The influence of manganese deficiency during gestation on the emergence of adult diseases. An experimental study on mice progeny.

Samreen Memon [1,*], Pashmina Shaikh[2], Kahaf Naz [3], Sana Kashif [4], Umbreen Bano[5] , Amber[6].

ABSTRACT:
Objective: To detect the possible role of Manganese deficiency in the development of adult diseases when exposed in utero.
Methodology: Total 24 adult female BALB/c female mice were divided into two equal size groups: group 01 was provided normal feeding, whereas group 02 received a Manganese-restricted diet. Animals were permitted to mate once desired levels were achieved, and pregnancy was assessed by examining vaginal area. The similar dietary regimen was maintained during the entire gestation. Pups were maintained on the same diet until 15 months of age. The behavior and locomotion of animals was kept under observation. At 05- and 15-months different blood parameters were examined. At 15 months age, the pancreas, kidneys and uterine tissue were excised for microscopic analysis.
Results: The group 2 animal group exhibited anomalous behavior and movement characteristics. In comparison to the control group, offspring in group G2 exhibited increased levels of blood glucose, serum insulin and cholesterol. H & E staining demonstrated modifications in the parenchyma of the pancreas and kidneys and were confirmed by immunohistochemistry. The uterus appeared normal in both groups as indicated by anti-MLH antibody staining.
Conclusion: In utero Manganese deficiency may eventually leads changes of vital organs and play a role in the development of diseases later in the life.

Key words: Manganese restriction; mice offspring; adult diseases; in-utero.

Cite as: Memon S, Shaikh P, Naz K, Kashif S, Bano U, Amber. The influence of manganese deficiency during gestation on the emergence of adult diseases. An experimental study on mice progeny. J Muhammad Med Coll. 2025;16 (1) pp-23-29.

Introduction:

The word epigenetics describes several processes that lead to heritable changes in gene expression through mitosis and meiosis, without modifying the DNA sequence 1. Key mechanisms of epigenetic changes include DNA methylation, histone modification, and non-coding RNA involvement 2. Early nutrition may play a crucial role in developmental programming through epigenetic modifications, potentially influencing individual susceptibility to the later onset of cardiovascular diseases, obesity, diabetes, and other non-communicable chronic conditions 3.

1. Professor, Department of Anatomy, Liaquat University of Medical & Health Sciences, Jamshoro,.
2. Associate Professor, Department of Anatomy, Liaquat University of Medical & Health Sciences, Jamshoro.
3. Lecturer, Department of Anatomy, Bilawal Medical College, Liaquat University of Medical & Health Sciences, Jamshoro.
4. Assistant Professor, Department of Anatomy, Chandka Medical College/ SMBBMU, Larkana.
5. Assistant Professor, Department of Anatomy, Liaquat University of Medical & Health Sciences, Jamshoro.
6. Assistant Professor, Department of Anatomy, Makran Medical College, Turbat, Baluchistan.

**=corresponding author* :
Email: samreen.memon@lumhs.edu.pk

Received: 11.06.2025 Revised 23.07.2025
Accepted 11.09.2025 Published online 7.10.2025

Micronutrients, encompassing trace elements, are integral to all biological and metabolic processes, including tissue development, cell signaling, motility, proliferation, and apoptosis 4. Manganese (Mn), is an essential micronutrient, in humans and animals, acts as an enzyme cofactor and metalloenzyme's component 5,6. It is required for synthesis of fatty acids, and 7 gluconeogenesis in liver by activating enzymes required for these processes 8,9. Manganese (Mn) is primarily sourced from dietary and water intake. The main site for its absorption is gut and once absorbed it is distributed to the liver, pancreas, and pituitary gland, where it is rapidly concentrated 10. Moreover, it has a critical role in the formation and initiation of various enzymatic activities. It is also involved in glucose and lipid metabolism, enhances protein, vitamin C, and vitamin B synthesis, catalyses haematopoiesis, regulates endocrine functions, and contributes to immune function improvement 11. It is also an important constituent of manganese superoxide dismutase (Mn-SOD) that may have a role in oxidant defense system occurring in mitochondria. Several studies have shown that the intolerance of glucose is reversible by supplementation of Mn, if it results from manganese deficiency 9,12. Despite its beneficial role in many adult processes, it is yet to be discovered that it might play a crucial part in developmental origin of adult disease when exposed in deficient amounts during in-utero life.

Objectives:

This study was conducted to detect the possible role of Manganese deficiency in the development of adult diseases when exposed in utero.

