



REVIEWER'S REPORT

Manuscript No.: IJAR-57681

Title: Association between Paraoxonase 1 Allelic Frequency Distribution, Adjusted Odds Ratios, and the Risk of Cardiovascular Diseases in a Moroccan population,

Recommendation:

Accept as it is

Accept after minor revision.....

Accept after major revisionYES

Do not accept (*Reasons below*)

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

Reviewer's ID: JPR-094

Detailed Reviewer's Report

Reviewer's Report

Overall Evaluation

The manuscript investigates the relationship between PON1 Q192R polymorphism, oxidative stress markers, lipid parameters, and cardiovascular disease risk among healthy, diabetic, and hemodialysis Moroccan subjects. The topic is clinically relevant because oxidative stress and PON1 dysfunction are implicated in diabetes, renal failure, and cardiovascular disease progression.

The study provides useful regional genetic epidemiological data from a Moroccan population and attempts to integrate biochemical markers with genotype-associated cardiovascular risk. However, the manuscript has substantial methodological, statistical, structural, and language-related limitations that significantly weaken the scientific validity and interpretation of the findings. Several conclusions are overstated considering the wide confidence intervals and lack of statistical significance for many odds ratios.

The manuscript requires ****major revision**** before it can be considered for publication.

Strengths

1. ****Clinically Relevant Topic****

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* The study addresses oxidative stress, PON1 polymorphism, diabetes, hemodialysis, and cardiovascular disease risk, which are important areas in cardiovascular and metabolic research.

2. ****Population-Specific Data****

* Provides data from a Moroccan population, which is underrepresented in genetic epidemiology literature.

3. ****Multifactorial Assessment****

* Combines biochemical markers (MDA, Vitamin E, lipid profile), enzyme activities (PON1 and arylesterase), and allelic frequency distribution.

4. ****Inclusion of Multiple Comparison Groups****

* Healthy controls, diabetic patients, and hemodialysis patients allow broader comparative analysis.

5. ****Use of Oxidative Stress Biomarkers****

* Measurement of MDA and Vitamin E adds mechanistic relevance to the study.

6. ****Ethical Approval Mentioned****

* Ethical approval and informed consent were appropriately stated.

Weaknesses

Major Weaknesses

1. Lack of True Genotyping Methodology

* The manuscript infers PON1 phenotypes using enzymatic activity ratios instead of direct molecular genotyping.

* No PCR, sequencing, or genotyping assay was performed to confirm Q192R polymorphism.

* Phenotypic classification alone may misclassify subjects.

2. Statistical Weaknesses

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* Most odds ratios have:

- * wide confidence intervals,
- * include unity (1.0),
- * and are statistically non-significant.
- * Despite this, authors repeatedly claim increased cardiovascular risk.
- * No multivariate regression model details are provided.

3. Overinterpretation of Results

- * Conclusions about “high cardiovascular risk” are not sufficiently supported.
- * Significant associations are weak and inconsistent across subgroups.

4. Small Sample Size for Genetic Subgroups

- * RR phenotype subgroup is extremely small (n=18), limiting statistical reliability.
- * Subgroup analyses by age and gender become underpowered.

5. Cross-Sectional Design

- * The study design cannot establish causality.
- * Cardiovascular outcomes are not prospectively assessed.

6. Confounding Factors Not Properly Controlled

Important confounders are insufficiently adjusted:

- * statin use,
- * hypertension,
- * obesity,
- * smoking,
- * dialysis duration,
- * diabetic duration,
- * medications.

7. Cardiovascular Disease Definition Unclear

- * The manuscript does not clearly define:
 - * cardiovascular disease criteria,
 - * diagnostic methods,
 - * adjudication process.

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8. Inconsistent Interpretation of Oxidative Stress Markers

- * Some MDA comparisons are non-significant but discussed as biologically meaningful.
- * Vitamin E interpretations are speculative.

Technical and Methodological Concerns

Study Design

- * Selection criteria for controls are not sufficiently rigorous.
- * Recruitment strategy may introduce selection bias.

Laboratory Methods

- * HPLC methods lack detailed validation parameters:
 - * calibration,
 - * sensitivity,
 - * reproducibility,
 - * inter-assay variability.

Statistical Analysis

Missing details:

- * adjustment variables,
- * power calculation,
- * Hardy–Weinberg equilibrium testing,
- * correction for multiple comparisons.

Odds Ratio Analysis

- * ORs are unstable due to small RR numbers.
- * Some ORs suggest no significant association despite strong narrative conclusions.

Example:

- * OR = 1.97 (95% CI: 0.63–6.21)
- * This is statistically inconclusive.

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Clarity and Language Issues

The manuscript contains extensive grammatical and typographical errors throughout.

Examples:

- * "give rises" instead of "gives rise"
- * "susceptibly" instead of "susceptibility"
- * "Measurement were performed"
- * "ration" instead of "ratio"
- * inconsistent tense usage.

