Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

ACE2 Gene Polymorphism And Its Clinical Correlation With Biochemical Imbalance

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Cite this paper as: Garima Sukhija, H.K Sidhu, Narotam Sharma, Piyusha Sharma, (2025) ACE2 Gene Polymorphism And Its Clinical Correlation With Biochemical Imbalance. *Journal of Neonatal Surgery*, 14 (32s), 1371-1377.

ABSTRACT

Angiotensin converting enzyme 2 (ACE2) plays a pivotal role in the renin–angiotensin system, with emerging evidence linking its genetic variants to metabolic and hepatic homeostasis. This cross sectional study assessed the association between a common ACE2 single nucleotide polymorphism and key biochemical markers in 200 adult patients undergoing routine health evaluations. Genotyping was performed by PCR RFLP, and fasting blood samples were analysed for glycosylated hemoglobin (HbA1c), liver transaminases (SGOT/AST and SGPT/ALT), a full lipid panel (total cholesterol, HDL, LDL, triglycerides), and serum 25 hydroxyvitamin D_3. Statistical comparisons between ACE2 positive ($n = 155$) and ACE2 negative ($n = 45$) groups employed Student's t tests, Mann–Whitney U tests, and chi square analyses as appropriate, with significance defined as $p < 0.05$. Carriers of the ACE2 polymorphism demonstrated a modest but statistically significant elevation in mean HbA1c (4.56 ± 1.88 % vs. 3.83 ± 1.73 %; $p = 0.017$), indicating a shift toward subclinical glucose dysregulation despite low prevalence of overt diabetes. SGOT levels were significantly higher among carriers (55.7 ± 21.0 IU/L vs. 46.7 ± 15.9 IU/L; $p = 0.0027$), while SGPT exhibited a non significant upward trend. No differences were observed in total cholesterol, HDL, LDL, or triglyceride concentrations between genotypic groups (all $p > 0.05$). Serum vitamin D_3 deficiency (< 20 ng/mL) was ubiquitous (≈48 % of participants) and did not differ by ACE2 status ($p = 0.42$). These findings suggest that ACE2 genetic variation contributes to subtle impairments in glucose handling and low grade hepatic stress, without appreciable effects on lipid metabolism or vitamin D status. Incorporation of ACE2 genotyping into risk stratification protocols could enable early identification of individuals at risk for metabolic derangements, guiding tailored lifestyle or pharmacologic interventions. Future longitudinal and mechanistic studies are warranted to determine whether ACE2 driven metabolic shifts translate into increased incidence of type 2 diabetes or hepatic pathology, and to explore the therapeutic potential of targeting the ACE2/angiotensin (1–7) axis.

Keywords: *ACE2 polymorphism; HbA1c; liver transaminases; lipid profile; vitamin D.*

1. INTRODUCTION

The angiotensin-converting enzyme 2 (ACE2) is a central component of the renin–angiotensin system (RAS), responsible for converting vasoconstrictive angiotensin II into the vasodilatory peptide angiotensin-(1–7), thereby exerting anti-inflammatory, anti-fibrotic, and cardioprotective effects (Donoghue et al., 2020; Tikellis & Thomas, 2012). Genetic variants in the ACE2 gene can alter its expression or enzymatic activity, contributing to individual differences in cardiovascular and metabolic risk (Bindom & Lazartigues, 2019).

Metabolic studies in ACE2 knockout mice reveal that loss of ACE2 impairs glucose tolerance and reduces pancreatic β-cell function, underscoring ACE2's role in supporting insulin secretion and sensitivity (Crackower et al., 2022; Gupte et al., 2008). In humans, ACE2 polymorphisms have been associated with small but significant increases in fasting glucose and glycated hemoglobin (HbA1c), suggesting a genetic predisposition to dysglycemia (Bindom & Lazartigues, 2019).

