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Effect of Multigenerational Undernutrition on the Haematological Profile of Adult Wistar Rats

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ABSTRACT

It has been suggested that diseases like hypertension, diabetes and coronary heart disease arise due to under nutrition in fetal life. We evaluated the effect of undernutrition on blood counts. Total leukocyte count (6.77 vs. 11.52 x 103/µL; p<0.0001, n=6, U vs. C) was significantly lower in the undernourished colony, MCV (60.05 vs.55.30 µm3; p<0.001, n=6, U vs. C) was higher but MCHC (27.50g/dl vs. 29.28g/dl; p<0.001, n=6, U vs. C) was lower in the undernourished colony. Platelet count (803.0 vs. 414.5 x 103/microliter, p<0.0001, n=6, U vs. C),Platelet distribution width (15.03 vs. 6.25 p<0,0001,n=6,U vs. C) and Platecrit (0.53 vs. 0.25,p<0.0001,n=6,U vs. C)were all higher in the undernourished colony. This is indicative of macrocytic anaemia, an inflammatory process and platelet anisocytosis and increased platelet mass.

KEYWORDS : Foetal Programming, Multigenerational Undernutrition, Complete Blood Count

Introduction

Developing countries like India are facing an epidemic of coronary heart disease, hypertension and Diabetes Mellitus (Echouffo-Tcheugui 2012).Human and animal studies have shown that one of the major reasons for this epidemic is impaired intrauterine growth (IUGR) and development (Godfrey and Barker 2000).Undernutrition in fetal life leads to "programming", this results in permanent changes in the development of a variety of tissue and organ systems and include metabolic, hormonal and cellular adaptations to the undernourished environment (McMillan and Robinson 2005). Barker and Yajnik have shown in the Indian population the role of undernutrition and has been labelled as the" Thin-Fat" Indian phenotype. Indians are especially vulnerable as they have one of the lowest birth weights, are insulin resistant and centrally obese. In Indians this thin fat phenotype is present at birth (Yajnik, Fall et al 2003).

Hardikar et al in their studies carried out in a multigenerationally undernourished Wistar rat model have shown the rats mimic the Indian phenotype. They are insulin and leptin resistant, centrally obese, and are also deficient in vitamin B12 and folate levels. Soft tissue growth is also hampered as seen by the lower weights of the muscle, heart, liver and the pancreas (Hardikar et al 2015).

Studies carried out in a variety of undernourished animals have also shown the effect of undernutrition on the hematopoietic environment in the bone marrow.These animals show a variety of changes in the marrow including hypocellularity, necrosis, and extracellular matrix modifications (Travlos GS (2006), Fried W et al (1978), Vituri CL(2000), Prestes-Carneiro et al (2006) and Borelli P et al(1995).This study has evaluated the role of multigenerational undernutrition on the haematological profile in female wistar rats.

Materials and Methods

The study was carried out in 10 week old adult female rats from the undernourished wistar rat colony. These animals received a diet that was deficient for calories (50% of the diet) as well as proteins, fiber, and other macro and micronutrients. (Hardikar et al 2015).The control group received a standard rat feed. Blood was collected by retro-orbital bleeding and serum was immediately separated and analysed for Complete Blood Count (CBC) on an auto analyser (Mindray BC2800).The study was ethically approved by the institutional ethics committee.

Statistical Analysis

All estimations were carried out in triplicates and values are expressed as +/- Std Error of Mean (SEM).The statistical significance was evaluated by the unpaired t test using Graph Pad Prism 6 version software.

Results

Table 1: Complete Blood Counts in Control and Undernourished (Thrifty Jerry) Adult Wistar Rats

Parameter	Control	Undernourished(Thrifty Jerry)
TLC (x 10^3/µL) ***	11.52 ± 1.01	6.77±0.5
Lymphocytes %	64.47±1.73	61.19±4.26
Granulocytes %	29.47±1.21	30.81±2.16
Monocyte %	4.88±0.47	4.40±0.27
RBC Count (x 10^6/µL)	7.63±0.46	7.80±0.27
Hb (g/dl)	12.83±0.51	12.30±0.46
RBC distribution width	15.72±0.73	16.40±0.12
Hematoctrit HCT	44.03± 1.38	43.45±0.89
MCV (µm^3) **	55.30±0.75	60.05±0.63
MCHC (g/dl)**	29.28±0.36	27.50±0.17
MCH (g)	16.17±0.27	16.48±0.20
Platelet Count (x 10^3/µL) ** ***	414±52.25	803±39.19
Mean platelet Vol (fL)	6.10±0.17	6.20±0.09
Platelet Distribution Width****	6.25±0.06	15.03±0.07
Plateletcrit ****	0.25±0.02	0.53±0.05

Significance: * p< 0.01, ** p<0.001,*/****P<0.0001**

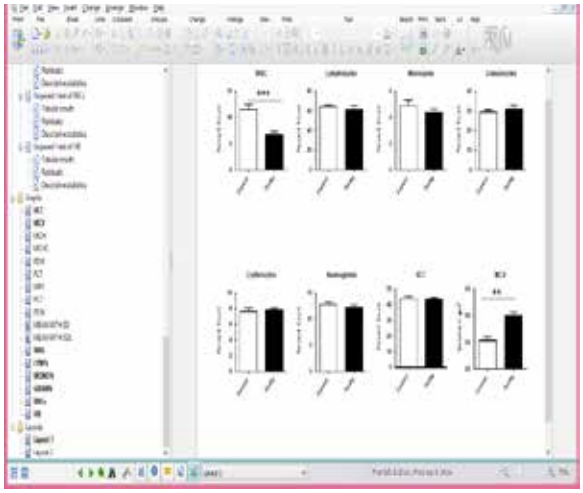

Figure 1: Blood Cell Count in Control and Undernourished (Thrifty Jerry) Adult Wistar Rat

