

# Assessment of Acute Phase Protein Serum hs-CRP Levels and its Role in Severity of Disease in Vitiligo Patients – A Hospital Based Case Control Study in M.Y. Hospital, Indore M.P.

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## ABSTRACT

**Introduction:** Vitiligo an acquired pigmentary disorder of the skin and mucous membranes characterized by well circumscribed, depigmented macules and patches resulting from selective destruction of melanocytes. CRP is an acute phase protein secreted by the liver in response to several inflammatory cytokines such as IL6. Since inflammatory and immune factors plays a key role in the pathogenesis of vitiligo, we aimed to assess the relationship between the serum level of hs-CRP and pathogenesis and severity of vitiligo.

**Material and methods:** The study was conducted in the Dept. of Biochemistry and Dept. of Dermatology and Venereology in MGM Medical College & M Y Hospital Indore after approval from ethical committee on 70 Confirmed & diagnosed cases of Vitiligo patients of age group 18 to 55 years attending Dermatology OPD in MY Hospital Indore during a period of April 2018 to April 2019. Patients were divided into three groups according to the area of skin affected. Healthy individual without vitiligo were taken as controls. Venous blood sample was analyzed for serum hs-CRP levels and liver function. Appropriate statistical tests were applied on Minitab Version 17.0 and p values < 0.01 was considered significant.

**Results:** In our study the mean serum hs-CRP in case group was higher  $12.09 \pm 11.64$  than in control group it was  $1.99 \pm 2.05$  with negative but statistically significant correlation with age of onset of disease and positive statistically significant correlation with duration of disease thus, serum hs-CRP level might be useful for evaluating the disease activity of vitiligo as Novel biomarker.

**Conclusion:** As high-sensitive C-reactive protein (hs-CRP) is an important sensitive diagnostic and prognostic marker in many systemic inflammatory diseases and very low concentrations of hs-CRP can be analyzed in the serum, its detection and serial measurements helps to provide a novel link to evaluate the disease activity and severity and response to treatment.

**Keywords:** Vitiligo, hs-CRP, Melanocyte Cytokines, Inflammation

idiopathic, circumscribed, milky white patches of different sizes and shapes affecting any part of body.<sup>4</sup>

Prevalence of Vitiligo worldwide affecting up to 2% of the population, out of which almost half manifests it before the age of 20 years<sup>1,5</sup> while in India is found to be 0.25-2.5% with highest rate ~8.8% observed in Gujarat and Rajasthan states.<sup>4</sup> Non segmental vitiligo is the most common type with patches of depigmentation is symmetrical on both sides of body while segmental vitiligo (SV) is relatively uncommon with unilateral dermatomal distribution.

CRP is an acute phase glycoprotein (normal levels of 0-0.5 mg/l) secreted by the liver in response to systemic inflammatory cytokines.<sup>6,7</sup> Autoimmune and inflammatory cytokine hypothesis is considered to play a key role in the pathogenesis of especially generalized vitiligo.<sup>8</sup> High sensitive C-reactive protein (hs-CRP) has been an important sensitive diagnostic and prognostic marker for heart diseases and many systemic inflammatory diseases, as very low concentrations of hs-CRP can be analyzed in the serum. A level of less than 1 mg/l is considered low risk, 1-3 mg/l medium risk, and more than 3 mg/l as high risk inflammation. For levels more than 10 mg/l the source of inflammation or infection should be sought and the test should be repeated after recovery.<sup>9</sup>

Vitiligo, because it is a pigmentary disorder of skin and mucous membrane causes social embarrassment to the

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patients associated with widespread social prejudices and taboos. Very often the patients are isolated from the community because of lack of scientific knowledge, ignorance and confusion with leprosy.<sup>10</sup> Vitiligo does not affect working capabilities and life expectancy but it affects quality of life as it causes cosmetic disfigurement resulting in psychological trauma to the patients<sup>3</sup>

Due to the increasing prevalence of vitiligo in India, the present study was conducted in central part of India with an objective to evaluate the serum hs-CRP and liver function tests in vitiligo cases and compare them with controls & correlate them with the severity of disease using VASI and VETF score.

## MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry and Department of Dermatology and Venereology of M.G.M. Medical College and M.Y. Hospital Indore, Madhya Pradesh. The study was approved and permitted by the institutional scientific and ethical committee. The period of study was from May 2018 to April 2019.

In present study included 70 clinically diagnosed cases of Vitiligo of age group 18-55 yrs of both gender attending Dermatology OPD in M.Y. Hospital and 70 apparently healthy controls matched for same age and sex were taken. The subjects were enrolled for the study after obtaining written consent.

### Inclusion criteria

1. Newly diagnosed patients with Vitiligo attending OPD.
2. Age group between 18-55 yrs.
3. Both gender
4. Morphological classification – Acrofacial, focal, mucosal, segmental and generalized.
5. Severity of disease- Mild, Moderate, Severe

### Exclusion criteria

1. Diabetes mellitus
2. Congestive heart failure
3. Chronic liver disease
4. Chronic kidney disease
5. Endocrine dysfunction
6. Malabsorption syndrome
7. Pregnancy
8. Chronic inflammatory & connective tissue disorder

The percentage of body surface area involved were evaluated and grading of the disease was done according to VASI (Vitiligo area severity index) is a valid quantitative scale coined by Hamzavi and coworkers and VETF (Vitiligo European task force).

Blood samples were collected in Clot activator tubes and serum was analyzed for Liver function test and hs-CRP. Particle enhanced immune-turbidimetric test with two point kinetic method was performed for estimating serum hs-CRP on Biosystem BA 400 Analyzer. Serum SGOT & SGPT were estimated by IFCC uv kinetic method, serum Total Bilirubin and Direct Bilirubin by Malloy Evelyn modified End point method, serum Alkaline Phosphatase by DGKC and SCE

method, serum Total Protein by Biuret endpoint and serum Albumin by Colorimetric Bromo-cresol green (BCG) End point method by using commercially available kits. All assays were done on Selectra Pro Automated Analyzer.

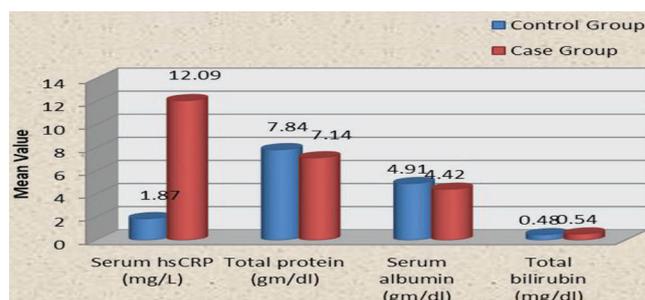
## STATISTICAL ANALYSIS

The data were tabulated on excel sheet & analyzed by appropriate statistical methods. Minitab Version 17.0 was used for calculating the p values. Results on continuous measurements were presented as Mean  $\pm$  SD. P value < 0.05(95% confidence interval) was considered significant and p value < 0.001 considered as highly significant. Comparison of mean between the two groups was done using Unpaired 't' test, comparison of means of more than two groups was done using One-way ANOVA followed by Post hoc Tukey test. Correlation between two parametric variables was done using Pearson Coefficient of Correlation.

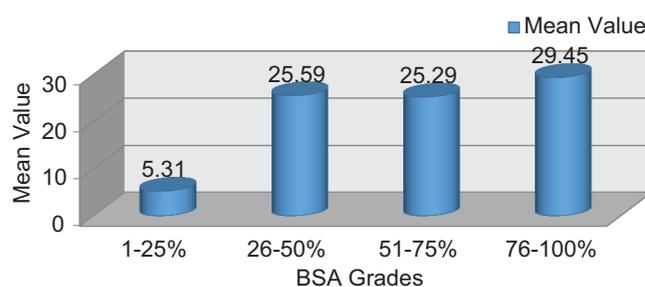
## RESULTS

In our study the mean age group of patients in the case group was  $32.20 \pm 13.25$  while in control group it was  $32.04 \pm 11.30$ . Maximum (37.1%) vitiligo patients & overall 49(35.1%) of the case and control group were in the age group 21-40 years. (Table 1).