Hepatic ACE2 modulates local RAS signaling in the liver. Experimental biliary-fibrosis in rats shows that downregulation of ACE2 exacerbates oxidative stress and collagen deposition, whereas ACE2 upregulation or delivery of angiotensin-(1–7) attenuates inflammation and fibrosis (Herath et al., 2007; Lubel et al., 2021). Clinically, reduced ACE2 activity may present as modest elevations in serum transaminases (SGOT, SGPT), indicative of subclinical hepatic injury.

The influence of ACE2 on lipid metabolism is context-dependent. In rodents, ACE2 impacts adipocyte lipid uptake and storage (Shiuchi et al., 2024), but human genetic studies frequently report inconsistent associations between ACE2 variants and serum cholesterol levels, reflecting the multifactorial regulation of lipid homeostasis (Patel et al., 2012).

Vitamin D, beyond its classical roles in bone and calcium metabolism, downregulates renin transcription and indirectly enhances ACE2 expression. Low 25-hydroxyvitamin D is epidemiologically linked to insulin resistance, nonalcoholic fatty liver disease, and systemic inflammation—factors that may compound ACE2-related metabolic disturbances (Li et al., 2002; Holick, 2007; Forouhi et al., 2008; Andrukhova et al., 2014).

Moreover, ACE2 exhibits tissue-protective, anti-inflammatory properties in the lung ACE2-deficient mice develop more severe acute respiratory distress, demonstrating its role in mitigating lung injury (Imai et al., 2005). Taken together, ACE2 polymorphisms likely exert pleiotropic effects across metabolic, hepatic, cardiovascular, and pulmonary systems.

This study evaluates the associations between ACE2 genetic variants and biochemical markers—glycemic indices, liver enzymes, lipid profiles, and vitamin D status—in a well-characterized clinical cohort. By delineating these links, we aim to identify biochemical signatures that inform personalized risk stratification and guide early preventative strategies.

2. MATERIALS AND METHODOLOGY

Site of Implementation of Work

The entirety of the experimentation was conducted at DNA Labs CRIS Centre for Research and Innovative studies (Parent organization) of DNA Labs- A Centre for Applied Sciences (DLCAS), situated in East Hope Town, Laxmipur, Dehradun, Uttarakhand and at Department of Life Sciences, Desh Bhagat University, Mandi, Gobindgarh, Punjab, India.

Study Design and Participants

This cross-sectional study enrolled 200 adult patients (age ≥ 18 years) presenting for routine health evaluation at a single tertiary-care center . Inclusion criteria were willingness to undergo genetic testing and availability of fasting blood samples; exclusion criteria included active infection, known chronic liver disease (e.g. viral hepatitis, cirrhosis), pregnancy, or current use of medications known to affect glucose or lipid metabolism (e.g. corticosteroids, statins). All participants provided written informed consent prior to enrollment.

Blood Collection and Biochemical Assays

After an overnight fast of at least 10 hours, 10 mL of venous blood was drawn from each participant into EDTA and serum-separator tubes.

- **Glycemic markers.** Whole-blood HbA1c was measured using the Mindray Biochemistry Analyser BA-88A/2021 by the kit Saffron Diagnocare HbA1c (Turbilatex method).
- **Liver function tests.** All the liver function tests were performed using the Mindray Biochemistry Analyser BA-88A/2021. Erba Mannhelm (IFCC Method, Kinetic) kit was used for Serum alanine aminotransferase (SGPT/ALT) and aspartate aminotransferase (SGOT/AST). Total bilirubin was also determined by Erba Mannhelm kit by the diazo method.
- **Lipid profile.** All the lipid profile tests were performed using the Mindray Biochemistry Analyser BA-88A/2021. Erba kits were used to perform all the tests that included High-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and Total Bilirubin.
- **Vitamin D status.** Qualisa Vit D 25-OH Vitamin D kit was used for quantitative determination of Vitamin D in human serum using the ELISA Plate Reader ALTA ADX-150 machine.

