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Effect of Hematology and Biochemistry during Lactation and Non-Lactation in Rhesus Monkey (*Macaca Mullata*)

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ABSTRACT

Lactation induces physiological changes in mammals, which can influence hematological and biochemical parameters. This study aims to compare the hematological and biochemical profiles of lactating and non-lactating Rhesus monkeys (Macaca mulatta) to better understand the impact of lactation on their metabolic and physiological status. The aims of this study were to verify if significant changes occur in hematological and biochemical parameters in Rhesus Macaques during Lactation period and Post lactation Hematological and biochemical parameters were evaluated in 12 Female Rhesus Macaques that were Lactating and Post Lactation .On Hematology analysis we could find the significant changes in neutrophil and Lymphocytes count between the two groups . Biochemistry analysis showed significant changes in Triglycerides level . Our findings provide reference data for researchers and contribute to a better understanding of primate biology in different reproductive states

Key words : Macaca Mulatta , Lactation , Hematology ,Biochemistry

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INTRODUCTION

The rhesus monkey (*Macaca mulatta*) is a widely used model in biomedical research due to its genetic and physiological similarities to humans (Rogers, 2022). Lactation, a critical phase of the reproductive cycle, imposes substantial metabolic demands on the mother, potentially altering hematological and biochemical parameters (Onasanya et al., 2015). Despite its significance, limited data exist comparing these parameters in lactating versus non-lactating rhesus monkeys. This study aims to address this gap by analyzing key hematological and biochemical markers, thereby establishing baseline reference values. Hematological and serum biochemical values in macaques are influenced by various physiological traits and housing conditions (Bakker et al., 2023). However, previous studies often rely on parametric assumptions to calculate reference intervals, report values from small sample sizes, or focus on the effects of specific conditions on normal ranges. Blood metabolic profiles are frequently used as indicators of nutritional status and overall health, making them critical tools for health assessment in animals (Washington et al., 2012). Young mammalian mothers face trade-offs between investing in their own growth

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and reproduction (Dittus et al., 2024). During pregnancy, a female's body undergoes dramatic metabolic changes that may predispose her to certain health disparities if these changes are not mitigated postpartum (Liu et al., 2014). Maternal insulin secretion increases during pregnancy to support glucose and amino acid transport to the fetus. However, the combination of increased insulin secretion, elevated circulating lipids, and consequent visceral adiposity often leads to insulin resistance in maternal cells (Mingrone et al., 2018). Serum biochemical constituents and hematological values are essential indicators of the metabolic state of nursing animals (Salem et al., 2017). Blood serves as a vital index of pathological or physiological alterations in the body (Salem et al., 2017). During lactation, up to 80% of blood metabolites are redirected to milk production (Piccione et al., 2009). The present study evaluates changes in hematological and biochemical values during and after the lactation period in rhesus mothers held in captivity.

MATERIALS AND METHODS :

Animals.

This study used 24 female Rhesus monkeys that were aged 8-13 years old and grouped into two groups lactating and Non lactating animals .The non Lactating animals are defined the animals that have given birth and has been weaned before the study (12 animals per group).All animals were bred and reared at the Primate Research Centre, National Institute of Immunology (New Delhi, India), and they were kept in accordance with Committee for the Purpose of Control and Supervision of Experimental Animals; The animals were maintained under standard environmental conditions (22 to 28 degree C, 55% to 65% humidity, 12:12-h dark: light photoperiod) and housed individually in stainless steel nonhuman primate cages. Daily each animal was fed 100 g commercial pellet primate feed (Golden Feeds, New Delhi, India) and 50 g-soaked chick peas (Cicer arietinum) in the morning, 2 slices of bread in the afternoon, and 350 g fruits and vegetables in the evening and had ad libitum access to water. Every 2 wk, they were supplemented with 5 ml oral B-complex multivitamins Vimeral (Virbac India)and calcium (Ostocalcium, Glaxo, Mumbai, India). The calcium and vitamin supplements were given thrice every week to the mothers in lactation and other group. All animals were tuberculosis-free, as determined by semiannual testing. The animals remained active, alert, and with no signs of clinical disorders or diseases

Blood sampling, hematologic and biochemical examinations.