Methodology:

/The experiments were performed at Sindh Agricultural University in Tando Jam and Liaquat University of Medical and Health Sciences in Jamshoro, Pakistan after ethical

approval (No. LUMHS/REC/-95), and was conducted in accordance with the guidelines of The National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 85-23, revised 1985). The animal food was prepared in accordance with the laboratory protocol. The Manganese-restricted diet, which solely limits Manganese intake, was formulated similarly to the control diet and utilised skimmed milk powder, with the manganese-restricted diet containing 0.0103 mg Mn/kg. All components were procured from the local market. The research protocols were employed as previously described. 13. Twenty-four female dams were allocated into groups, each comprised of twelve animals and housed in conventional polypropylene cages under a 12-hour light-dark cycle, at a temperature of 22±2°C and relative humidity of 55±10%. The initial weight of both groups was assessed before the experiment, showing no statistically significant difference (Table-1). Group 1 control mice were provided unrestricted access to basal diet, whereas Group 2 mice were granted unrestricted access to a Manganese-restricted diet for a duration of 12 weeks. Plasma manganese values were checked on blood samples taken by puncturing orbital vein, using an Atomic Absorption Spectrophotometer (USA) (Table 2).Upon confirming manganese levels, the animals were allowed matting with two into one ration (female to male), and once a positive vaginal plug was detected it was regarded as day 1 of gestation. The identical diet was implemented during gestation. At delivery, litter size was standardized to ten (10) per group, and all litters were provided same dietary regimen until they reached 15 months of age. Pups were evaluated for body weight, behaviour, and mobility, in addition to biochemical tests of manganese, cholesterol, high-density lipoprotein, insulin, and glucose at 5 months and 15 months before sacrifice. Subsequent to sacrifice, dissection of litter was conducted, and tissues were procured, including pancreas, kidney, adipose tissue, and uterus, for H&E staining, and immunohistochemistry.

Morphological assessment of the offspring: Litters were evaluated for the behaviour in accordance with the previously established scale. 13 The movements of pups were categorized into 10 grades, as described previously 13.

Biochemical evaluations: Samples were taken at five and fifteen months to evaluate glucose levels and serum insulin utilising the ELISA assay kit method on a Spectrophotometer (Hitachi 902, Roche Diagnostics, USA). Blood cholesterol and HDLc levels were measured with an enzymatic colorimetric technique with commercially available kits on the Microlab Chemistry Analyser (Micro Lab 300 Spectrophotometer, Roche USA).

Haematoxylin and Eosin (H&E) staining: Slides underwent 80°C oven drying for 25–30 minutes prior to treat with xylene, alcohol, and finally distilled water. Slides underwent a 3% hydrogen peroxide treatment for 10 minutes to inhibit nonspecific antibodies, followed by extensive rinsing. After antigen retrieval, tissues underwent EDTA buffer treatment for 8 minutes at 120°C and were cooled for an additional 10 minutes. The residual EDTA was removed from the slides by complete washing with PBS. Primary antibodies (Anti-Islet 1 antibody) [1B1], Ksp-Cadherin (Kidney-specific Cadherin), and (anti-MLH-1 antibody) were applied at room temperature for 01 hour. Once the slides were washed and antibody was removed, the tissues were incubated in the dark with secondary antibody for 10 to 15 minutes. The secondary antobody was removed by washing with PBS buffer. The nuclei were stained using DAB applied for 5 minutes. Counterstaining with haematoxylin was performed before the slides were washed. Slides were prepared using DPX mounting medium.

Statistical analysis: The statistical software SPSS 22.0 (IBM, Inc., USA) was employed for data analysis. Continuous variables were evaluated via the Student t-test, with findings expressed as mean ± SD. Categorical variables were evaluated with the Chi-square test, with findings displayed as frequency and percentage. A p-value of ≤0.05 was considered significant in all cases.

Results:

Regarding the body weight of female mice before experiment, there was no significant difference observed (P=0.083). However, Serum Mn of female mice before mating revealed statistically significant difference between two groups (P=0.049) as shown in table 1.

Table No 1: Body weight and serum Manganese levels of dams before the start of the experiment.

