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Original Research Paper

Haematological Profile in Chronically Undernourished Thrifty Rats at 28 Days of Age

Medical Science

KEYWORDS : Foetal Programming, Multigenerational Undernutrition, Complete Blood Count, Post-weaning profile

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ABSTRACT

Foetal programming due to undernutrition is a known risk factor for the worldwide epidemic of Type 2 diabetes and cardiovascular disease.Total leukocyte count (6.9 vs. 12.7x 103/µL ; p<0.0001, n=6, U vs. C) and Lymphocytes (36.5% vs. 67.7%; p<0.0001, n=6, U vs. C) was significantly lower but granulocytes was significantly higher (21.1% vs. 69.9%; p<0.0001, n=6, U vs. C) in the undernourished colony, RBC content (7.7 vs. 9.4 x 1012/L;, p< non significant,n=6, U vs. C) Hb (11.5g/dl vs 14.8g/dl; p<0.0001, n=6, U vs. C) and Hematocrit (39.05% vs. 47.11%; p<0.0001, n=6, U vs. C) were less but with a high RBC distribution width (11.99 vs. 13.36,p<0.0001,n=6,U vs. C). Platelet count (424.3 vs. 602.3 x 103/microliter, p<0.001, n=6, U vs. C) and Mean platelet volume (5.9 vs. 6.1 fL p< 0.01, n=6, U vs. C) were lower in the undernourished colony. This is indicative of a state of anaemia, an inflammatory process and a hypoplastic bone marrow.

Introduction

Developing countries like India face a burden of Type 2 Diabetes Mellitus, micro- and macro-vascular disease and cancer (Echouffo-Tcheugui 2012). There is increasing evidence from worldwide studies that have shown that these diseases occur due to impaired intrauterine growth (IUGR) and development (Godfrey and Barker 2000) .Experimental studies in a variety of animals have shown that this occurs due to "programming", wherein an insult during the intrauterine life causes permanent changes in the development of a variety of tissue and organ systems. These alterations include a variety of cellular, structural, chemical, metabolic and hormonal adaptations to the undernourished environment during fetal life (McMillan and Robinson 2005).A variety of clinical studies have confirmed these observations. The studies carried out by David Barker and C.S.Yajnik provide evidence for the role of undernutrition in fetal programming especially in the Indian scenario. The Thin-Fat Indian phenotype as seen in the Pune Maternal Nutrition Studies has highlighted the role of body composition. Indian babies have the third lowest birth weight in the world, are insulin resistant, centrally obese but have a lower soft tissue weight. This phenotype is present at birth and is probably programmed through many generations of undernutrition (Yajnik, Fall et al 2003).

Similar observations by have been made in a Wistar rat model (Thrifty Jerry) that has been chronically undernourished for more than 50 generations. The rats were insulin resistant, centrally obese, had higher homocysteine, endotoxin and leptin levels but lower adiponectin, vitamin B12 and folate levels.Exposure to streptozotocin, a selective β cell toxin showed an eight-fold increased susceptibility to diabetes. These animals also showed a reduction in soft tissue growth especially in muscle, heart, liver and the pancreas (Hardikar et al 2015).

A restricted diet has also shown a disturbed hematopoietic environment in the bone marrow leading to hypocellularity, necrosis, and extracellular matrix modifications in undernourished animals (Travlos GS (2006), Fried W et al (1978), Vituri CL(2000), Prestes-Carneiro et al (2006) and Borelli P et al(1995).However, the effect of multigenerational undernutrition on the haematological profile has not been evaluated. In this study we looked at the effect of multigenerational undernutrition on the blood cell count in 28 day pups.

Materials and Methods

The study was carried out in pups from a multigenerationally (50 generations) undernourished wistar rat colony. The colony was undernourished for proteins, fats, fiber, vitamins and received a 50% isocaloric diet. (Hardikar et al 2015).The control group received a standard rat feed. Blood was collected from 28 day pups by retro-orbital bleeding and serum was immediately separated and analysed for Complete Blood Count (CBC) on an autoanalyser (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 GraphPad Prism 6 version software.

Results

Table 1: Complete Blood Counts in Control and Undernourished (Thrifty Jerry) Wistar Rat Pups (28 Day)

Parameter	Undernourished	Control
TLC (x 10^3/µL) ***	6.9 ± 0.7016	12.7 ± 1.519
Lymphocytes % ****	36.5 ± 4.280	67.7 ± 4.020
Granulocytes % ****	69.9 ± 3.301	29.13 ± 3.861
Monocyte %	3.05 ± 0.1258	3.16 ± 0.1764
RBC Count (x 10^6/µL)	7.74 ± 0.7441	9.39 ± 0.2817
Hb (g/dl) ****	11.5 ± 0.4359	14.8 ± 0.3180
RBC distribution width ***	11.99 ± 0.1370	13.36 ± 0.2881
Platelet Count (x 10^3/µL) **	424.3 ± 42.89	602.3 ± 51.34
Mean platelet Vol (fL)*	5.960 ± 0.05099	6.329 ± 0.09440
Hematoctrit HCT ***	39.05 ± 1.256	47.11 ± 0.6277
MCV (µm^3)	53.93 ± 0.6537	53.04 ± 1.271
MCHC (g/dl)	30.60 ± 0.3256	31.26 ± 0.2010
MCH (g)	15.8 ± 0.43	16.1 ± 0.53

