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ORIGINAL RESEARCH ARTICLE CLINICAL

Immunophenotyping of normal individuals classified on the basis of human *dosha prakriti*

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ABSTRACT

Background: Human variations related to immune response and disease susceptibility is well-documented in Ayurveda. *Prakriti* (body constitution) is the basic constitution of an individual established at the time of birth and distinguishes variations, into three broad phenotype categories such as *vata*, *pitta* and *kapha*. Variation in immune response is often attributed to and measured from the difference in cluster differentiation (CD) markers expressed in lymphocytes. Currently, there are no reports available on the expression of CD markers related to *prakriti*. **Objective:** This is a pilot study performed to evaluate a panel of lymphocyte subset CD markers in dominant *prakriti* individuals. **Materials and Methods:** Immunophenotyping was carried out using whole blood from a total of healthy 222 subjects, who are grouped into *kapha* (n = 95), *pitta* (n = 57) and *vata* (n = 70) *prakritis*. CD markers such as CD3, CD4, CD8, CD14, CD25, CD56, CD69, CD71 and HLA-DR were analyzed using flow cytometry method. Differences between groups were analyzed using one-way ANOVA or Kruskal-Wallis analysis of variance (ANOVA) and multiple comparisons between groups were performed by Bonferroni or Mann-Whitney U test with corrections for type I error respectively. Significance was evaluated by ANOVA and Pearson's correlation. **Results:** We observed a significant difference ($P < 0.05$) in the expression of CD markers such as CD14 (monocytes), CD25 (activated B cells) and CD56 (Natural killer cells) between different *prakriti* groups. CD25 and CD56 expression was significantly higher in *kapha prakriti* samples than other *prakriti groups*. Similarly, slightly higher levels of CD14 were observed in *pitta prakriti* samples. **Conclusion:** Significant difference in the expression of CD14, CD25 and CD56 markers between three different *prakriti* is demonstrated. The increased level of CD25 and CD56 in *kapha prakriti* may indicate ability to elicit better immune response, which is in conformity with textual references in Ayurveda.

Key words: Cluster differentiation 14, cluster differentiation 25, cluster differentiation 56, immunophenotyping, *prakriti*

INTRODUCTION

Variation in humans, in anatomical, physiological, immunological, psychological, disease susceptibility, disease prognosis and response to treatment forms the basic tenets of personalized medicine. Studies have indicated an increase in clinical potential of pharmacogenetics to identify genetic variants that affect treatment efficacy.[1] Recently, the studies on personalized medicine have relied on understanding of the molecular/genetic variations with respect to disease for the implementation of preventive health-care strategies and drug therapies to healthy individuals or during the various stage of the disease.[2] Thus, it aims to prevent diseases, maintenance of health, early detection and diagnosis of disease, customized screening for risk and treatment of the diseases is important for the development of tailored treatment to improve the quality-of-life after treatment.

Indian traditional medicinal system, Ayurveda largely represents personalized medicine by emphasizing on the concept of *prakriti*. *Prakriti* is the specific type of body

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Received: 18-Jul-2013
Revised: 25-Sep-2013
Accepted: 07-Oct-2013

Access this article online	
Quick Response Code:	Website: www.jaim.in
	DOI: 10.4103/0975-9476.128857

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constitution of an individual produced by the relative proportions of the three *doshas* (humors) such as *vata*, *pitta* and *kapha*, which is fixed at the time of conception and remains same throughout the life.[3-5] There are seven different types of *prakriti* manifested by different combination of these three *doshas* attributing to specific phenotypic characters and are documented in the ancient Ayurvedic literature viz., *Charaka Samhita* and *Ashtanga Hridaya*.[6-10] Ayurveda also explains the predisposition and susceptibility of human subjects with different *prakriti* to various diseases. For example, individuals with *vata*, *pitta* and *kapha prakriti* are generally predisposed to neurological disorders, digestive malfunctions and respiratory illness respectively.[8,11-13]