Body weight (grams) of dams initial findings			
	Mean	SD±	P -value
Group 1. Control mice	31.03	3.63	0.083
Group 2. Manganese (0.0103mg/Kg)	32.47	5.06	
Serum Manganese (µg) of female mice before pregnancy Baseline findings			
	Mean	SD±	P -value
Group 1. Control mice	1.14	0.10	0.0491
Group 2. Manganese (0.0103mg/Kg)	0.37	0.14	

Table No 2: Body weight of the dams during pregnancy

Body weight (grams) of female mice during pregnancy			
	Mean	SD±	p value
1st week			
Group 1. Control mice	39.87	1.37	0.071
Group 2. Manganese (0.0103mg/Kg)	39.02	1.33	
2nd week			
Group 1. Control mice	41.55	0.79	0.0531
Group 2. Manganese (0.0103mg/Kg)	40.02	1.32	
3rd week			
Group 1. Control mice	41.02	1.31	0.01
Group 2. Manganese (0.0103mg/Kg)	39.23	1.29	

Body weight (g) measurement in offspring of control and Mn restricted group is shown in table no 3. Mean ± SD body weight of MnR group showed significant body weight loss compared to control mice. Low body weight was note from 2nd to 15th month in Mngroup-2. (P=0.0001).

Behavior of controls showed no observable deficits (grade 0), in contrast the experimental mice show the various grades of behavioral abnormalities, the grading is shown in

table 4, abnormalities of abnormal gait, mobility problems, immobility, tense and nervous behavior on handling and marked distress with shaking, vocalization and aggressive behavior (p=0.0001).

Table No 3. Body weight (grams) of litters (n=20)

Month	Group 1. Control	Group 2. Mn (0.0103mg/Kg)	P-value
1st	1.61±0.06	1.34±0.08	0.087
2nd	3.40±0.18	2.64±0.33	0.001
3rd	5.84±1.30	4.24±1.12	0.001
4th	7.84±0.71	6.80±0.64	0.01
5th	9.78±0.98	9.23±0.77	0.034
6th	14.60±0.93	12.78±1.61	0.001
7th	16.6±0.60	15.62±0.79	0.001
8th	22.92±6.20	17.07±1.21	0.001
9th	28.91±1.24	22.46±6.65	0.001
10th	30.78±1.18	28.36±1.31	0.001
11th	33.10±1.04	30.78±1.18	0.001
12th	34.87±0.82	33.10±1.04	0.001
13th	35.14±0.68	31.82±2.96	0.001
14th	36.99±1.27	34.83±0.4	0.001
15th	38.53±0.97	36.99±1.27	0.001

Table No 4: Behavior of litters (n=20)

Month	Group 1. Control	Group 2. Mn (0.0103mg/Kg)	P-value
1st	0	6	0.0001
2nd	0	6	0.0001
3r	0	6	0.0001
4th	0	6	0.0001
5th	0	6	0.0001
6th	0	6	0.0001
7th	0	6	0.0001
8th	0	6	0.0001
9th	0	6	0.0001
10th	0	6	0.0001
11th	0	6	0.0001
12th	0	5	0.0001
13th	0	5	0.0001
14th	0	5	0.0001
15th	0	5	0.0001

Grade 0.No observable deficit, Grade 1. Slightly abnormal gait, Grade 2. Markedly abnormal gait, Grade 3. Significant mobility problems, Grade 4. Immobility >24 hours, Grade 5. Tense & nervous on handling, Grade 6. Marked distress on handling (shaking, vocalizing, aggressive)

Movement grading of controls showed no observable defects (grade 0). Compared to controls, the experimental mice showed various grades of movement abnormalities as shown in table 5, abnormalities of reduced movements, little or no investigation around cage, shelter seeking movements, no movement around cage, typically isolated and nearly moribund.

Low serum Mn was found in the group 2. Serum Mn was calculated as 0.21±0.01 and 0.24±0.05 µg at 5th and 15th months respectively (p=0.0001) as shown in table 6. Similarly, Mean blood glucose was higher in group 2 offspring. At 5th and 15th months; the mean blood glucose in control and Mn group were noted as 119.54 and 170.9 mg/dl and 117.5 and 161.8 mg/dl respectively (p=0.0001) as shown in table 8. Mean ± SD serum insulin at 5th and 15th months was observed as 9.36, and 9.92 mIU/L (p=0.003) and 8.92 and 11.54 mIU/L respectively (p=0.0001). At 5th and 15th months; the cholesterol was noted as 118.7, and 164.9 mg/dl and 115.0, and 160.3 mg/dl respectively (p=0.0001). Mean ± SD HDLc was low in pups of Mn limited fed dams in comparison to normal diet off springs. At 5th and 15th months; the cholesterol was noted as 35.1 and 27.6 & 36.6and 28.7 mg/dl respectively (p=0.0001). Table 6.