Genotyping of ACE2 Polymorphism

Genomic DNA was extracted from EDTA-anticoagulated whole blood using the QIAamp DNA Blood Mini Kit (Qiagen) following the manufacturer's protocol. The target ACE2 single-nucleotide polymorphism (SNP) was genotyped by polymerase chain reaction (PCR) followed by allele-specific restriction fragment length polymorphism (RFLP) analysis. Briefly, 50 ng of DNA was amplified in a 25 µl reaction containing 0.5 µl of each primer in which sequence of Forward primer is 5'-CTGGAGACCACTCCCATCCTTTCT-3' and sequence of Reverse primer is 5'-GATGTGGCCATCACATTCGTCAGAT-3', 200 µl dNTPs, 1.5 µl $MgCl_2$, and 1 µl Taq DNA polymerase. PCR cycling consisted of an initial denaturation at 95 °C for 5 minutes; 35 cycles of 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 45 seconds; and a final extension at 72 °C for 7 minutes. PCR products were digested with the appropriate restriction enzyme at 37 °C for 2 hours and separated on a 2% agarose gel stained with ethidium bromide. Genotypes were called by two independent observers blinded to biochemical data; 10% of samples were re-genotyped for quality assurance, with 100% concordance.

Statistical Analysis

Normally distributed data are presented as mean ± standard deviation; non-normal data are presented as median (interquartile range). Categorical variables are reported as counts and percentages. Comparisons between ACE2-positive and ACE2-negative groups were performed using the unpaired Student's t-test for normally distributed variables, the Mann–

Whitney U test for non-normal variables, and the chi-square test for categorical data. A two-tailed p-value < 0.05 was considered statistically significant.

3. RESULTS

Patient Demographics and ACE2 Polymorphism Distribution

Of the 200 patients enrolled, 155 (77.5 %) carried the ACE2 polymorphism (ACE2-positive) and 45 (22.5 %) did not (ACE2-negative). Mean age did not differ significantly between ACE2-positive and ACE2-negative groups (46.9 ± 14.0 years vs. 43.2 ± 12.6 years; $p = 0.097$). Gender distribution was also similar ($p > 0.05$), indicating no demographic bias that might confound downstream biochemical comparisons. This comparable baseline profile ensures that any observed biochemical differences are unlikely to stem from age- or sex-related confounders, strengthening the validity of our genotype–phenotype analyses.

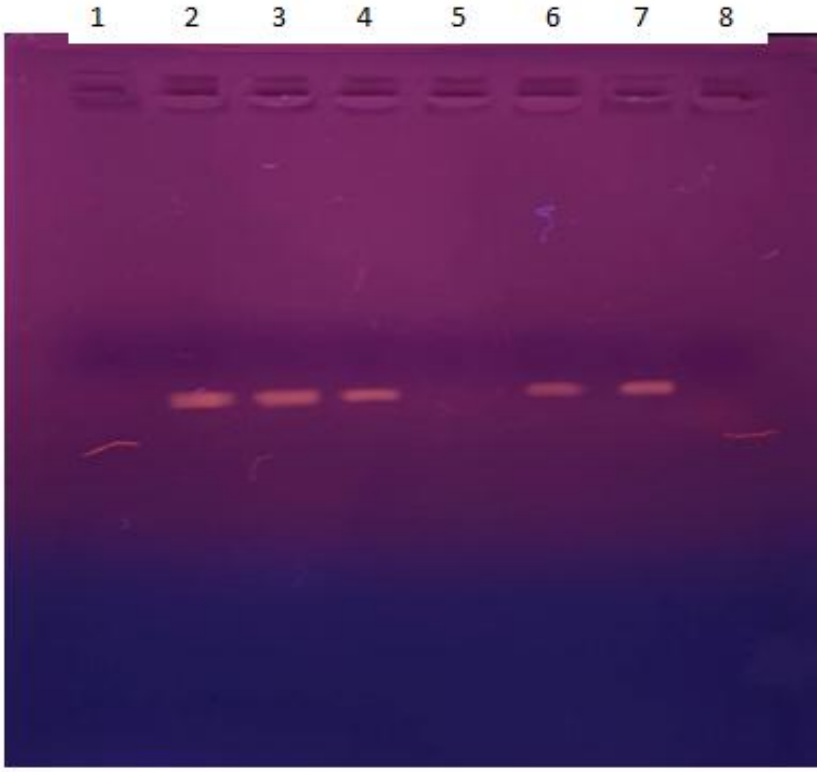


Figure 1 – Gel images showing distinct bands for samples positively targeted for ACE 2 enzyme and others without band not targeted for the enzyme