Circulating and resident immune cells collectively and coordinatively mount an immune response and depending on the number of factors, the extent of which may vary among individuals. These may also be attributed to predisposition and susceptibility of an individual to pathogen specific response.[14] Several reports are available on the enhancement of immunity by Ayurvedic herbal formulations.[15] Gautam *et al*.,[16] and Somarathna *et al*.,[17] have shown an increase in immune capacity by Ayurvedic herbal formulations through elevating the expression profile of cluster differentiation (CD) markers. Moreover, Ayurveda treats every individual as unique and the treatment is based on the concepts of *prakriti*. Studies by Bhushan *et al*.,[18] and Ghodke *et al*.,[19] have shown specific differences in genetic polymorphisms between different *prakriti* which in turn is related to drug metabolizing ability. Similarly, Prasher *et al*.,[20] have reported the variation in gene expression and biochemical profiles of the three constitutional *prakriti* types. Such reports are scanty and clear evidences to show the differences among the different *prakritis* are required. Hence, the present study was performed to evaluate the ability to initiate the immune response in dominant *prakriti* individuals by analyzing various CD markers and relate to the concepts laid down in Ayurveda.

MATERIALS AND METHODS

Ethical statement

Screening and sample collection was performed at the center named as Shri Dharmasthala Manjunatheshwara College of Ayurveda (SDMCA). Ethical consent was obtained from Institutional Ethical Committee of SDMCA, Udupi.

Study design

In this cross-sectional pilot study, volunteers for *prakriti* assessment were recruited from various educational institutions from Udupi to Dakshina Kannada districts including Mangalore. *Prakriti* camps were conducted in various institutes with the consent from Head of the institutions. The students at these camps were made aware of *prakriti* determination by an Ayurvedic physician. Consenting volunteers were registered and informed consent was obtained. *Prakriti* assessment of each subject was performed in three stages. In the first stage**,** *prakriti* was assessed by senior *vaidya (ayurvedic physician)* based on his clinical skill and experience by applying classical Ayurvedic parameters of *prakriti* determination. In the second stage**,** another *vaidya* carried out same subject's *prakriti* assessment through Ayusoft *prakriti* assessment tool. Ayusoft is a logical tool, which consists of a comprehensive questionnaire and was formulated based on information from original Ayurvedic literature to qualitatively and quantitatively determine the *prakriti*.[21] Finally, a third *vaidya* who was blinded to the *prakriti* assessment of both senior *vaidya* and Ayusoft, compared the *prakriti* analysis. The role of third *vaidya* was to assess concordance amongst senior *vaidya* and Ayusoft assessments, since the *prakriti* assessment by *vaidya* and Ayusoft were performed independent of each other. Third vaidya also shortlisted the subjects, who showed *prakriti* percentage of 60% and above as per Ayusoft and in agreement with senior *vaidya's* assessment. Subjects who did not satisfy either criterion were excluded from the study. The criterion of 60% or above of single *prakriti* which was considered as dominant is primarily based on feasibility and consensus. It is well-recognized amongst Ayurvedic clinicians that single any *dosha prakriti* with very high percentage of one *dosha*, are extremely rare. Most of the individuals possess dual-*dosha prakritis* and therefore 60% as minimum cut off was maintained. To acquire the desired sample size with a much higher cut-off would be unviable due to (a) required sample size and (b) duration of the study.

Healthy, non-smoking, non-alcoholic male subjects between the age group of 20-30 were screened for the *prakriti* assessment (mean age 23 ± 4 years). Health status of the individual is considered on the basis of Ayurvedic literature as determined by Ayurvedic physicians. Accordingly, to consider one as healthy, individual should have signs of desire for food, easy digestion of ingested food, proper excretion of feces, urine, flatus, proper functioning of eyes, ears, nose, tongue and skin, comfortable sleep, easy awakening, attainment of strength, complexion and equilibrium of fire (both digestive and metabolic). Complete medical history including history of chronic illnesses or surgery, general physical examination with blood pressure, pulse rate, height, weight and routine auscultation was recorded to rule out overt cardiac or respiratory anomaly and history of infection within the past 3 months. The blood samples were collected following the World Health Organization (WHO) guidelines and international standard

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methodology. We have ensured the consistency in blood draw by following methodology reported in the literature.[22] Briefly, blood was drawn from selected individuals in the morning (7 AM-9 AM) before intake of any food in a BD Vacutainer® tubes (BD, Franklin Lakes, NJ, USA) by a senior technician as per WHO guidelines.[23] Samples were immediately transferred to the laboratory in an insulated container and immunophenotyping was performed using flow cytometry.