Table No 5. Movement of litters (n=20)

Month	Group 1. Control	Group 2. Mn (0.0103mg/Kg)	P-value
1st	5	9	0.0001
2nd	4	8	0.0001
3rd	3	8	0.0001
4th	2	8	0.0001
5th	2	7	0.0001
6th	1	7	0.0001
7th	1	7	0.0001
8th	1	7	0.0001
9th	1	7	0.0001
10th	1	7	0.0001
11th	1	6	0.0001
12th	1	6	0.0001
13th	1	6	0.0001
14th	1	5	0.0001
15th	1	5	0.0001

Grade 1. Moving quickly around cage, Grade 2. Frequently standing at sides of cage, Grade 3. Active investigation into surroundings, Grade 4. Reduced movement around cage, Grade 5. Little to no investigation around cage, Grade 6. Seeks shelter, Grade 7. Moves around cage when stimulated, Grade 8. No movement around cage, Grade 9. May be moribund, Grade 10. Typically isolated from cage mates

Histological findings

Pancreas-The H & E-stained histological examination of pancreas shows normal tissue architecture. Both endocrine and exocrine parts and pancreatic ducts are visible in Photomicrographs 1 (A). However, Photomicrographs 1 (B) show small sized Islet of Langerhans and atrophy in group 2

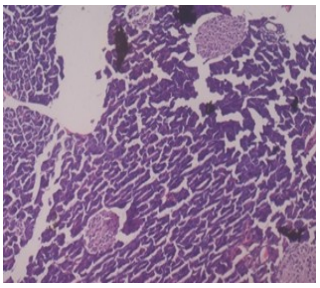
Kidneys-The kidney section show glomeruli, renal tubules and interstitial tissue in control group with normal architecture as shown in photomicrograph 2 (A). However, microscopic assessment of Kidney section in Mn restricted group shows infiltrated and abnormal caliber tubules, hypervascularity, a few atrophic tubules and fibrosed glomeruli [photomicrograph 2 (B&C)].

Table No 6: Biochemical parameters.

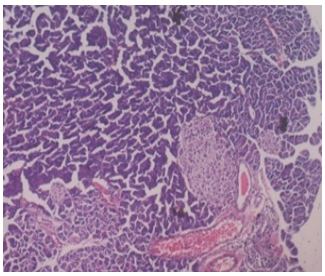
Serum Manganese (µg) of mice offspring (n=20)			
At 5th month			
	Mean	SD±	p value
Group 1. Control	0.91	0.17	0.0001
Group 2. Mn (0.0103mg/Kg)	0.21	0.01	
At 15th month			
Group 1. Control	1.03	0.04	0.0001
Group 2. Mn (0.0103mg/Kg)	0.24	0.05	
Blood glucose (mg/dl) of mice offspring (n=20)			
At 5th month			
Group 1. Control	119.54	31.92	0.0001
Group 2. Mn (0.0103mg/Kg)	170.99	3.20	
At 15th month			
Group 1. Control	117.54	28.10	0.0001
Group 2. Mn (0.0103mg/Kg)	161.84	7.40	
Serum Insulin (mIU/L) of mice offspring (n=20)			
At 5th month			
Group 1. Control rats	9.36	1.27	0.003
Group 2. Mn (0.0103mg/Kg)	9.92	0.84	
At 15th month			
Group 1. Control rats	8.92	0.73	0.0001
Group 2. Mn (0.0103mg/Kg)	11.54	1.52	
Blood Cholesterol (mg/dl) of mice offspring (n=20)			
At 5th month			
Group 1. Control	118.76	32.25	0.0001
Group 2. Mn (0.0103mg/Kg)	164.90	6.57	
At 15th month			
Group 1. Control	115.02	31.01	0.0001
Group 2. Mn (0.0103mg/Kg)	160.35	7.53	
Blood HDLc levels (mg/dl) of mice offspring (n=20)			
At 5th month			
Group 1. Control	35.17	6.63	0.0001
Group 2. Mn (0.0103mg/Kg)	27.60	1.61	
At 15th month			
Group 1. Control	36.63	6.82	0.0001
Group 2. Mn (0.0103mg/Kg)	28.74	0.52	

Uterus- The microscopic sections of uterus show bicornuate uterus (Photomicrograph-3 A). The histological examination of uterus in group 2 shows the mononuclear lymphocytic infiltration around endometrial glands (Photomicrograph-3 B)

Photo Micrograph-1 histology findings of Control (A) and experimental manganese group of Pancreas (B).