After overnight fasting, the animals were physically restrained at the front of a squeeze cage to anesthetize using Ketamine Hydrochloride. A hindlimb was brought forward and held firmly, and the site was cleaned with alcohol. A 4-ml blood sample was drawn from the saphenous vein using a 23-gauge needle. The needle was removed from the syringe, and the blood sample was slowly expressed into a vial to minimize the risk of hemolysis. A 1-ml aliquot of blood was thoroughly mixed in a vial containing ethylenediaminetetraacetic acid (EDTA) and used for estimating hematologic indices. For the estimation of various biochemical parameters, the remaining 3 ml of whole blood was allowed to clot for 1 hour at room temperature and then refrigerated to facilitate clot retraction. The serum was collected within 2 hours of bleeding and stored at –20°C for biochemical analysis, which was conducted the following day. Hematologic indices were estimated using an automated hematology analyzer (Genuri 5-Part Hematology Analyzer). The parameters measured included: Neutrophils (Neu%) ,Lymphocytes (Lym%),Monocytes (Mon%),Eosinophils (Eos%),Basophils (Bas%),Red Blood Corpuscles (RBC, 10^{12}/L),Hemoglobin (HGB, g/dL),Hematocrit (HCT, %),Mean Corpuscular Volume (MCV, fL),Red Cell Distribution Width (RDW-CV, %),Platelet Distribution Width (PDW-CV, %) Plateletcrit (PCT, mL/L).Serum biochemistry was estimated using an automated serum biochemistry analyzer (TransAsia Biochemistry Analyzer, India). The following parameters were measured: Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Transaminase (SGOT), Urea, Creatinine, Uric Acid, Glucose (GLU), Triglycerides (TGY), Cholesterol (CHO), Calcium (Ca), Protein (PRO), Albumin (ALB)

Statistics :

Results are presented as Mean±SD. Differences between the two groups were tested using one-way and two-way ANOVA with Bonferroni post-hoc tests using GraphPad Prism (Version 10). The value of $P<0.05$ was considered significant.

Results : The changes in the hematologic values of Lactating and Non lactating females are shown in