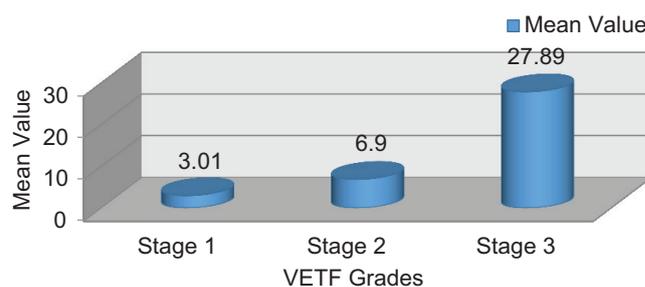
In case group, 41 (58.5%) patients were females and 29



Graph-1: Biochemical parameters in control and case group



Graph-2: Comparison of mean serum hs-CRP across BSA grade VASI score



Graph-3: Comparison of mean serum hs-CRP across VETF grades

Age	Control Group	Case Group	Total
18-20 years	22(31.4%)	21(30.0%)	43(30.7%)
21-40 years	23(32.8%)	26(37.1%)	49(35.1%)
41-60 years	25(35.7%)	23(32.9%)	48(34.2%)
Total	70	70	140
Mean $\pm$ SD	32.04 $\pm$ 11.30	32.20 $\pm$ 13.25	
't' value, df; -0.075, df=138			

**Table-1:** Distribution of patients according to age

Parameters	Control Group (n=70) (Mean $\pm$ S.D)	Case Group (n=70) (Mean $\pm$ S.D)	't' value	P value
Serum hsCRP (mg/L)	1.87 $\pm$ 1.87	12.09 $\pm$ 11.64	-7.252, df=138	<0.001*
Total protein (gm/dl)	7.84 $\pm$ 0.46	7.14 $\pm$ 0.73	6.800, df=138	<0.01*
Serum albumin (gm/dl)	4.91 $\pm$ 0.52	4.42 $\pm$ 0.46	5.912, df=138	<0.01*
Total bilirubin (mg/dl)	0.48 $\pm$ 0.10	0.54 $\pm$ 0.22	-2.153, df=138	<0.01*

**Table-2:** Biochemical parameters in control and case groups

BSA/VASI		No.	hs -CRP (mg/dl) [Mean $\pm$ SD]	F value	P value
1-25%	mild	49	5.31 $\pm$ 5.01	94.070	<0.001*
26-50%	moderate	2	25.59 $\pm$ 4.41		
51-75%	severe	6	25.29 $\pm$ 6.69		
76-100%	very severe	13	29.45 $\pm$ 5.21		

**Table-3:** Comparison of mean hs-CRP according to VASI SCORE/ BSA

(41.4%) patients were males and in the control group it was 38 (54.2%) and 32(45.7%) respectively. Out of 70 patients significant proportion of patients (46 patients) had onset of vitiligo below age of 20 years and 22 (31.4%) patients had a positive family history of vitiligo and 7 (10.0%) patients were having children affected with vitiligo.

The mean serum hs-CRP in control group was 1.99  $\pm$  2.05, while in the case group it was 12.09  $\pm$  11.64 with statistically significant p value (p<0.001), showing a higher serum hs-CRP level in case group. (Table 2 & Graph 1)

The mean total bilirubin in control and case group was 0.48  $\pm$  0.10 and 0.54  $\pm$  0.22 respectively (p<0.01\*). The mean total protein in control group was 7.84  $\pm$  0.46, while in the case group it was 7.14  $\pm$  0.73. In control group the mean serum albumin was 4.91  $\pm$  0.52 while in the case group it was 4.42  $\pm$  0.46 with p <0.01, showing a slightly lower total protein & serum albumin in case group.

The mean direct bilirubin in control and case group 0.25  $\pm$  0.07 & 0.27  $\pm$  0.10 (p=0.158). The mean SGOT was in control group 22.08  $\pm$  8.88 and in case group 24.14  $\pm$  8.08 (p=0.189). The mean SGPT in control group was 22.06  $\pm$  6.85, while in the case group it was 24.76  $\pm$  12.71 (p=0.119). The mean alkaline phosphatase in control group was 200.41  $\pm$  79.45, while in the case group it was 207.15  $\pm$  144.06 (p=0.733,) showing a comparable mean between the control and case group.