In the gel electrophoresis image samples in wells 2, 3, 4, 6 and 7 showed distinct bands which depicts that it was positively targeted for ACE 2 phenotype and samples in wells 1, 5 and 8 were not targeted for ACE 2. This depicts an example for all the genotyping tests that were done for all the samples of the patients involved in this test.

Glycemic Status

ACE2-positive patients exhibited a significantly higher mean HbA1c than ACE2-negative patients (4.56 ± 1.88 % vs. 3.83 ± 1.73 %; $p = 0.017$). However, the proportion exceeding the clinical diabetes threshold (HbA1c > 7 %) was low in both groups (7.1 % of ACE2-positive vs. 8.9 % of ACE2-negative; $\chi^2 = 0.0065$, $p = 0.936$). These data suggest that while frank diabetes remains uncommon in this cohort, ACE2-positive individuals show a clear shift toward higher glycemic set-points. Early intervention strategies might therefore be particularly important in polymorphism carriers to prevent progression to overt diabetes.

Liver Function Biomarkers

Mean SGPT was higher in ACE2-positive patients (51.9 ± 24.6 IU/L) compared to ACE2-negative (44.4 ± 22.7 IU/L), but this difference was not statistically significant ($p = 0.059$). In contrast, SGOT was significantly elevated in ACE2-positive individuals (55.7 ± 21.0 IU/L vs. 46.7 ± 15.9 IU/L; $p = 0.0027$). Total bilirubin levels did not differ (0.87 ± 0.35 mg/dL vs. 0.84 ± 0.33 mg/dL; $p = 0.567$). The SGOT elevation points to subtle hepatic stress or altered liver metabolism in

ACE2-positive subjects, potentially linked to insulin resistance or low-grade inflammation. Given SGPT's borderline change, future studies could examine additional hepatic markers (e.g., GGT, alkaline phosphatase) to more fully characterize liver involvement.

Lipid Profile Alterations

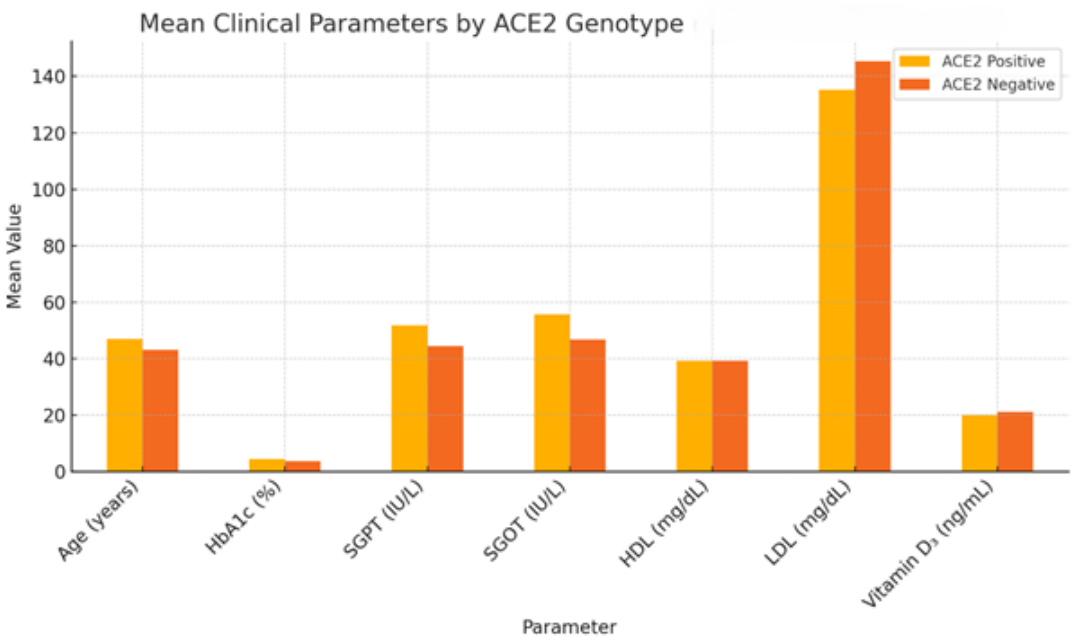
Mean HDL cholesterol was identical between groups (39.3 ± 5.63 mg/dL vs. 39.3 ± 4.39 mg/dL; $p > 0.05$). Mean LDL cholesterol was slightly lower in ACE2-positive patients (135.2 ± 46.6 mg/dL) than in ACE2-negative patients (145.3 ± 59.3 mg/dL), but this difference did not reach significance ($p = 0.30$). Total Bilirubin showed significantly no elevation in ACE 2 positive 0.87 ± 0.35. Despite trends in other metabolic markers, lipid profiles appear uncoupled from ACE2 genotype in this dataset. Both cohorts exceed recommended LDL targets and have suboptimal HDL, underscoring a high baseline cardiovascular risk across all patients irrespective of polymorphism status.