There are also:

- * formatting inconsistencies,
- * duplicated references,
- * citation formatting errors,
- * spacing issues,
- * inconsistent statistical notation.

The manuscript requires professional English language editing.

Key Scientific Points

Positive Findings

- * Reduced PON1 activity in hemodialysis patients is biologically plausible.
- * Increased oxidative stress markers in diabetic and hemodialysis subjects align with prior literature.
- * Higher R allele frequency in hemodialysis patients may warrant further investigation.

Problematic Findings

- * The study repeatedly interprets non-significant ORs as evidence of increased risk.
- * Phenotype-based inference without genotyping weakens genetic conclusions.
- * Several subgroup analyses appear exploratory rather than hypothesis-driven.

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Significance

The study has moderate potential significance because:

* PON1 polymorphism remains relevant in oxidative stress and cardiovascular research.

* Data from North African populations are limited.

However, current methodological limitations substantially reduce translational impact.

At present, the study contributes mainly as:

* preliminary observational evidence,

* hypothesis-generating research,

rather than definitive evidence of cardiovascular risk association.

Specific Recommendations for Improvement

Major Revisions Required

Methodology

1. Include direct genotyping confirmation if samples are available.
2. Clearly define cardiovascular disease criteria.
3. Provide detailed multivariate adjustment methods.
4. Include power analysis.

Statistics

5. Reanalyze ORs with proper adjustment.
6. Avoid claiming associations when confidence intervals cross 1.
7. Correct for multiple subgroup comparisons.

Results

8. Simplify repetitive subgroup analyses.
9. Present clearer tables with exact p-values.

Discussion

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10. Reduce speculative mechanistic interpretations.
11. Better distinguish statistically significant vs non-significant findings.
12. Discuss limitations more transparently.

Language

13. Extensive English editing is mandatory.
14. Correct grammar, formatting, and reference inconsistencies.

Recommendation

Final Recommendation: **Major Revision**

Justification

The manuscript addresses an important clinical and biochemical topic and includes valuable regional data. However, major concerns regarding:

- * statistical interpretation,
- * lack of molecular genotyping,
- * overstatement of conclusions,
- * small subgroup sizes,
- * insufficient adjustment for confounders,
- * and poor language quality

currently prevent acceptance in its present form.

Substantial methodological clarification, statistical revision, and language editing are necessary before reconsideration for publication.

Major Revision Justification

The manuscript addresses an important topic involving Cardiovascular Genetics, oxidative stress, and PON1 Q192R Polymorphism in diabetic and hemodialysis patients from a Moroccan population. However, the manuscript contains substantial methodological, statistical, linguistic, structural, and interpretational weaknesses that currently limit its scientific reliability and reproducibility. Major revision is required before the manuscript can be considered for publication.

REVIEWER'S REPORT**Major Issues with Reasons (Line-by-Line)**

Line(s)	Issue	Reason for Major Revision
7-10	Aim is poorly structured and vague	The objective mixes phenotype, allelic frequency, and cardiovascular complications without clearly defining primary endpoint or hypothesis.
11-14	Study design inadequately described	No mention whether study is case-control, cross-sectional, or observational analytical study.
15-17	Laboratory methods insufficient	HPLC procedures lack calibration, validation, reproducibility, and quality-control details.
18-29	Conclusions overstated	Odds ratios are mostly statistically non-significant because confidence intervals include 1.0, yet authors claim association.
20-21	OR interpretation inappropriate	OR 1.97 (0.63–6.21) is not statistically significant. Authors incorrectly imply strong association.
21-22	Another non-significant OR	OR 1.37 (0.62–3.04) also crosses 1.0; conclusions should be cautious.
27-29	Causal language used in observational study	“Suggest development of cardiovascular complications” implies causality without longitudinal evidence.
38-52	Literature review outdated and repetitive	Introduction relies heavily on old references and lacks recent genomic or mechanistic insights.
48-49	Biological description oversimplified	Q allele described as “low activity” and R allele “high activity” without acknowledging substrate dependency of PON1 activity.
50-52	Contradictory interpretation	Authors state R allele increases risk but later discussion inconsistently describes protective effects in some contexts.
53-56	Aim repetition	Similar objective already mentioned in abstract.
67-74	Sampling strategy unclear	No information on randomization, matching, recruitment bias, or power analysis.
75-78	Healthy controls not properly matched	Controls differ substantially from diseased groups in medication and comorbidities, creating confounding bias.
79-83	Clinical heterogeneity in diabetic group	Presence of hypertension, nephropathy, insulin use, and surgery history introduces confounders not adjusted statistically.
84-90	Hemodialysis group	Previous transplantation and valvular disease may