In our study we have found that according to VASI score

mean hs-CRP in mild vitiligo was 5.31  $\pm$  5.01, in moderate to severe vitiligo it ranges between 25.29  $\pm$  6.69 and in very severe type it was 29.45  $\pm$  5.21 (Table 3 and Graph 2) which was statistically very significant with p value <0.001\*.

The mean hs-CRP in VETF stage 1 (mild) was 3.01  $\pm$  3.24 mg/dl, in Stage 2 (moderate) it was 6.90  $\pm$  5.43 mg/dl and in Stage 3 (severe) it was highest 27.89  $\pm$  5.70 mg/dl which was statistically significant difference (p<0.001).

## DISCUSSION

As several mechanisms of melanocytes destruction in vitiligo have been proposed but autoimmune and inflammatory cytokine hypothesis are widely accepted theories which plays a key role in the vitiligo pathogenesis. TH17 cells is found increased in the serum and epidermis of vitiligo patients with active lesions<sup>11,12</sup> and also raised levels of pro-inflammatory cytokines GM-CSF, TNF $\alpha$ , IL-1 $\beta$ , IFN- $\gamma$  and IL-10 by innate cell like natural killer (NK) cells.<sup>13,14</sup>

In our study out of 70 patients, 58.5% patients were females while 41.4% patients were males indicating female preponderance of vitiligo and were very similar with the finding by Sehgal VN et al., and Akay BN et al., which showed more females affected as compared to male.<sup>15,16</sup>

This may be because of because women notice the change in appearance and approach the doctors sooner than men due to cosmetic reasons.

our study revealed that 7 (10.0%) patients were having

children affected with vitiligo. Also 22 (31.4%) patients were having affected close relatives indicating a higher familial incidence and is similar to a study reported 34% by Garsaud, et al.,<sup>17</sup> while in India it was 19% by Agarwal S, et al.,<sup>18</sup> indicating complex transmission and polygenic inheritance of disease.

The majority of the patients 34 (48.5%) had age of onset of vitiligo less than 20 years consistent with the most reports by Koranne RV, et al., and Behl PN, et al.,<sup>19,20</sup> This shows that the disease starts at a younger age in the Indian population. However, Howtiz, et al., showed the age of onset of vitiligo between 40-60 years.<sup>21</sup>

The mean total bilirubin was found raised slightly which may be due to the complex interaction of inflammatory, cytotoxic, and immunological factors in vitiligo patients inducing the systemic disturbances. The mean total protein and serum albumin was lower in vitiligo patients could be due to dietary deficiency associated with psychological disturbances like depression and anxiety and social stigma and associated cytokine mediated metabolic defects at cellular level.

In our study the mean serum hs-CRP in case group was higher  $12.09 \pm 11.64$  than in control group  $1.99 \pm 2.05$ . Findings of our study was in concordance with the study by Reza Ghaderi<sup>22</sup> who found raised levels of hs-CRP in patients with generalized vitiligo.

In our study the serum hs-CRP levels has statistically significant correlation with age of onset of disease indicating that early the age of onset of vitiligo, more will be the levels of hs-CRP while it has positive, statistically significant correlation with duration of disease indicating that longer the duration of disease more will be the levels of hs-CRP.

The pair wise comparison between VASI and VETF and hs-CRP was done showing a positive, statistically significant correlation between VASI and hs-CRP (p value <0.001\*) indicating that as the body surface area affected by vitiligo increases (grade 3), the hs-CRP levels increases thus, serum hs-CRP level might be a useful index for evaluating the disease activity of vitiligo as Novel biomarker.

## CONCLUSION

In our study vitiligo patients have markedly raised serum levels of hs-CRP as compared to controls and shows a positive correlation with the disease severity. We have also found a positive significant correlation between hs-CRP and duration of disease and a negative correlation between hs-CRP and age of onset of disease signifies that, early the age of onset of disease more will be the levels of hs-CRP.

Further studies by dermatologist, cosmetologist and medical practitioner are essential to detect the association. We suggest screening of all the patients of vitiligo for serum hs-CRP estimation at initial visits. Further large scale prospective studies are needed to establish the cause effect relationship.

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