Fig 1 A,B,C and Table 1

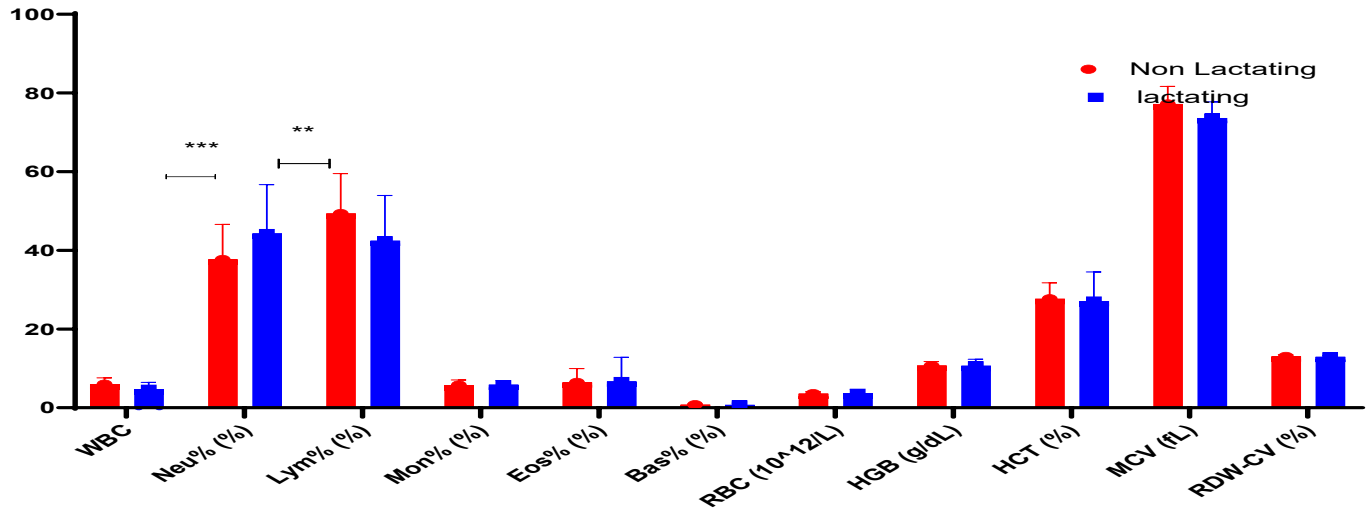


Figure 1A

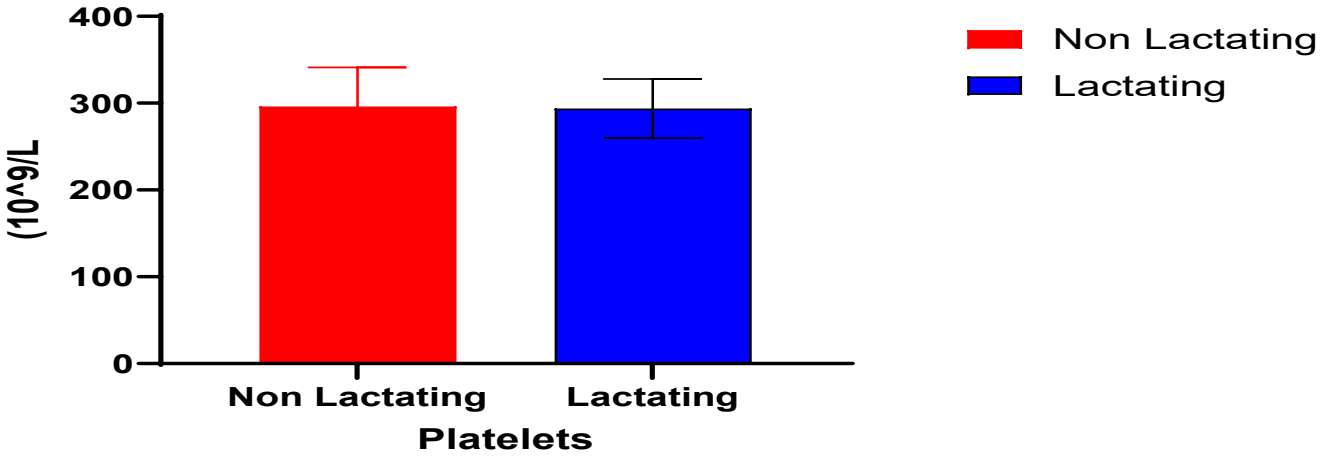


Figure 1B

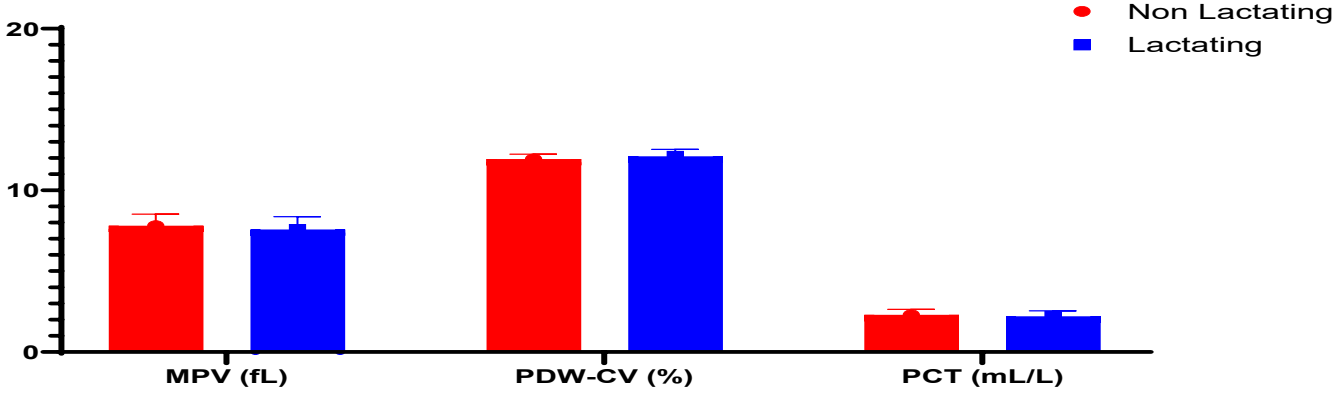


Figure 1C

	Non-Lactating		Lactating		Percentage Change
	Mean	SD	Mean	SD	
WBC	5.95	+/-1.63	4.71	+/- 1.73	-20.84033613
Neu% (%)	37.66666667	+/-8.91	44.29230769	+/-12.4	17.5901974
Lym% (%)	49.35	+/-10.14526311	42.46153846	+/-11.49852	-13.95838205
Mon% (%)	5.791666667	+/-1.288733228	5.846153846	+/-1.043724	0.940785824
Eos% (%)	6.433333333	+/-3.511366824	6.661538462	+/-6.155558	3.547229985
Bas% (%)	0.758333333	+/-0.21514618	0.738461538	+/-0.435007	-2.620456485
RBC (10^12/L)	3.590833333	+/-0.516957152	3.673846154	+/-0.935749	2.311798218
HGB (g/dL)	10.725	+/-1.037588989	10.66153846	+/-1.676076	-0.591715991
HCT (%)	27.69166667	+/-4.064806455	27.13076923	+/-7.383132	-2.025509864
MCV (fL)	77.19166667	+/-4.481975687	73.62307692	+/-4.136052	-4.623024614
RDW-CV (%)	13.025	+/-0.504750163	12.96153846	+/-0.295912	-0.487228714
PLT (10 9/L)	296.0833333	+/-44.97768807	293.8461538	+/-34.08774	-0.755591162
