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Analysis of C-Reactive Protein and Matrix Metalloproteinase-2 and 9 Levels: A Comparison between Arthritis Patients and Snail Consuming Subjects

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Abstract: ***The subjects who consumed snails, the prevention of arthritis was observed as per the declining levels of biomarkers viz. C-reactive protein (CRP), matrix metalloproteinases-2 (MMP-2) and MMP-9. The objective of this study was to analyse the level of CRP), matrix metalloproteinases-2 (MMP-2) and MMP-9 in arthritis patients compared to subjects who consumed snails. A total of 100 subjects, half of the patients were presented with OA (group A) and rest subjects did not show OA (group B) who consumed the flesh of the snails in their diet. For CRP (mg/ml), a highly significant (P<0.0001) decreased level was observed in group B (3.06 ± 1.13) when compared to group A (8.55 ± 4.55). For MMP-2 (ng/ml), a highly significant (P<0.0001) decreased level was observed in group B (498.60 ± 17.08) when compared to group A (545.58 ± 61.99). For MMP-9 (ng/ml), a highly significant (P<0.0001) decreased level was observed in group B (20.83 ± 2.77) when compared to group A (25.96 ± 5.33). In conclusion, the natural products of edible flesh of the snails and mussels are preventing arthritis as per the reducing levels of CRP, MMP-2 and-9.***

Keywords: Anti-inflammation, CRP, MMP-2, MMP-9, Natural products, Snails and mussels

1. Introduction

C-reactive protein (CRP) is a pentameric protein synthesized by the liver during inflammation, which is closely related to many disorders and osteoarthritis (OA) is very common. [1, 2, 3] Moreover, increasing level of CRP is a symptomatic biomarker in OA as per many investigators. [2, 3, 4] During OA, the CRP level exceed standard value (<6 mg/L). [4]

Generally, enzymes such as matrix metalloproteinases-2 (MMP-2) and MMP-9 were elevated in the case of osteoarthritis (OA) patients when compared to healthy controls. [5] Recently, Pulik et al. [6] reported that MMPs are the important biomarkers to identify the pathogenesis of OA. The articular cartilage comprises of chondrocytes and extracellular matrix (ECM), made up of two main components viz. type II collagen fibrils and aggrecan proteoglycans. [7] On the other hand, matrix metalloproteinases (MMPs) synthesized by chondrocytes and synovial fibroblasts are included the main enzymes responsible for this degradation. [8]. Many studies stated that MMP expression is elevated in cartilage and synovial tissues among OA patients. [9-11] It was recorded that therapies targeting MMPs offered a capable approach for this disorder and in recent years, there have been major developments in MMP-targeted therapies associated with the genetic background, which included the discovery of new synthetic and natural products, and potential biomarkers. [12]

The onset of cartilage destruction is due to mechanical stresses, which either directly damages chondrocytes or triggers them to create abnormal levels of MMPs and reactive oxygen species (ROS) responsible for cartilage breakdown and the release of microcrystals, osteochondral fragments, and products of ECM degradation into the joint cavity. [12]

Moreover, Makarov [13] observed that nuclear factor-kappa B (NF-kappa B) performed an essential regulatory function for many disorders viz. inflammation, hyperplasia, and tissue destruction in RA. While Simmonds et al. [14] studied the role of NF-kappa B during OA and inflammation. A collection of elements viz. comprising proinflammatory cytokines, chemokines, and lipid mediator, which is tasked with binding to chondrocytes and managing the immune cell activity by triggering signal transduction pathways, particularly the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) pathway. [15] Thus, an increased quantity of metalloproteinases would be created when the synthesis of type II collagen is suppressed. [16]

About 100% of the natural compounds could slow down the progression of OA by targeting the oxidative stress signaling pathway. Many studies were conducted *in vitro* only or *in vitro* followed by *in vivo* study where the success rate in cartilage regeneration or inhibition of the chondrocytes damage. [15, 16] Some studies also revealed that natural products like curcumin inhibits MMP-9 to prevent OA. [17] But the study is lacking to know the inhibitory activities of these markers after the consumption of snails and mussels.

In the present study, we estimated to know the level of CRP, MMP-2 and-9 in arthritis patients compared to subjects who consumed snails.

2. Materials and Methods

Study design
This research was a comparative and analytical study.

Study groups
In the present study, the study groups were considered as per our earlier study. [18]

Biomarkers for arthritis
According to the history of inflammation in patients, the biomarker especially C-reactive protein (CRP), matrix metalloproteinases-2 (MMP-2) and MMP-9 for group A and group B separately were analysed. The CRP value was expressed in terms of mg/L by using Kit as per protocol by many investigators. [19, 20, 21] The parameters like MMP-2 and-9 were expressed in terms of ng/ml estimated by using kit (Elabscience® Human MMP-2 and MMP-9 ELISA Kits, catalogue No. E-EL-H1445 and E-EL-H6075).