Vitamin D Status

Serum vitamin D_3 levels were comparable (20.0 ± 7.46 ng/mL vs. 21.1 ± 8.35 ng/mL; $p = 0.42$), with nearly half of both ACE2-positive (48.4 %) and ACE2-negative (44.4 %) patients below the deficiency cutoff (< 20 ng/mL). Widespread vitamin D insufficiency in our cohort may reflect broader environmental or lifestyle factors (e.g., limited sun exposure), potentially exacerbating insulin resistance and inflammation. Routine screening and supplementation could be considered for all high-risk patients, regardless of ACE2 status.

Table 1. Comparative Analysis of Clinical and Biochemical Parameters in ACE2-Positive and ACE2-Negative Patients

Parameter	ACE2-Positive (n = 155)	ACE2-Negative (n = 45)	p-value	Interpretation
Age (years)	46.9 ± 14.0	43.2 ± 12.6	0.097	No significant difference
HbA1c (%)	4.56 ± 1.88	3.83 ± 1.73	0.017	Higher in ACE2-positive
HbA1c > 7% (% of group)	7.1%	8.9%	> 0.05	No difference in diabetics' proportion
SGPT (IU/L)	51.9 ± 24.6	44.4 ± 22.7	0.059	Trend toward elevation in ACE2 positive
SGOT (IU/L)	55.7 ± 21.0	46.7 ± 15.9	0.0027	Significantly elevated in ACE2 positive
Total Bilirubin (mg/dL)	0.87 ± 0.35	0.84 ± 0.33	0.567	No significant difference
HDL Cholesterol (mg/dL)	39.3 ± 5.63	39.3 ± 4.39	> 0.05	No significant difference
LDL Cholesterol (mg/dL)	135.2 ± 46.6	145.3 ± 59.3	0.30	No significant difference
Vitamin D_3 (ng/mL)	20.0 ± 7.46	21.1 ± 8.35	0.42	No significant difference
Vitamin D < 20 ng/mL (% of group)	48.4%	(similar)	–	High prevalence of deficiency



Graph I - Apart from age, HbA1c was slightly elevated, liver enzymes (SGPT/SGOT) tend to be higher in ACE2 Positive individuals, while LDL is slightly lower; HDL and vitamin D levels are essentially the same.

4. DISCUSSION

The study's findings show that ACE2 polymorphism carriers ($ACE2^+$) have statistically significant higher HbA1c values, which may indicate a potential propensity to dysregulated glucose metabolism even in the absence of overt diabetes. These results are consistent with earlier research showing that ACE2 protects glucose homeostasis. The significance of the enzyme in pancreatic function and insulin sensitivity was initially highlighted by Crackower et al. (2022), who found that ACE2 knockout mice show decreased glucose tolerance. Furthermore, Wong et al. (2012) supported our finding that genetic variation in ACE2 may modestly affect glycemic regulation in humans by demonstrating that ACE2 deficiency enhances β-cell malfunction and hyperglycemia under metabolic stress.