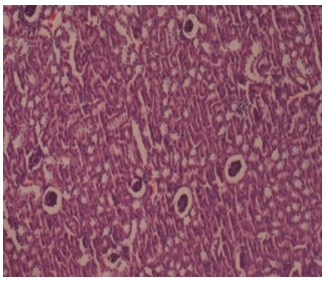


A. Pancreatic section showing Islets of Langerhans of control group

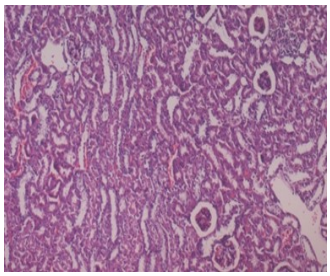


B. Two islets are seen one is smaller in size and Atrophic appearance of islets

Photo Micrograph-2 Histology findings of Control (A) and experimental manganese group of Kidney (B).

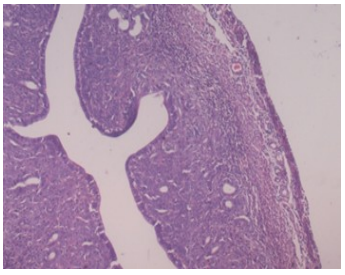


A. Normal kidney tissue section showing Glomeruli and tubules

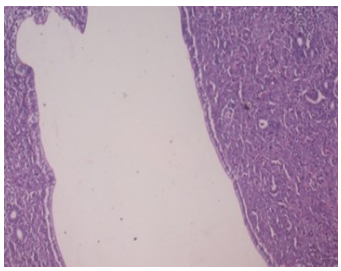


B: Two to three tubules are dilated, one to two glomeruli are fibrosed, mononuclear lymphocytic infiltration of interstitium One glomeruli completely fibrosed (loaded with spindle shaped cells), tubules also dilated.

Photo Micrograph-3 Histology findings of Control (A) and experimental manganese group of Uterus (B).



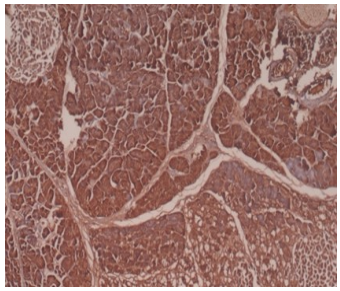
A. Normal lining of uterine cavity, endometrial glands and normal looking myometrium



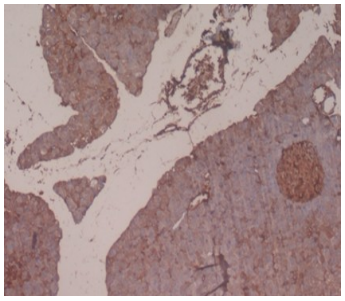
B: Uterus Findings: Mononuclear lymphocytic infiltration around endometrial glands

Immunohistochemistry (IHC)

Pancreas: The IHC findings of control mice show normal Anti-Islet 1 antibody [1B1] as shown in Photomicrograph 4. The IHC findings of Group 2 mice showing normal islet of Langerhans (Photomicrograph 5). (Anti-Islet 1 antibody) [1B1]

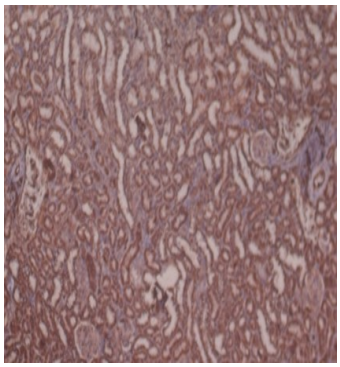


Photomicrograph 4. Pancreatic section shows normally appearing Islets of Langerhans. Group 1- IHC (Formalin/PFA-fixed paraffin embedded sections) - Anti-Islet 1 antibody [1B1

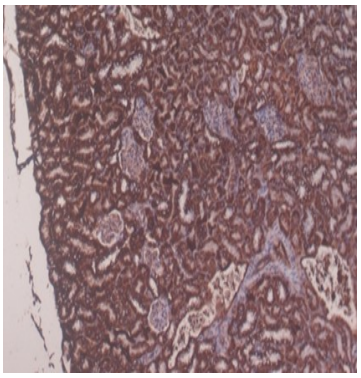


Photomicrograph 5. Pancreas Findings: islets are two in number and normal in appearance IHC (Formalin/PFA-fixed paraffin embedded sections) - Anti-Islet 1 antibody [1B1]

Kidneys: The IHC findings of control mice show normal Ksp-Cadherin distribution as shown in Photomicrograph 6. The IHC findings of group 2 mice (Ksp-Cadherin staining) show the glomeruli are fibrosed and dilated renal tubules (Photomicrograph 7). Fibrosed glomeruli with dilated renal tubules are also visible in Photomicrograph 7.

