 premenopausal women experiencing menopausal symptoms and 100 age-matched asymptomatic controls) at Al-Hilla Teaching Hospital in Iraq. Using ELISA techniques, the authors evaluated peripheral levels of both neuropeptides and mapped them against clinical self-reports of vasomotor, sleep, mood, and sexual symptoms.

The underlying scientific premise is relevant and timely. While extensive research links the hypothalamic kisspeptin-neurokinin B-dynorphin (KNDy) neuronal network to menopausal thermoregulation and gonadotropin control in postmenopausal women, relatively few empirical studies examine peripheral neuropeptide elevations during the earlier, premenopausal transition.

The manuscript's core findings indicate that both kisspeptin (2.84 ± 0.91 mIU/mL vs 2.43 ± 0.76 mIU/mL, $p < 0.04$) and neurokinin B (68.7 ± 21.4 pg/mL vs 59.8 ± 18.6 pg/mL, $p < 0.017$) are significantly higher in symptomatic premenopausal women than in controls. Furthermore, specific symptoms demonstrate preferential associations with one or both peptides, and a moderate positive correlation ($r = 0.383$, $p < 0.001$) exists between circulating kisspeptin and NKB.

However, despite these interesting preliminary data, the manuscript suffers from significant methodological errors in the laboratory protocol description, internal statistical reporting discrepancies,

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inconsistent textual statements, and severe formatting/typographical flaws. Major revisions are required to resolve these issues before the paper can be considered for publication.

2. Strengths of the Manuscript

1. **Clinical Relevance and Timing:** Investigating KNDy-related biomarkers specifically within premenopausal women experiencing early transitional symptoms addresses an important gap in the literature, where postmenopausal cohorts predominate.
2. **Clear Case-Control Design Structure:** The clinical study design, age-matching approach, and well-defined participant exclusion criteria (e.g., excluding diabetes, endocrine disorders, and recent hormone therapy) provide a solid baseline framework.
3. **Plausible Neuroendocrine Grounding:** The findings logically align with emerging clinical interest in NK3R antagonists and the established physiology of KNDy hyperactivation following subtle alterations in sex-steroid feedback.

3. Revisions & Critical Weaknesses**A. Methodological Flaw: Erroneous Cell Culture Protocol in Serum Assay Description**

Lines 178–183 contain a critical protocol error. While describing the sandwich/competitive ELISA assays for measuring human serum kisspeptin and NKB, the text suddenly introduces an in vitro cell culture procedure involving A549 lung carcinoma cells ("A549 cells were seeded in 96-well plates with 200 µl of a cell suspension at a density of 1×10^5 cells/ml per well... cell viability was evaluated by...").

- **Required Correction:** A standard ELISA performed on collected patient serum samples does not involve seeding human lung cancer cells or evaluating cell viability. This passage appears to be a copy-paste error from an unrelated cell biology manuscript. The authors must completely remove lines 178–183 and replace them with the correct standard ELISA incubation, washing, substrate addition, and stop-solution protocol for serum detection.

B. Statistical and Reporting Discrepancies**1. Conflicting Correlation Data:**

- In the Abstract (lines 25–26) and Results Section text (line 261), the Pearson correlation between kisspeptin and NKB is reported as $r = 0.383$, $p < 0.001$.
- In Table 6 (line 266), the p-value is listed as $p < 0.032$.
- In the Discussion (lines 357–358), the p-value is reported as $P = 0.042$.
- **Required Correction:** The exact p-value for the correlation coefficient ($r = 0.383$) must be calculated and standardized across all sections (Abstract, Results Table 6, and Discussion).

REVIEWER'S REPORT**2. Inconsistent Table Classifications & Annotations:**

- In Table 4 (Night Sweats), the p-value is reported as \$0.23\$, which is correctly marked as non-significant in the statistical test column (\$1.20\$), but the Significance column incorrectly prints "S" (Significant) instead of "NS".
- In Table 4 (Decrease in Libido), \$p = 0.05\$ is labeled as "S", whereas the main text states it only "approached significance".
- In Table 5 (Night Sweats), the mean values (\$72.5\$ vs \$64.0\$, \$p = 0.02\$) are marked as "NS" (Non-Significant) in the table legend, contradicting the text and p-value.
- **Required Correction:** Audit all tables (Tables 1–6) against raw statistical outputs to ensure that p-values, t-test statistics, and "S" / "NS" designators match precisely.

C. Contradictory Statements in Text and Conclusion

1. **Direction of Correlation in Conclusion:** In line 379 of the Conclusion, the authors state: *"Kisspeptin concentrations correlated inversely with the frequency of hot flashes, vaginal dryness, and mood disturbance"*. However, the actual study findings (Table 4, lines 237–239) show that kisspeptin levels were **significantly higher** (positively associated) in women with these symptoms (\$3.02\$ vs \$2.53\$ mIU/mL for hot flashes).
 - **Required Correction:** Fix the word "inversely" in the conclusion to "positively" or state that kisspeptin levels were significantly elevated in symptomatic women.
2. **Personal Laboratory Claim in Discussion:** In line 311, the authors write: *"In vivo and in vitro studies performed in our laboratory confirmed the participation of increased NKB signaling..."* citing Gombert-Labedens et al. (2025). Unless the current authors are the primary authors of that cited review paper, attributing third-party literature to "our laboratory" is inappropriate. Frame this purely as a literature citation.

4. Minor Revisions & Language/Formatting Corrections**1. Title & Formatting Fixes:**

- Fix missing space in title: "Neurokinin Bwith" $\rightarrow$ "Neurokinin B with".
- Fix title typographical placement in line 222: "Neurokinin Bbetween" $\rightarrow$ "Neurokinin B between".
- Fix line 229: "andpsychological/sleep-related" $\rightarrow$ "and psychological/sleep-related".

2. **Kit Catalog & Reagent Description:** In lines 164–169, kit catalog references contain residual draft notes (e.g., "(Cat. (primer kit No. EEL080...)", "Cat. Lawson as follows: LS-F39694 | 2.)"). Rephrase to standard citation formatting for assay kits (Company, City, Country, Catalog #).

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3. **Table Formatting:** Ensure units are clearly defined in table headers (e.g., Kisspeptin in mIU/mL and NKB in pg/mL).

Final Recommendation: Revisions. The dataset provides valuable clinical insights into early premenopausal neuroendocrine alterations. However, publication cannot be granted until the cell culture text error is excised, statistical tables are verified, and narrative contradictions are corrected.