Immunophenotyping analysis

Immunophenotyping using fluorochrome labeled antibodies to CD3, CD4, CD8, CD14, CD19, CD25, CD56, CD69, CD71 and HLA-DR were carried out to analyze their distribution across different *prakritis* by employing fluorescence activated cell sorter (FACS Calibur; Becton Dickinson; BD, San Jose, CA). The analysis was performed following "lyse no wash" procedure as per the manufacturer's instructions. In brief, 50 μl of anti-coagulated whole blood in falcon tubes was mixed with fluorochrome labeled antibodies (20 μl) and incubated for 30 minute in dark at room temperature. Subsequently, red blood cell lysis buffer (1X: 450 μl) and liquid counting beads (20 μL of BD™ liquid counting beads, catalog number − 335925, BD, San Jose, CA) were added. The samples were mixed thoroughly and acquired after 12-15 minute of incubation at room temperature. Cell quest Pro software version 5.2 (BD, San Jose, CA) was used throughout for data acquisition and analysis.

Statistical analysis

The cell count distribution of each CD marker across the *prakriti* samples was statistically analyzed using the statistical package for the social sciences version 11.5 (IBM SPSS Statistics, USA) and GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego, California, USA). The differences in absolute count of all CD marker's expression were ascertained by applying parametric or non-parametric analysis of variance (ANOVA). The difference in expression of CD19, CD25, CD56, CD14 and CD69 between *prakriti* was assessed by Kruskal-Wallis ANOVA with Mann-Whitney U multiple comparison analysis corrected for type I error. Pearson's correlation analysis was performed using R version 2.15.2, Base graphing package to calculate the relation between the percentage of *prakriti* and absolute count of significant CD markers. The $P \leq 0.05$ was considered as significant.

RESULTS

Estimation of CD markers in dominant *prakriti*

The distribution of dominant *prakriti* was found to be highest in *kapha* with 42.79% followed by *vata* 31.53% and *pitta* 25.67%. Immunophenotyping was carried out in whole blood from a total of 222 subjects, categorized into different dominant *prakriti* such as *kapha* (n = 95), *pitta* (n = 57) and *vata* (n = 70). In the present study, the total number of cells in a defined volume expressing CD3, CD4, CD8, CD19, CD14, CD25, CD56, CD69, CD71 and HLA-DR was analyzed. Statistical analysis revealed that there was a significant difference ($P < 0.05$) in the expression of CD markers such as CD14 (monocytes), CD25 (activated B-cells) and CD56 (Natural killer cells [NK cell]) between different *prakriti* groups of subjects [Figure 1]. There was a statistically significant difference between *prakriti* groups in absolute CD14 expressions (H[2] = 6.22, P = 0.044) with a mean rank of 99.08 (median = 114) for *kapha*, 119.96 (median = 147) for *pitta* and 121.46 (median = 139) for vata *prakriti*. The median expression for CD14 was found to be higher in *pitta* as compared to *kapha* and *vata prakritis*. However, Mann-Whitney U test multiple group comparison analysis showed insignificant P value ($P > 0.017$ [corrected α error]) in all possible comparison analysis. Kruskal-Wallis ANOVA revealed a significant (H[2] = 11.15, P = 0.003) difference in the expression of CD25 among *prakritis*. The median expression of CD25 in case of *kapha* found to be highest (136) with a mean rank of 127.48, followed with *pitta prakriti* (121) mean rank of (105.46) and it was least for *vata prakriti* (106.5) which showed a mean rank of 105. Further, multiple comparison analysis between *kapha* and *vata prakritis* revealed a significant difference in expression with Mann-Whitney test with U = 2343 (Z = −3.238) and P = 0.001 and this difference was observed to-be moderate (r = −0.21). The median CD56 expression also showed a high level of expression in the case of *kapha* (122.4) followed with *pitta* (111.65) and then by *vata* (96.81) samples. Between three dominant *prakriti* group comparison, a statistical significant difference was observed (H[2] = 6.318, P = 0.042). Mann-Whitney U test showed *kapha prakriti* with higher expression of CD56 as compared to *vata* with P = 0.016. On the other hand, there were no significant differences ($P > 0.05$) in the expression of CD3, CD4, CD8, CD19 CD69, CD71 and HLA-DR between different *prakritis* [Table 1]. The mean expression of different lymphocyte subset CD markers in total population (n = 222) was found to be comparable with the earlier studies, where reference ranges for different lymphocyte subset have been reported in healthy Indian population.[24-26]

The inference on the effect of *doshas* with respect to the percentage for expression of CD14, CD25 and CD56 was further evaluated by correlation analysis. Pearson correlation analysis is performed to assess the relationship between percentage values of *doshas* (*kapha, pitta* and *vata*) and CD marker expression. Analysis of CD25 and CD56 marker

