Original Article

# Decoding the link associated with areca nut chewing and cardiovascular disease using hsCRP as a biomarker

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## Abstract

**Background:** Chewing of areca nut is associated with various oral and systemic ill effects. The deleterious oral effects of areca nut have been widely documented to range from reactive lesions, periodontal health compromise to oral pre-cancer and cancerous states and have been described widely in the literature. The reported systemic effects of areca nut chewing are relatively less documented but reported in literature. Areca nut chewing may predispose to cardiovascular disease due to a systemic inflammatory response from the elevated levels of circulating inflammatory mediators. High-sensitivity C-reactive protein (hsCRP) is a systemic inflammatory biomarker to assess the risk of cardiovascular disease.

**Materials and Methods:** This cross-sectional, observational study was conducted among areca nut chewers ($n = 50$) and non-chewers ($n = 50$). Areca nut chewers were considered as chewers/cases. Blood samples were collected from the participants and hsCRP levels were studied. The data were analysed using SPSS software, version 21 for statistical significance. Chi-square test was used to compare categorical variables. Mann–Whitney *U* test was done to analyse continuous variables. The level of statistical significance was set at $P$ value $< 0.05$.

**Results:** hsCRP mean was higher among the chewers ($2.3 \pm 3.7$) compared to non-chewers ($0.9 \pm 1.3$). The difference in hsCRP levels between the study group was statistically significant ($P = 0.002$).

**Conclusion:** This study demonstrates the link between areca nut and cardiovascular disease using hsCRP as biomarker.

**Keywords:** Areca nut, biomarker, cardiovascular disease, hsCRP, systemic inflammation

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## INTRODUCTION

The word "areca" is derived from "adakka" (Malay)/"adakeya" (Indian). Areca nut finds mention in ancient Greek, Sanskrit and Chinese literature. Use of areca nut is indigenous to Southeast Asia and Polynesian Islands.[1-3] Alkaloids are an important component of the areca nut. The four major important alkaloids are arecoline, arecaidine, guvacaine and guvacoline.[4]

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Chewing of areca nut has been associated with various oral and systemic ill effects. The deleterious oral effects of areca nut have been widely documented to range from reactive lesions, periodontal health compromise to oral pre-cancer and cancerous states and have been described widely in the literature.[5,6] The mechanism by which areca nut causes systemic effects may be attributed



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to the release of various inflammatory mediators, such as interleukin-6 and tumour necrosis factor-α (TNF-α), resulting in chronic inflammation, diabetogenic potential, neurochemical modulation and by its effect on sympathetic and parasympathetic nervous system. Areca nut chewing may be a predisposing factor for cardiovascular disease due to the systemic inflammatory response from the elevated levels of circulating inflammatory markers. Depending on the duration and degree of use of areca nut or its product, the extent of severity of the systemic effects varies.[7-9]

C-reactive protein (CRP) is an acute-phase reactant synthesised by the liver. Serum levels of CRP are increased in response to inflammation, infection, etc. Compared to CRP, high-sensitivity C-reactive protein (hsCRP) is more specific and ideal biomarker to assess the cardiovascular risk. hsCRP is also released by liver under the stimulation of cytokines such as TNF-α, interleukin-1 and interleukin-6. hsCRP is considered to identify the risk for cardiovascular disease, diabetes mellitus, obesity, metabolic syndrome and coronary artery disease.[10,11] The present study was aimed to evaluate the cardiovascular risk associated with areca nut chewing by analysing the serum hsCRP levels.

## MATERIALS AND METHODS

The cross-sectional study was approved by the Institutional Ethical Committee (20141125) and included 100 participants between 21 and 60 years of age (chewers 50, non-chewers 50) after obtaining informed consent. Individuals with the habit of chewing areca nut in any form were included in the study as cases. Age- and sex-matched subjects without any habits were considered as controls. Patients with history of smoking, alcohol intake, systemic diseases, infections, cardiac ailments, long-term medications, autoimmune diseases, immunocompromised patients, and local or systemic inflammatory diseases were excluded from the study. Structured case sheet was used to record the demographic details and habit history of the patient. Intra-oral examination was done to include only patients without oral hard and soft tissue lesions. Five millimetres of venous blood was withdrawn from the antecubital vein of cases and controls and stored in anticoagulant coated tubes. Blood samples were centrifuged at 3000 rpm for 15 min and stored at −80°C until further analysis. hsCRP was quantitatively analysed by particle-enhanced immunonephelometric assay in Nephstar® protein analyser.