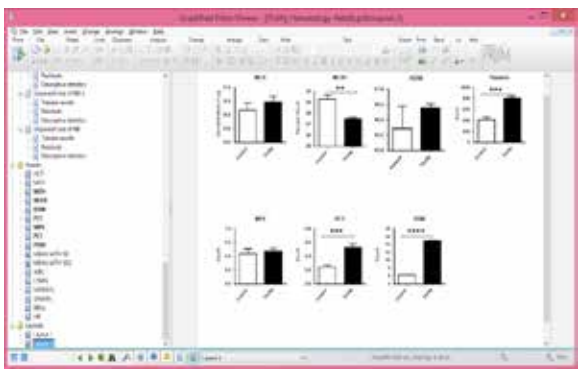

Figure 2: Blood indices and platelet volumes in control and undernourished (Thrifty Jerry) Wistar Rat pups (28 days)

Discussion

It has been has shown that diseases like hypertension,coronary heart disease and type 2 diabetes mellitus occur due to impaired intrauterine growth during fetal development.(Godfrey and Barker 2000).This has been called as "programming" leading to permanent changes in the structure, function and the metabolism of the adult. These adverse health outcomes in adult life have been confirmed by a variety of epidemiological studies (McMillan and Robinson 2005). Yajnik et al have described the "Thin-Fat" Indian phenotype and demonstrated the role of undernutrition in the development of this phenotype (Yajnik,Fall et al (2003), Yajnik and Deshmukh,(2012).Most studies have focused on the effects of undernutrition on renal, cardiovascular, and endocrine abnormalities. Hypocellularity,necrosis & and extracellular matrix modifications have been identified to be the adverse effects of undernutrition on the hemopoietic environment of the bone marrow (Travlos GS (2006), Fried W et al (1978), Vituri CL(2000), Prestes-Carneiro et al (2006) and Borelli P et al(1995).The role of undernutrition on the blood cell counts have not been clearly delineated. This study evaluated the role of multigenerational undernutrition on the complete blood counts in the adult wistar rat model (Thrifty Jerry).

The measurements for Complete blood count in the adult female rats of the undernourished wistar rat colony (Thrifty Jerry) show adaptations to undernutrition leading to altered bone marrow function. (Table 1 & Figs 1 & 2).

A decreased TLC count is indicative of an decreased synthetic ability of the myelopoietic system.However,the differential counts of the leukocytes remain unaltered.Interestingly,the studies carried out by Hardikar et al have shown a state of (chronic) inflammation as revealed by the higher globulin and endotoxin levels in these rats Hardikar et al (2015).

RBC counts, Haemoglobin estimation, Hematocrit and RBC distribution width (RDW) were unaltered in the undernourished colony. However the blood indices were significantly altered. Mean Corpuscular Volume (MCV) was significantly higher in the undernourished colony. This is because the undernourished animals have a deficient folate and B_{12} diet leading to macrocytosis (Hardikar et al 2015).Incidentally Mean Corpuscular Haemoglobin (MCH) was not significantly different from the control colony. Mean Corpuscular Haemoglobin Concentration (MCHC) was however significantly less in the undernourished colony indicative of a picture of iron deficiency or hypochromic anemia. However,no significant differences were observed in the hematocrit and haemoglobin values, though they were marginally lower in the undernourished colony.

Platelet count, Platelet distribution width (PDW) and Plateletcrit (PCT) were significantly higher in the undernourished group. These animals thus showed a picture of thrombocytosis.Secondary thrombocytosis is known to be a reactive process caused due to infection, inflammation, iron deficiency, tissue damage, post surgical trauma, cardiovascular pathology or after splenectomy. Chiarello P(2011), Griesshammer et al(1999). PDW and PCT are known to reflect the total platelet mass.PCT is the number of circulating platelets in a unit volume of blood analogous to the hematocrit for erythrocytes.Ibrahim Akpinar et al (2014).It has been shown that PCT is correlated with C-reactive protein(CRP) levels in patients with chronic inflammatory diseases such as tuberculosis Sahin et al(2012).PDW is known to be an indicator of platelet activation and a specific activation marker for coagulation Vagdatli et al(2010).The undernourished animals have been shown to have an active inflammatory process as revealed by increased globulin levels.The increased Platelet Count,PCT & RDW thus appear to be due to this inflammatory process Hardikar et al (2015). It is also known that slow coronary flow as seen on coronary angiography as a delayed opacification of distal vessels may occur due to platelet activation and inflammation.Hardikar et al have reported congestion of the coronary blood vessels and occasional infarcts in these animals.Undernutrition has been observed to be associated with a state of chronic inflammation. It has been seen to be present before birth via modifications in the immunoepigenome of the undernourished parents Claire Bourke et al (2016). Whether these observations are due to increased platelet numbers and or increased platelet activation remains to be evaluated.

We have however not carried out bone marrow studies to evaluate the changes observed in this colony. Whether these effects are due to the effects of undernutrition on the marrow or epigenetic changes thereof remains to be determined. Hardikar et al have observed fewer (pro-) insulin 2 gene transcripts in their studies of the adult rat pancreas in the undernourished colony. Gene suppression as seen with an increased abundance of KMT1A, a histone-3 lysine-9 methyltransferase was observed. Similarly, studies with chromatin immunoprecipitation (ChIP) and TaqMan based real time PCR of immunoprecipitated DNA showed suppression of pro-insulin gene transcription. Gene expression and ChIP studies in this undernourished colony would highlight the epigenetic changes due to undernutrition on haemopoiesis.

Conclusion

Multigenerational undernutrition for more than 50 generations causes macrocytic anaemia with increased platelet counts and increased platelet activation probably due to a state of inflammation.

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