MPV (fL)	7.816666667	+/-0.706892474	7.576923077	+/-0.794936	-3.067082175
PDW-CV (%)	11.94166667	+/-0.293747985	12.10769231	+/-0.429072	1.390305429
PCT (mL/L)	2.308333333	+/-0.328794861	2.213076923	+/-0.338462	-4.126631481
N/L Ratio	0.76		1.04		

(Fig 1 A, B, C and Table-1) Haematology Parameters of Lactating and Non lactating Animals used in the study n=12)

On hematology we could find there was decrease in WBC ,Lymphocytes , Basophils , MCV, MPV , MPV and PCT counts However we could stastitical significant changes ($P< 0.05$) only in Neutrophils and Lymphocytes count within non lactating and Lactating animals . There was increase in Neutrophils and decrease in Lymphoctes count in Lactating mothers and we could not find any significant changes in hematology counts within Non lactating and Lactating animals .

BIOCHEMISTRY ANALYSIS :

The changes in the Biochemistry values of Lactating and Non lactating females are shown in Table 2 and Fig 2 A,B,C and the biochemical values are shown in Table 2.

Fig.2 A

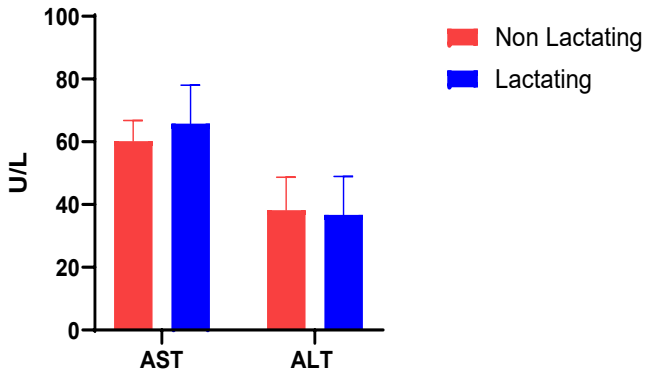
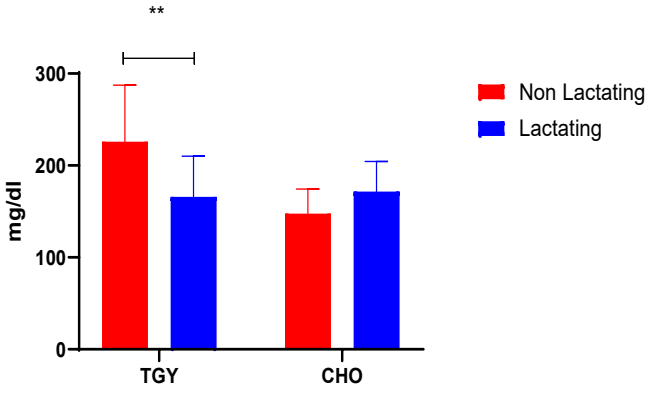