The American Heart Association and Centres for Disease Control (AHA/CDC) Working Group has classified hsCRP levels low (<1 mg/L), intermediate (1–3 mg/L) and high (>3 mg/L) risk groups for cardiovascular disease.[12]

The data were analysed using SPSS software, version 21 for statistical significance. Chi-square test was used to compare categorical variables. Mann–Whitney *U* test was done to analyse continuous variables. *P* value <0.05 was considered statistically significant.

## RESULTS

Among the study participants, 72% were males and 28% were females. 16% were in the age group of 21–30 years, 34% were between 31 and 40 years, 34% belonged to 41–50 years, and 16% were in the age group of 51–60 years [Figure 1]. Women [2.1 (±1.6) mg/L] had higher mean hsCRP levels compared to men [1.5 (±1.1) mg/L]. The difference was not statistically significant in terms of age and gender ($P > 0.05$).

Among the study group, 26% of chewers and 43% of non-chewers had less than 1 mg/L of hsCRP levels, 16% of chewers and 1% of non-chewers had 1–3 mg/L of hsCRP levels, and 8% of chewers and 6% of non-chewers had more than 3 mg/L of hsCRP levels [Figure 2]. The mean hsCRP levels among chewers were 2.3 (± 3.7) mg/L and non-chewers were 0.94 (±1.3) mg/L. hsCRP mean was higher among the chewers compared to non-chewers. The difference in hsCRP levels between the study group was statistically significant ($P = 0.002$) using Mann–Whitney *U* test [Figure 3, Table 1].

## DISCUSSION

The evidence for the link between areca nut chewing and various systemic diseases remains inconclusive owing to the paucity of published data. Alkaloids are the key players in the pathway to various systemic effects of areca nut. Local and systemic inflammation from areca alkaloids, chiefly arecoline, is responsible for the development of metabolic and cardiovascular diseases.[7] Areca nut has been reported

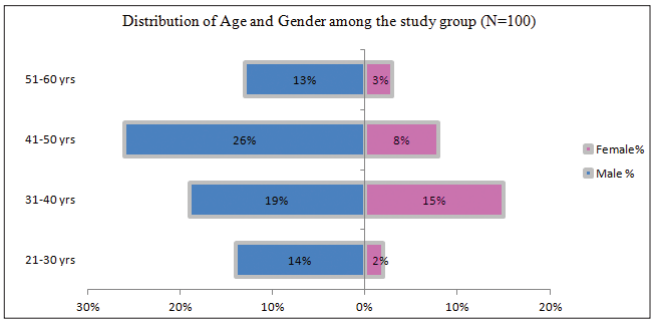


**Figure 1:** Age and gender distribution among the study population (*n* = 100)

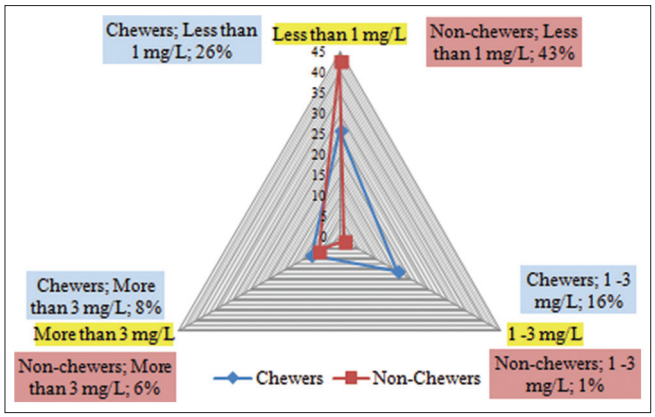


**Figure 2:** Distribution of hsCRP levels among the study group ($n = 100$)

**Table 1: Mean hsCRP in the study group**

| | Chewers ($n$=50) | Non-chewers ($n$=50) | *U* | *P* |
|---|---|---|---|---|
| Mean | 2.3 | 0.9 | 809 | 0.002* |
| Standard deviation | 3.7 | 1.3 | | |
| Median | 0.9 | 0.4 | | |
| Interquartile range | 1.8 | 0.5 | | |