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

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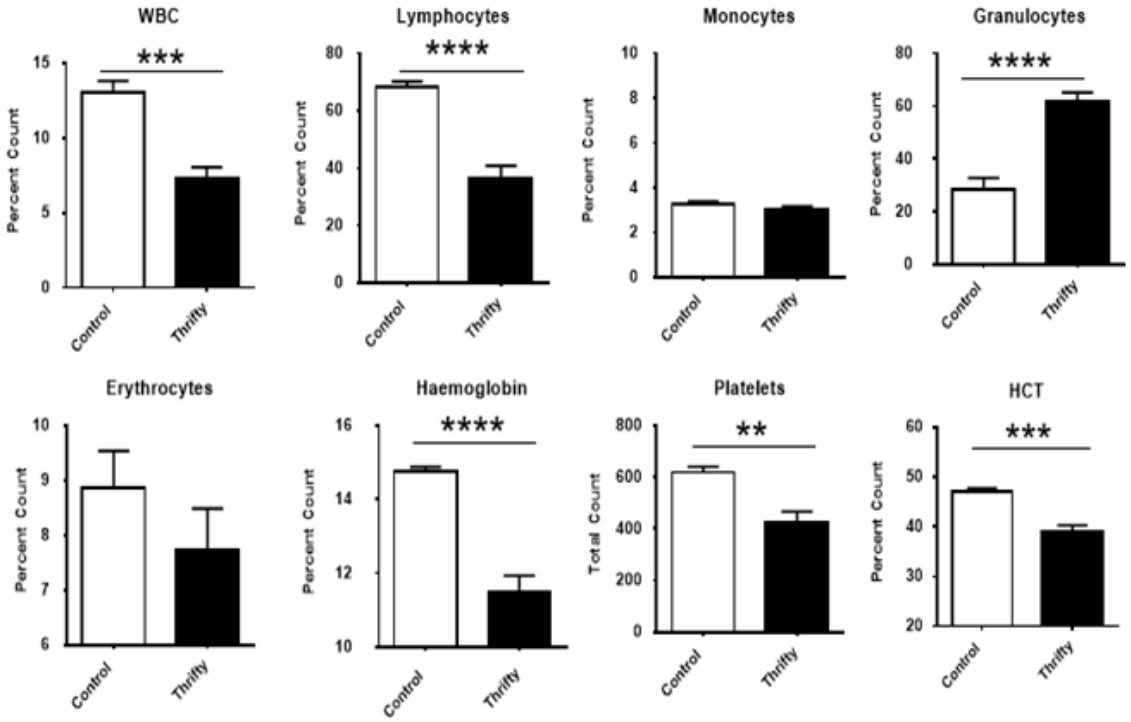

Figure 1: Blood Cell Count in Control and Undernourished (Thrifty Jerry) Wistar Rat pups (28 days)