Fig.2B



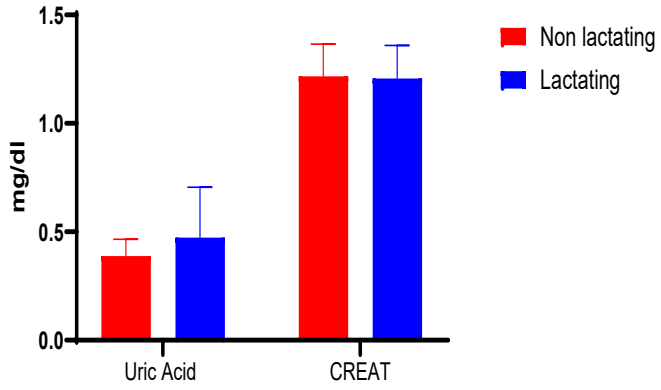


Fig 2C

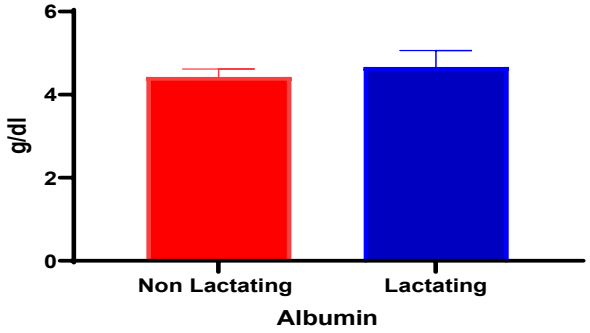


Fig. 2D

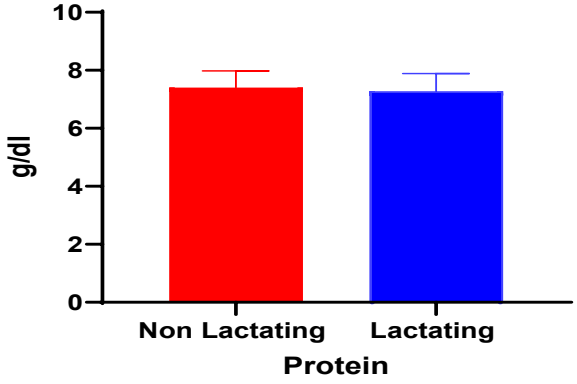


Fig. 2 E

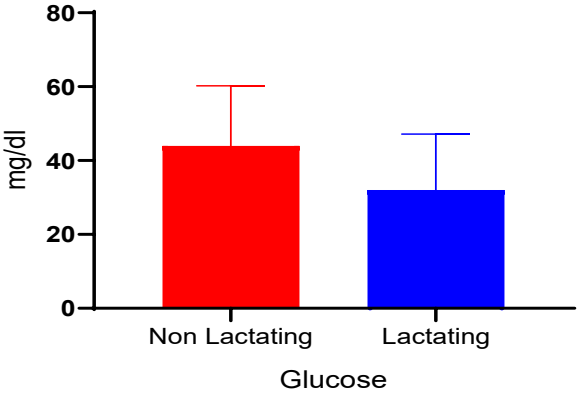


Fig. 2F

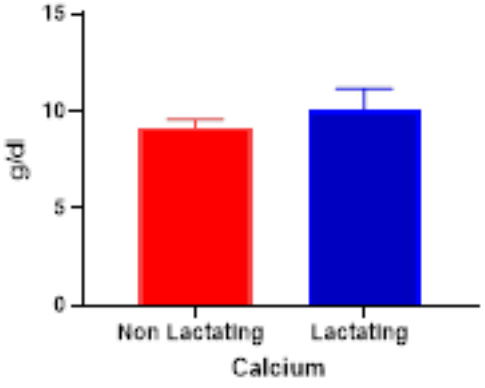


Fig .2G

(Fig 2 A, B, C, D, E,F ,G and Table-2) Biochemical Parameters of Lactating and Non lactating Animals used in the study n=12)