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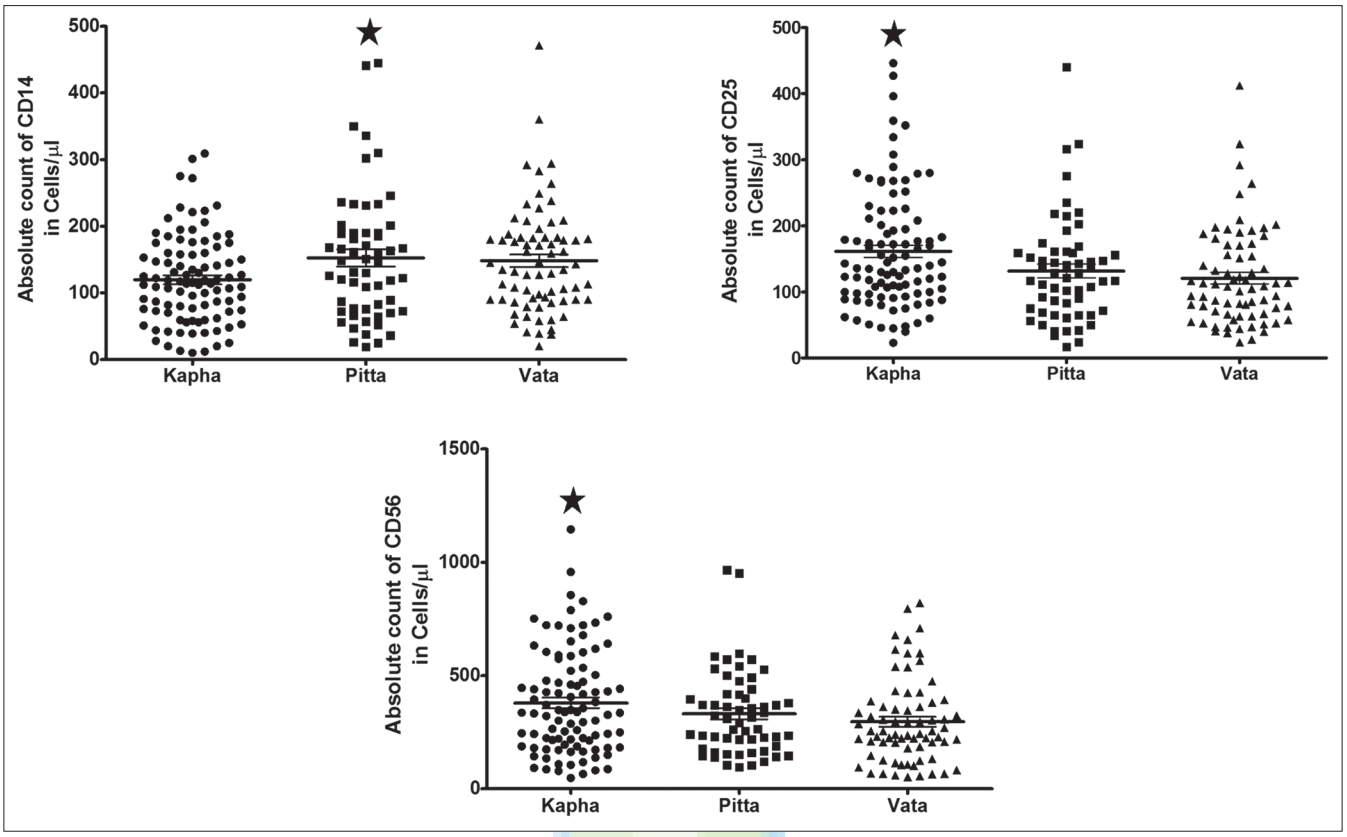

Figure 1: Expression of significant cluster differentiation markers across three *prakriti*. Scatter plot for expression of CD14, CD25 and CD56 which are significant in expression between three dominant *prakriti* ($P < 0.05$). Significance with the highest mean value was represented in * sign

Table 1: The expression distribution of different CD markers in three dominant *prakritis*