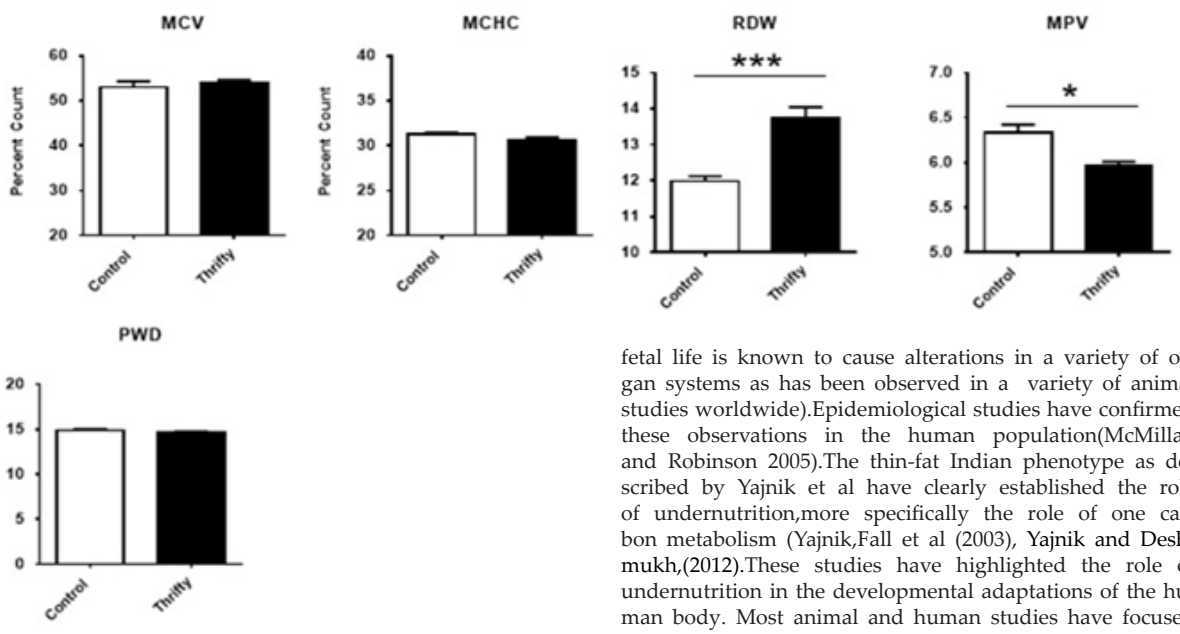

Figure 2: Blood indices and platelet volumes in control and undernourished (Thrifty Jerry) Wistar Rat pups (28 days)

Discussion

The "fetal origins of adult diseases" was first put forth by David Barker and colleagues (Godfrey and Barker 2000).It stated that intrauterine factors, particularly nutrition, act in early life to program the risks for adverse health outcomes in adult life.Under-nutrition during key stages of fetal life is known to cause alterations in a variety of organ systems as has been observed in a variety of animal studies worldwide).Epidemiological studies have confirmed these observations in the human population(McMillan and Robinson 2005).The thin-fat Indian phenotype as described by Yajnik et al have clearly established the role of undernutrition,more specifically the role of one carbon metabolism (Yajnik,Fall et al (2003), Yajnik and Deshmukh,(2012).These studies have highlighted the role of undernutrition in the developmental adaptations of the human body. Most animal and human studies have focused on the role of undernutrition in the development of Diabetes Mellitus as well as renal, cardiovascular, and endocrine abnormalities. Few studies have looked at the role of undernutrition on the hematopoietic environment. This study evaluated the role of multigenerational undernutrition on the haematological disturbances in the wistar rat model (Thrifty Jerry).

We carried out measurements for Complete blood count in 28 day pups of the chronically undernourished wistar rat colony(Thrifty Jerry).The complete blood count reflects the

adaptations to undernutrition that is indicative of a hypoplastic/altered bone marrow function. (Table 1 & Figs 1 & 2).

A decreased TLC with a decreased lymphocyte % is indicative of the reduced immunity. Increased granulocyte % is clearly indicative of an inflammatory process. Biochemical and histological studies in the pancreas in the adult undernourished rats have shown higher globulin and endotoxin levels confirming a state of inflammation in these rats.

Decreased RBC, Haemoglobin count and Hematocrit reflects an anaemic state. A decreased RBC distribution width is indicative of a mixed population of small and large RBC's- a state of anisocytosis and probably poikilocytosis (variation in shape).This is again a classical feature of iron deficiency anaemia. This is because the undernourished animals have a deficient folate and B_{12} diet and therefore increased homocysteine levels. In adults even after supplementation, these levels were corrected but still remained much lower than the controls (Hardikar 2015).

A decreased platelet count and low mean platelet volume is indicative of the presence of older platelets and therefore decreased production in the marrow. We have however not carried out bone marrow studies to see if this leads to a greatly suppressed marrow or hematopoietic failure. These studies thus highlight that deficiency of macronutrients especially protein and micronutrients cause a hypoplastic bone marrow.

Whether these effect are due to undernutrition or epigenetic changes remains to be determined. Hardikar et al in their studies in adults have shown fewer (pro-) insulin 2 gene transcripts in the adult rat pancreas in the same thrifty jerry colony. Increased abundance of KMT1A, a histone-3 lysine-9 methyltransferase that causes gene suppression was also observed. Similarly studies with chromatin immunoprecipitation (ChIP) and TaqMan based real time PCR of immunoprecipitated DNA showed suppression of pro-insulin gene transcription. Similar studies with respect to the hematopoietic system would detail the epigenetic changes, if any, in this colony not only in these pups but also in the adults. This study has also not evaluated the role of supplementation of folate and B_{12} in these 28 day pups.Hardikar et al have shown that supplementation in the adults have improved the B_{12} and folate status but not to the level of controls. It thus remains to be determined whether these changes are due to a lack of macro and micronutrients in the diet or epigenetic changes thereof, or both. This would require a more detailed study in the future.

Conclusion

Multigenerational undernutrition for more than 50 generations in 28 day rat pups causes a hypoplastic bone marrow leading to anaemia, decreased platelet and lymphocyte count but an increased granulocyte count indicative of a state of inflammation.

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