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Line(s)	Issue	Reason for Major Revision
	heterogeneous	influence oxidative stress and lipid parameters.
91-92	Ethical approval incomplete	Ethics approval number/reference not provided.
94-102	Questionnaire not validated	No information on reliability or validation of questionnaire.
99-102	Blood pressure methodology outdated	WHO 1999 criteria are obsolete; current hypertension guidelines should be used.
104-113	Blood collection details incomplete	No fasting duration, sample stability, freeze-thaw cycles, or assay variability provided.
118-125	Paraoxonase assay insufficiently standardized	Units and extinction coefficient formatting unclear; assay reproducibility absent.
131-135	Arylesterase assay incomplete	Missing intra/inter-assay coefficient of variation.
138-143	Phenotype classification confusing	Sentence construction unclear and phenotype categorization poorly written.
145-150	Statistical analysis inadequate	No multivariate regression model description, adjustment variables, or normality testing mentioned.
146	Use of outdated software	SAS version 6.11 is extremely outdated and raises reproducibility concerns.
147	Data presentation inappropriate	“mean +/- standard deviation of number cases” grammatically and statistically incorrect.
148-149	Multiple comparison correction absent	Numerous subgroup analyses performed without correction for type I error.
154-158	Borderline p-values overstated	p = 0.08 reported as biologically meaningful despite lack of significance.
159-170	Excessive subgroup comparisons	Numerous comparisons increase false-positive risk.
172-182	Interpretation inconsistent	Slight increase in diabetic PON1 activity contradicts expected oxidative-stress physiology without mechanistic explanation.
183-203	Vitamin E analysis confusing	Contradictory statements regarding significance and direction of associations.
Table 1	Poor table formatting	Inconsistent symbols, unclear annotations, and overlapping comparison markers reduce readability.
212-216	OR calculations unreliable	Small RR subgroup size leads to unstable estimates with wide confidence intervals.

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Line(s)	Issue	Reason for Major Revision
217-222	Allelic frequency interpretation weak	No Hardy–Weinberg equilibrium analysis reported.
223-236	Triglyceride findings confounded	Statin use differs across groups but not adjusted in analysis.
249-254	Chi-square details absent	Degrees of freedom and exact p-values not reported.
256-283	Gender subgroup analysis underpowered	Sample sizes too small for meaningful sex-stratified inference.
285-307	Age subgroup analysis statistically weak	Very wide confidence intervals indicate insufficient power.
309-346	Tables 5 and 6 poorly organized	OR definitions inconsistent and table layout confusing.
348-366	Discussion repetitive	Repeats results instead of interpreting mechanisms critically.
367-370	Contradictory literature interpretation	Authors cite studies showing protective R allele effects but conclude opposite without reconciliation.
379-384	Mechanistic explanation inaccurate	Statement linking arginine directly to free radical production is oversimplified and biologically misleading.
391-392	Overstatement of findings	Manuscript “confirms” association despite statistically weak results.
394-399	Unsupported oxidative stress claims	No direct oxidative stress markers beyond MDA sufficiently support broad conclusions.
401-410	Gender discussion speculative	Claims about obesity and hypertension in women not supported with presented data.
414-430	Confounding by medication not controlled	Statin therapy acknowledged but not adjusted statistically.
432-435	Mediterranean diet explanation speculative	No dietary data collected to support this claim.
436-439	Conclusion not aligned with statistics	Most ORs are non-significant; conclusions should be tempered.
References	Several citation inconsistencies	Formatting errors, duplicated references, outdated citations, and inconsistent journal styles present.
References 17-19	Suspicious future-dated references	References from 2025–2026 appear inconsistent relative to study context and require verification.
Entire	Extensive grammatical	Language quality substantially impairs scientific

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Line(s)	Issue	Reason for Major Revision
manuscript	and typographical errors	clarity and readability.

Statistical Concerns Requiring Major Revision

Most confidence intervals cross 1.0, indicating lack of statistical significance.

No correction for multiple testing.

No Hardy–Weinberg equilibrium analysis.

No sample size or power calculation.

Multivariate adjustment variables not defined.

Small RR subgroup causes unstable OR estimates.

Excessive subgroup analyses increase false discovery risk.

Methodological Concerns

Cross-sectional design cannot infer causality.

Phenotype determination used instead of direct genotyping confirmation.

Confounding factors:

statin therapy,

hypertension,

obesity,

dialysis duration,

nephropathy,

smoking/alcohol history.

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No detailed inclusion/exclusion standardization.

Language and Formatting Problems

The manuscript requires extensive English editing because of:

grammatical errors,
typographical mistakes,
inconsistent terminology,
incomplete sentences,
incorrect statistical notation,
formatting issues in tables and references.

Examples:

“give rises” → “gives rise”

“susceptibly” → “susceptibility”

“Measurement were performed” → “Measurements were performed”

Recommendation

Decision: Major Revision

The manuscript requires:

Comprehensive statistical reanalysis.
Clearer study design description.
Proper interpretation of non-significant odds ratios.
Reduction of speculative conclusions.
Improved methodology reporting.
Extensive English language editing.

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Updated literature review with recent evidence.

Better table formatting and consistency.

Inclusion of Hardy–Weinberg equilibrium analysis and power calculation.

Stronger discussion of limitations and confounding variables.