*Mann–Whitney *U* test; *P* value <0.05 – considered significant

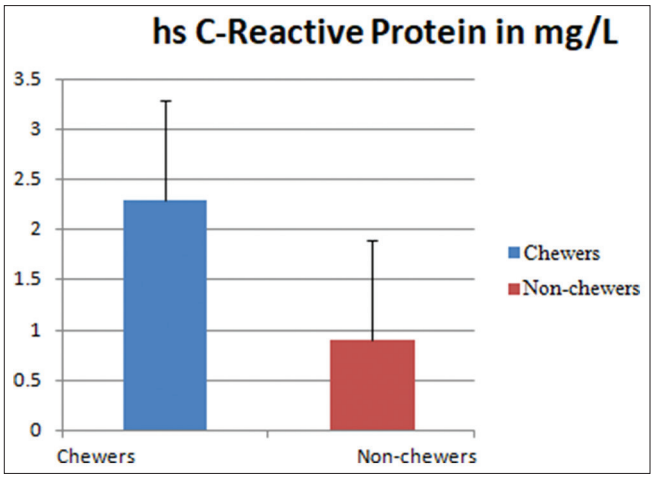


**Figure 3:** Mean hsCRP levels among chewers and non-chewers

to increase the secretion of inflammatory cytokines such as interleukin-6, interleukin-8, interleukin-1β and TNF-α contributing to inflammation in a time- and dose-dependent manner.[13] The inflammatory process due to fresh areca nut was found to be higher than that from processed forms.[14] In-vitro studies have illustrated the release of pro-inflammatory cytokines due to the mobilization of calcium from immune cells which contribute to the chronic inflammation.[15]

hsCRP can be used as a biomarker in cardiovascular disease as it causes activation of complements, mediates the absorption of low-density lipoprotein (LDL) by macrophages, induces the expression of plasminogen activator inhibitor-1, induces the oxidation of LDL, induces the expression of cell adhesion molecules and decreases the production of nitric oxide.[16,17]

In our study, women had higher hsCRP levels compared to men. Similar finding was reported by Lai *et al*.[18] who observed that hsCRP levels were elevated in women than in men and proved a stronger association of metabolic syndrome in women than in men. Oestrogen may be attributed to the difference in hsCRP levels among male and female.[19]

Our results were in concordance with that of Shafique *et al*.[8] who reported that areca nut chewers had elevated CRP levels more than 10 mg/dL and areca nut chewing has a significant association with systemic inflammation.

The results of our study were similar to the observation of Lin *et al*.[20] who reported that current and former areca nut chewers had higher risk of cardiovascular disease than never chewers. The association of heart disease and betel quid chewing was also studied by Guh *et al*.[21] who demonstrated that the habit of betel quid chewing was independently associated with heart disease. Our findings were also consistent with that of Tsai *et al*.[22] who documented areca nut chewing to be an independent risk factor for coronary artery disease. Arecoline, the alkaloid of areca nut, inhibits adipogenic differentiation, induces lipolysis in adipocytes, blocks HDL receptors and inhibits the uptake of LDL uptake by the liver.[7] Computational molecular docking studies have reported that arecoline binds with the active site of LDL receptors of endothelial cells and has higher affinity than that of LDL cholesterol. This enhances the atherogenic activity and deposition of cholesterol in blood vessel wall.[17]

In our study, hsCRP measurement was not repeated after 2 weeks due to logistical constraints, as per the guidelines of AHA/CDC working group. The AHA/CDC working group recommends the assay to be done twice in 2 weeks' interval either in fasting or non-fasting state.[9] Also, larger sample size can better prove such potential links by addressing confounding factors. Although hsCRP is an independent biomarker for cardiovascular disease, use of other biomarkers such as interleukin IL-6, IL-7 and TNF-α would be corroborative.

## CONCLUSION

hsCRP protein as a biomarker to predict cardiovascular diseases has been documented in literature. This study demonstrates the link between areca nut and cardiovascular disease using hsCRP as biomarker. The importance of

awareness on the effects of areca nut on human health necessitates implementation of cessation programs to demystify the socio-cultural belief irrespective of the socio-economic groups.

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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## Conflicts of interest

There are no conflicts of interest.

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