On biochemistry analysis we could find percentage decrease in SGPT, UREA ,GLU ,TRIG, in lactating animals and there was percentage increase in SGOT Uric acid ,Calcium ,Cholesterol and Albumin in lactating animals .However we could find statistically significant difference in Triglycerides count within non lactating and Lactating animals($p<0.05$) . There was decrease in Triglycerides count in Lactating mothers and we could not find any statistically significant changes in Serum Biochemistry parameters within Non lactating and Lactating animals .

Table 2

	NON-LACTATING		LACTATING		Percentage Changes
	Mean	SD	Mean	SD	
SGPT	38.23333333	+/-10.46651	36.72222	+/-12.23885	-3.95234524
SGOT	60.16666667	+/- 6.608443	65.8	+/-12.25561	9.36288088
UREA	24.40833333	+/-10.03471	20.61538	+/-4.665806	-15.53958346
CRE	1.216666667	+/-0.148406	1.206923	+/-0.152172	-0.800849342
GLU	43.975	+/-16.27464	32.05385	+/-15.09893	-27.10892553
TRIG	225.9833333	+/-61.39508	165.8077	+/-44.30857	-26.62835016
UA	0.3875	+/-0.078407	0.473077	+/-0.232285	22.0843871
CA	9.166666667	+/-0.411943	10.07692	+/-1.109169	9.93003636
PRO	7.4025	+/-0.57468	7.282308	+/-0.60589	-1.62366768
CHO	147.5	+/-26.83451	171.6923	+/-32.72966	16.40155932
ALB	4.4275	+/-0.189695	4.668462	+/-0.396292	5.442394128

DISCUSSION

We report here the baseline values of clinical chemistry and hematology parameters of rhesus monkeys during lactation and non-lactation periods. This study provides valuable baseline data on the hematological and biochemical profiles of lactating and non-lactating rhesus monkeys. These findings are crucial for designing experiments and ensuring the well-being of primates used in research. During pregnancy and lactation, mothers require additional nutritional supplements to nourish their infants. Inadequate nutrition can lead to a decline in the health of macaques held in captivity. This study was undertaken to determine whether the nutrition provided during lactation is sufficient to maintain the proper health of mothers and their infants. Based on hematological parameters, we did not observe significant changes between lactating and non-lactating mothers, except for a significant increase in neutrophil count and a decrease in lymphocyte count in lactating animals. The neutrophil-to-lymphocyte (N/L) ratio was higher in lactating animals than in non-lactating ones. The increase in the N/L ratio is likely due to the release of the hormone cortisol, which is elevated when animals experience stress (Titisari et al., 2019). Cortisol causes the bone marrow to release more neutrophils, increasing their numbers in blood circulation. Thus, stress-induced neutrophilia appears to be attributed to the redistribution of neutrophils into the systemic circulation (Zini et al., 2011). Lactating macaques in captivity are prone to lactation stress, which indirectly increases the N/L ratio. On analyzing key biochemical parameters, we observed a significant decrease in triglyceride levels in lactating animals compared to non-lactating ones. As reported previously, plasma triglyceride levels in lactating rats are lower, while plasma cholesterol levels are elevated during lactation. This is because triglyceride-rich particles are rapidly cleared from the circulation during lactation, likely through conversion to IDL/LDL-size particles by mammary gland lipoprotein lipase to supply lipids for milk production (Hamosh et al., 1970). Milk triglycerides and cholesterol are derived from both lipoproteins and de novo synthesis in the mammary gland. From this study, we propose that proper nutrition plays an essential role in maintaining the health of lactating females and their infants. Additionally, providing stress-free environmental conditions for lactating females will help reduce cortisol levels, thereby preventing pathological changes in the mothers' bodies.

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