CD marker	*Kapha* (*N*=95)	*Pitta* (*N*=57)	*Vata* (*N*=70)	ANOVA, *F* value, *P* value	Kruskal-Wallis ANOVA, *H* value, *P* value
CD3[a]	1592.04±524.58	1575.61±635.27	1553.05±518.05	0.1, 0.9	-
CD4[a]	773.22±263.59	751.21±340.29	727.22±231.60	0.56, 0.57	-
CD8[a]	647.80±250.54	678.56±325.14	649.27±303.61	0.23, 0.79	-
CD4 by CD8[a]	1.27±0.43	1.20±0.39	1.22±0.40	0.53, 0.58	-
CD19[a]	236.98±128.95	236.54±121.55	240.20±126.47	0.17, 0.98	-
CD25[b]	136 (110)	121 (89.5)	106.5 (105)	-	11.15, 0.003*[£]
CD56[b]	336 (334)	308 (234)	248.5 (196)	-	6.31, 0.042*[£]
CD14[b]	114 (88)	147 (115.5)	139 (93)	-	6.22, 0.044*
CD69[b]	167 (116)	188 (133.5)	159 (142.5)	-	1.22, 0.54
CD71[a]	120.61±58.89	112.70±59.53	114.37±57.35	0.399, 0.67	-
HLA-DR[a]	500.61±162.50	508.57±203.94	517.28±166.27	0.183, 0.83	-

CD=Cluster differentiation, ANOVA=Analysis of variance, [a]CD marker expression values are represented with mean±standard deviation and analyzed by ANOVA, [b]CD marker expression values are represented in median (interquartile range) and analyzed by Kruskal-Wallis ANOVA, *P<0.05 and is considered as significant, [£]Mann-Whitney test P<0.017 and expression is significantly different beween *kapha* versus *vata*

expression for *kapha prakriti* revealed moderate positive correlation of 0.23 and 0.16 respectively, as opposed to *vata prakriti* wherein a negative correlation (Pearson $r = -0.20$ and -0.16 respectively) with $P < 0.05$ was observed. CD14 expression analysis with the percentage of individual *prakriti* compared to *kapha prakriti* showed a negative correlation (Pearson $r = -0.19$) with P 0.05. The Pearson correlation matrix is shown in Figure 2.

DISCUSSION

In this study, we have attempted to evaluate the quantitative estimation of the dominant percentage of *prakriti* which could be further coupled to the traditional assessment of *prakriti*. In the absence of such data with respect to *prakriti* stratified Indian population, we have for the first time shown here the differences in CD marker expressions. This study may provide base line values for different CD

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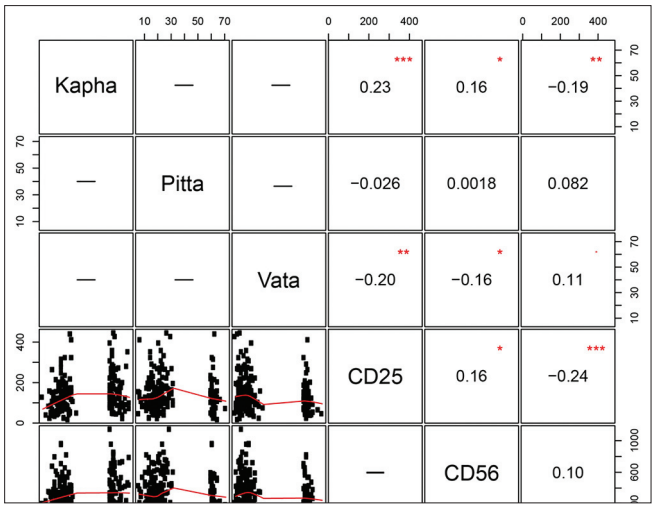

Figure 2: Pearson correlation matrix to find correlation between percentage of *prakriti* and cluster differentiation 14, CD25 and CD56 expression. Data represented as the percentage values of *kapha*, *pitta* and *vata* obtained from the Ayusoft against, the absolute count of CD marker of the population studied. The values inside the boxes are the Pearson's correlation *r* values and statistical significance is represented by * sign

markers within dominant *prakriti* and could be used for understanding *prakriti* based disease manifestation, etiology and improving the treatment modalities by an Ayurvedic practitioner.

Inter individual variations to elicit innate or acquired immune response is well-documented and it is attributed to the number of factors including the proportion of immune cells in circulation.[24-28] The preservation of health and control of disease manifestations are the primary objectives of Ayurveda and these in part, can be achieved by enhancing the immunity.[29-31] Ayurveda also suggests differences immune response in individuals which is based on their *prakriti*.[32,33] An increase in innate immunity was observed when herbal formulations were used for natural healing[17] and this has been attributed to increase in the expression of CD antigens. Our results show a significant difference in expression of CD14, CD25 and CD56 between the dominant *prakritis* in normal individuals.