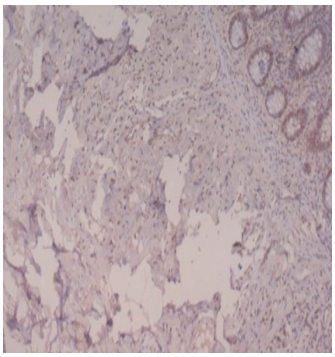


Photomicrograph 6. Normal Kidneys Findings: Normal appearance of glomeruli and tubules Group 1- IHC (Formalin/ PFA-fixed paraffin embedded sections) - Ksp-Cadherin (Kidney-specific Cadherin)

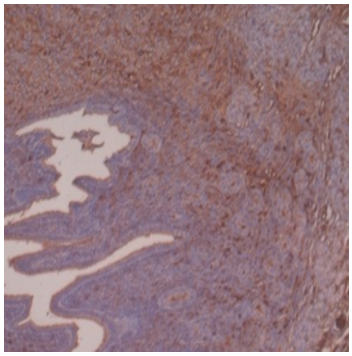


Photomicrograph 7. Kidneys Findings: 3 to 4 glomeruli are fibrosed. Tubules are also dilated Group 3- IHC (Formalin/PFA-fixed paraffin embedded sections) - Ksp-Cadherin (Kidney-specific Cadherin)

Uterus: The IHC findings of control mice show normal uterus as shown in Photomicrograph 8. The IHC findings of group 2 mice show normal endometrium (Photomicrograph 9).



Photomicrograph 8. Normal Uterus- Findings: nuclear staining of endometrial glands, myometrium in secretory phase. Group 1- IHC anti MLH-antibody



Photomicrograph 9. Uterus Findings: cavity is bicornuate, endometrial glands are normal in appearance, and cytoplasmic staining is seen Group 2- IHC anti MLH- antibody

Discussion:

Sir Waddington (1942) defined epigenetics as "physical nature of genes". He considered epigenetic as an interaction between genes and their environment with resultant phenotypic changes [14]. Animal studies support the role of environmental epigenetic in disease susceptibility. Recent studies proved the relation between disease susceptibility, environmental exposure and germline mutations on two specific parts of the genes which are coding and promoter regions [14]. In present study, the manganese deficiency resulted in low body weight in the offsprings when measured at 5- and 15-months age. Although past animal-based research mentioned no effects on body weight. However, these findings are in conjunction with previous epidemiological data regarding maternal intake of less nutrient diet and weight in the offspring. This might be due to the fact that maternal diet containing decreased amounts of micronutrients such as Mn has an effect on offspring BMI and fat forming cells. Another finding of this study was raised glucose levels in pups of low manganese fed female mice lly. The findings suggest the role of Mn in blood glucose homeostasis. High blood glucose levels in progeny of dams provided with the MnR diet throughout gestation, suggested role of this trace element in regulating blood glucose at different levels. An interesting finding of elevated insulin along with glucose in litters of MnR dams suggests metabolic abnormality of *insulin resistance*. the findings of current study are supported by previous studies [15-17] which have reported the role of trace minerals on the glucose homeostasis and insulin secretion. Mn plays role in glucose homeostasis through unknown mechanism. Mn deficiency in guinea pigs resulted in glucose intolerance and this metabolic defect was improved by Mn supplementation [18]. A previous study reported raised Mn content of liver in streptozocin induced diabetes mellitus, the reasons remain unknown [18]. Mn may have role in glucose metabolism through arginase enzymes, is one suggested mechanism, because the arginase may accelerate the amino acid metabolism through urea cycle. And disturbed blood amino acid levels might be causing the insulin alterations and glucose intolerance [19]. Mn in diabetics has highly con-

troversial role, some reported its blood level is normal while others reported Mn level may be low or high [20,21].