Statistical analysis
Categorical variables were calculated and expressed in percentage frequency distribution and continuous variable expressed as Mean ± SD and comparison were performed between group A and group B patients as per student 't' test by using statistical tool. P<0.05 considered as significant.

3. Results

Table 1 evaluates the frequency distribution of CRP (mg/ml) in serum of group A and B participants. In group A, the majority of patients were observed >5 (35, 70.0%) and ≤5 (15, 30.0%) while in group B, all the subjects were observed ≤5 (50, 100.0%) in serum.

Table 1: C- reactive protein distribution among participants

CRP (mg/l)					
Group A	Frequency	%	Group B	Frequency	%
≤5	15	30.0	≤5	50	100.0
>5	35	70.0	>5	0	0.0
Total	50	100.00	Total	50	100.00

Table 2 evaluates the frequency distribution of MMP-2 (ng/ml) in serum of group A and B participants. In group A, the majority of patients were observed >500 (35, 70.0%) and ≤500 (15, 30.0%) while in group B, the majority of subjects were observed ≤500 (30, 60.0%) and >500 (20, 40.0%) in serum.

Table 2: Matrix metalloproteinase-2 distribution among participants

MMP-2 (ng/l)					
Group A	Frequency	%	Group B	Frequency	%
≤500	15	30.0	≤500	30	60.0
>500	35	70.0	>500	20	40.0
Total	50	100.00	Total	50	100.00

Table 3 evaluates the frequency distribution of MMP-9 (ng/ml) in serum of group A and B participants. In group A, the majority of patients were observed >20 (43, 86.0%) and ≤20 (7, 14.0%) while in group B, the majority of subjects were observed ≤20 (27, 54.0%) and >20 (20, 46.0%) in serum.

Table 3: Matrix metalloproteinase-9 distribution among participants

MMP-9 (ng/l)					
Group A	Frequency	%	Group B	Frequency	%
≤20	7	14.0	≤20	27	54.0
>20	43	86.0	>20	23	46.0
Total	50	100.00	Total	50	100.00

Table 4 evaluates comparative analysis of mean ± SD of CRP, MMP-2 and-9 between arthritis patients (group A) and snails and mussels consumed (group B) subjects. For CRP (mg/ml), a highly significant (P<0.0001) decreased level was observed in group B (3.06 ± 1.13) when compared to group A (8.55 ± 4.55). For MMP-2 (ng/ml), a highly significant (P<0.0001) decreased level was observed in group B (498.60 ± 17.08) when compared to group A (545.58 ± 61.99). For MMP-9 (ng/ml), a highly significant (P<0.0001) decreased level was observed in group B (20.83 ± 2.77) when compared to group A (25.96 ± 5.33).

Table 4: Comparative analysis of mean CRP, MMP-2 and-9 and between the groups

Parameters (Mean ± SD)	Group A (n = 50)	Group B (n = 50)	P-value
CRP (mg/ml)	8.55 ± 4.55	3.06 ± 1.13	0.0001
MMP-2 (ng/ml)	545.58 ± 61.99	498.60 ± 17.08	0.0001
MMP-9 (ng/dl)	25.96 ± 5.33	20.83 ± 2.77	0.0001

4. Discussion

It is well-known fact that the CRP is elevated due to inflammation in OA. [4, 22, 23, 24] Moreover, this biochemical marker is a suitable indicator to predict the risk factors. [4] On the other hand, in response to inflammatory signals, CRP levels fluctuate quickly. As soon as the underlying cause is identified and treated, CRP levels rise sharply and fall quickly. Ongoing inflammation from persistent infections or inflammatory disorders may be indicated by chronic elevation. [25] There is a close similarity for CRP elevation in the arthritis patients when compared to snails and mussels consumed subjects.

Likewise, MMP-2 and-9 are also the potential biomarkers during rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) as per examined by Chang et al. [26] In the present study, it was recorded the elevation of both the markers in arthritis patients compared to snails and mussels consumed subjects. Our study is supported by an earlier study where these markers concentrations were found in higher level. [5]

In our earlier study, it was mentioned that many biomarkers depleted who consumed snails and mussels and many edible snails flesh are potential to prevent OA among subjects. [18, 27] It is confirmed that snails consumed subjects significantly improved the biomarkers viz. CRP, MMP-2 and-9 in our study.

5. Conclusions

The present study revealed that the prevention of arthritis was significantly higher as per the reduction concentrations of biomarkers such as of CRP, MMP-2 and-9 among subjects

who consumed natural bioactive compounds especially flesh of snails and mussels. It is evidenced that these natural products of edible flesh of the snails and mussels are preventing oxidative stress and well-known anti-inflammatory effects.

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Conflict of interest
As per authors no conflict of interest is declared.

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