The CD14 surface antigen is preferentially expressed by monocytes, macrophages, granulocytes (weak expression), B-cells, dendritic cells and hepatocytes and mediates the innate immune response to bacterial lipopolysaccharide. Ayurvedic principles[34] and anecdotal clinical experiences suggest that *pitta prakriti* has a higher tendency to develop hypersensitive skin responses, in particular, flares (red rashes). Accordingly, our study indicates that CD14 expression is higher in *pitta* dominant individuals, thereby suggesting a causal relationship. Consistent with this, Ferrero *et al.*,[35] and Merlin *et al.*,[36] have reported an increase in the hypersensitivity reaction with higher CD14 expression in *in vivo* mouse model.

The mammalian immune system protects the host from a broad range of pathogenic microorganism while avoiding excessive immune reactions that would be deleterious to the host. Interleukin 2-Rα (CD25) is a 55 kDa transmembrane polypeptide, expressed mainly by activated B-cell, T-cell and NK-cells. The deficiency of CD25 in humans results in recurrent infections and lymphocyte infiltration in multiple tissues.[37] The CD25 expression on B-cells appears to function both on innate and adaptive immune response mechanism[38] and in our study, we demonstrate a higher expression of CD25 in individuals with *kapha prakriti*. Ayurvedic literature also suggests that individuals with *kapha prakriti* show strong immune response as compared to *vata prakriti*.[32,33]

CD56 also known as neural cell adhesion molecule, a member of a large family of cell surface glycoprotein and mainly expressed by the NK-cells.[39] Depending on the context, they serve as a signaling receptors and help in cellular adhesion, migration, proliferation, apoptosis, differentiation, survival and cellular plasticity. NK-cells are the large granular, innate effector lymphocytes, which provide defense against microbial-infected or malignant cells through Fas or Perforin/granzyme pathway.[14,40] In our study, the *kapha prakriti* showed a higher level of expression of CD56 as compared to *vata*, which is concordance to the earlier report.[32,33]

As the selected samples were from healthy population, we did not observe any significant difference in expression of other CD markers which are mainly activated by T-cells and B-cells. From the perspective of *prakriti*, the differences between two single *dosha prakritis* will reveal only slight to moderate differences. For example, features such as dry skin of *vata* and soft skin of *pitta* may overlap, making distinction difficult. Prominent *prakriti* features, such as, premature graying of hair in *pitta* or extremely dry skin of *vata*, are immediately observable and assume decisive value. It is possible that the CD markers of only the few dominant *prakriti* features show detectable differences. Strong immunity in *kapha prakriti*, marked tendency to develop bright red hypersensitivity rashes in *pitta prakriti* and low immunity in *vata* relative to *kapha* are clear and decisive *prakriti* features in clinical Ayurveda. Identifying *prakriti* specific markers of specific diseases by unearthing and cataloguing *prakriti* specific genes in physiologically stable states may provide as a reference to facilitate genetic sub-grouping of human populations. The limitations of the present study is that the *prakriti* assessment carried out for (a) only healthy male individuals in the age group of 20-30 and (b) only

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few selected CD markers were tested and further studies are warranted for any definitive conclusions.

CONCLUSION

In the present study, we have shown that significant differences in the expression of CD14, CD25 and CD56 subset with their constitution type or *prakriti*, which is in conformity with co-relations to textual references in Ayurveda.

ACKNOWLEDGMENTS

The authors are thankful to all the volunteers who have participated in this study. We thank Innovation in Science Pursuit for Inspired Research, for providing INSPIRE fellowship to HR. We gratefully acknowledge the office of the principal scientific advisor to the government of India (PSA-GOI) for financial support.

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How to cite this article: Rotti H, Guruprasad KP, Nayak J, Kabekkodu SP, Kukreja H, Mallya S, Nayak J, Bhradwaj RC, Gangadharan GG, Prasanna BV, Raval R, Kamath A, Gopinath PM, Kondaiah P, Satyamoorthy K. Immunophenotyping of normal individuals classified on the basis of human dosha *prakriti*. J Ayurveda Integr Med 2014;5:43-9.

Source of Support: We gratefully acknowledge the office of the principal scientific advisor to the government of India for financial support, **Conflict of Interest:** None declared.

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