This study also revealed higher mean blood cholesterol in offspring of MnR diet fed dams. Evidence based findings suggest the high blood cholesterol in offsprings of experimental mice. Raised blood cholesterol is also an abnormality that points towards the metabolic syndrome with hypercholesterolemia similar to *insulin resistance*. Similarly, HDLc was low in progeny of Mn restricted diet fed mothers. Offsprings of Mn restricted diet fed female mice shows various grades of behavioral and movement abnormalities. Experimental mice offspring show the abnormalities of abnormal gait, mobility problems, immobility, tense and nervous behavior on handling and marked distress with shaking, vocalization and aggressive behavior (p=0.0001). Mn induced neurotoxicity is reported in previous studies [22,23]. The findings of gait and movement abnormalities in female pregnant mice of present study are supported by above studies. John Couper (1837) was the first to report Mn induced neurotoxicity as whispering speech, limb tremors, muscle weakness, salivation and stooped posture in male working in Mn plant, symptoms were collectively termed as the "Manganism". Manganism resembles to the Parkinson's disease but through damaging brain areas other than basal nuclei [24,25]. Pine et al reported the Mn disrupts the normal function of the endocrine system, in particular the sexual hormones [26]. Lee et al reported an increase in serum LH, FSH, and testosterone levels, sperm production, and efficiency of spermatogenesis in rats given Mn in diet [27]. Control group showed normal histological examination of pancreas, adipose tissue, kidneys and uterus. Experimental group showed small size Islet of Langerhans's, normal adipose tissue with kidney abnormalities. Kidney tissue shows dilated renal tubules with mononuclear and lymphocytic infiltrations. Hypervascularity, a few atrophic tubules and fibrosed glomeruli are visible. Renal tubules are dilated, with mononuclear lymphocytic infiltration; some tubules are atrophic, & hypervascularity is visible and interstitial mononuclear lymphocytic infiltration. In a previous study, it was concluded that Mn could lead to acute kidney failure [28]. Uterus showed the normal appearing histological architecture. Similarly, the Immunohistochemical analysis of tissue sections in Mn restricted diet off springs showed some atrophic islets of Langerhans abnormal renal tubules and fibrosed glomeruli with hyalinization and dilation of renal tubules. Some atrophic renal tubules were also visible. The uterus IHC anti MLH- antibody showed the cytoplasmic staining and normally appearing uterine lining, glands and myometrium.

Conclusion:

This study mentions that Mn depletion during intra uterine life could lead to long term complications and adult diseases especially metabolic disorders and renal defects.

Limitations: As this was an animal-based laboratory study, further studies are warranted to explore association between trace element deficiency during intrauterine life and origin of adult diseases later in life.

Acknowledgement:

Highly thankful to Liaquat University of Medical & Health Sciences for providing the funding for this project. Also acknowledge the support of staff and faculty at Sindh Agricultural University, Tando Jam and Diagnostic and research laboratory, LUMHS, Jamshoro

Conflict of Interest:

The authors declare no conflict of interest.

References:

1. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet. 1986 May 10;1(8489):1077-81. doi: 10.1016/s0140-6736(86)91340-1. PMID: 2871345.
2. Chen H-Y, Hsu M, Lio C-WJ. Micro but mighty—Micronutrients in the epigenetic regulation of adaptive immune responses. Immunol Rev. 2022; 305: 152–164. doi:10.1111/imr.13045
3. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, Webb P, Lartey A, Black RE; Lancet Nutrition Interventions Review Group, the Maternal and Child Nutrition Study Group. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet. 2013 Aug 3;382(9890):452-477. doi: 10.1016/S0140-6736(13)60996-4. Epub 2013 Jun 6. Erratum in: Lancet. 2013 Aug 3;382(9890):396. PMID: 23746776.
4. Avila DS, Puntel RL, Aschner M. Manganese in health and disease. Met Ions Life Sci. 2013;13:199-227. doi: 10.1007/978-94-007-7500-8_7. PMID: 24470093; PMCID: PMC6589086.
5. Leach RM, Lilburn MS. Manganese metabolism and its function. World Rev Nutr Diet. 1978;32:123-34. doi: 10.1159/000401764. PMID: 104445.
6. Erikson, K. & Aschner, M. (2019). 10. MANGANESE: ITS ROLE IN DISEASE AND HEALTH. In P. Carver (Ed.), Essential Metals in Medicine: Therapeutic Use and Toxicity of Metal Ions in the Clinic (pp. 253-266). Berlin, Boston: De Gruyter. doi: 10.1515/9783110527872-010
7. Klimis-Tavantzis DJ, Leach RM Jr, Kris-Etherton PM. The effect of dietary manganese deficiency on cholesterol and lipid metabolism in the Wistar rat and in the genetically hypercholesterolemic RICO rat. J Nutr. 1983 Feb;113(2):328-36. doi: 10.1093/jn/113.2.328. PMID: 6822906.
8. Rognstad R. Manganese effects on gluconeogenesis. J Biol Chem. 1981 Feb 25;256(4):1608-10. PMID: 7462214.
9. Everson GJ, Shrader RE. Abnormal glucose tolerance in manganese-deficient guinea pigs. J Nutr. 1968 Jan;94(1):89-94. doi: 10.1093/jn/94.1.89. PMID: 5638643.
10. Deng Q, Liu J, Li Q, Chen K, Liu Z, Shen Y, Niu P, Yang Y, Zou Y, Yang X. Interaction of occupational manganese exposure and alcohol drinking aggravates the increase of liver enzyme concentrations from a cross-sectional study in China. Environ Health. 2013 Apr 15;12:30. doi: 10.1186/1476-069X-12-30. PMID: 23587294; PMCID: PMC3639182.
11. Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis. Mol Aspects Med. 2005 Aug-Oct;26