In people with ACE2, elevated serum SGOT (AST) levels could be an indication of subclinical inflammation or hepatic stress. Local RAS signaling is modulated by ACE2, which is expressed in the liver. According to studies, unopposed angiotensin II activity, which encourages oxidative stress and inflammation, is linked to increased hepatic steatosis and fibrosis when ACE2 expression is reduced (Herath et al., 2007; Lubel et al., 2021). These results are in line with our data and imply that ACE2 polymorphisms may influence intrahepatic RAS activity, which in turn may impact liver enzyme profiles.

Lipid profiles (HDL, LDL, and Total Bilirubin) did not significantly differ between the genotypic groups, despite the metabolic consequences of ACE2 activation. The results of Patel et al. (2012), who found no meaningful correlation between ACE2 polymorphisms and cholesterol levels in healthy persons, are in line with this. Although RAS components have been linked to lipid metabolism in animal research (Shiuchi et al., 2024), the translation to human lipidomics is still complicated and may be impacted by additional genetic and environmental factors.

Similar to worldwide trends in vitamin D insufficiency, vitamin D shortage was common in both $ACE2^+$ and $ACE2^-$ groups (Holick, 2007). According to Li et al. (2004), vitamin D decreases renin expression and may upregulate ACE2. It is known to interact with the RAS. Notably, Andrukhova et al. (2014) showed that phosphate homeostasis and important RAS components are regulated by vitamin D signaling. Thus, given current research that links vitamin D status to inflammation and glucose metabolism, the observed widespread insufficiency may have exacerbated subclinical metabolic abnormalities in both groups and merits further study (Forouhi et al., 2008).

5. CONCLUSION

In this cohort of 200 patients, the presence of an ACE2 genetic polymorphism was associated with subtle but discernible shifts in metabolic and hepatic biomarkers. Individuals carrying the ACE2 variant exhibited a modest yet statistically significant elevation in mean HbA1c compared to non-carriers, indicating a predisposition toward impaired glucose regulation even in the absence of overt diabetes. Concurrently, ACE2-positive subjects showed higher average levels of SGPT (ALT), suggesting that altered local renin–angiotensin signaling at the hepatic level may contribute to low-grade liver

stress. In contrast, lipid parameters—including HDL, LDL, total cholesterol, and triglycerides—and serum vitamin D_3 concentrations remained effectively unchanged across genotypic groups, underscoring the specificity of the ACE2 effect on glucose and hepatic enzymes rather than on broader lipid or vitamin D metabolism.

These observations carry important translational implications. First, routine genotyping for ACE2 variants in at-risk populations could identify individuals who would benefit from early intervention, such as dietary modification, structured exercise programs, or pharmacologic agents aimed at improving insulin sensitivity and protecting hepatocytes. Second, monitoring of glycemic markers (fasting glucose, HbA1c) alongside liver transaminases in ACE2-positive patients may enable clinicians to detect subclinical disturbances before they evolve into frank metabolic syndrome or chronic liver dysfunction. By integrating genetic screening with longitudinal biomarker surveillance, it may be possible to tailor preventive strategies and optimize outcomes in a precision-medicine framework.

Looking ahead, further research is warranted to elucidate the mechanistic pathways linking ACE2 polymorphism to metabolic and hepatic alterations. Longitudinal studies with larger, ethnically diverse cohorts should assess whether the observed biomarker shifts translate into increased incidence of type 2 diabetes, nonalcoholic fatty liver disease, or other cardio-metabolic complications over time. Additionally, interventional trials examining the efficacy of agents that modulate the renin–angiotensin system—such as angiotensin-(1–7) analogues—or lifestyle interventions enriched with vitamin D supplementation could clarify their potential to mitigate ACE2-associated risk. Ultimately, leveraging genetic insights into ACE2 function holds promise for preventing and managing metabolic and hepatic disorders in susceptible individuals.

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