(4-5):353-62. doi: 10.1016/j.mam.2005.07.003. PMID: 16099026; PMCID: PMC6309959.
12. Li L, Yang X. The Essential Element Manganese, Oxidative Stress, and Metabolic Diseases: Links and Interactions. Oxid Med Cell Longev. 2018 Apr 5;2018:7580707. doi: 10.1155/2018/7580707. PMID: 29849912; PMCID: PMC5907490.
13. Bano, U., Memon, S., Shahani, M. Yaqoob, Shaikh, P., Gul, S. Epigenetic effects of in utero bisphenol A administration: Diabetogenic and atherogenic changes in mice offspring. Iranian Journal of Basic Medical Sciences, 2019; 22(5): 521-528. doi: 10.22038/ijbms.2019.29909.7357
14. Buha A, Manic L, Maric D, et al. The effects of endocrine-disrupting chemicals (EDCs) on the epigenome-A short overview. Toxicology Research and Application. 2022;6. pp 1-9. doi:10.1177/23978473221115817.
15. Rogers RE, Chai S, Pask AJ, Mattiske DM. Prenatal exposure to diethylstilbestrol has long-lasting, transgenerational impacts on fertility and reproductive development. Toxicol Sci. 2023 Aug 29;195(1):53-60. doi: 10.1093/toxsci/kfad066. PMID: 37471692; PMCID: PMC10464516.
16. Liu J, Yu C, Doherty TM, Akbari O, Allard P, Rehan VK. Perinatal nicotine exposure-induced transgenerational asthma: effects of reexposure in F1 gestation. FASEB J. 2020;34:11444-11459. doi: 10.1096/fj.201902386R
17. Farsetti A, Illi B, Gaetano C. How epigenetics impacts on human diseases. Eur J Intern Med. 2023 Aug;114:15-22. doi: 10.1016/j.ejim.2023.05.036. Epub 2023 Jun 3. PMID: 37277249.
18. Ogura M, Matsutani M, Asai K, Suzuki M. Glucose controls manganese homeostasis through transcription factors regulating known and newly identified manganese transporter genes in Bacillus subtilis. J Biol Chem. 2023 Aug;299(8):105069. doi: 10.1016/j.jbc.2023.105069. Epub 2023 Jul 17. PMID: 37468100; PMCID: PMC10448178.
19. Zakaria NF, Hamid M, Khayat ME. Amino Acid-Induced Impairment of Insulin Signaling and Involvement of G-Protein Coupling Receptor. Nutrients. 2021 Jun 29;13(7):2229. doi: 10.3390/nu13072229. PMID: 34209599; PMCID: PMC8308393.
20. Julia G. Debertin, Elizabeth A. Holzhausen, Douglas I. Walker, Brismar Pinto Pacheco, Katherine A. James, Tanya L. Alderete, Laura Corlin. Associations between metals and metabolomic profiles related to diabetes among adults in a rural region. Environmental Research. 2024; Volume 243, 117776. doi: 10.1016/j.envres.2023.117776.
21. Du S, Wu X, Han T, Duan W, Liu L, Qi J, Niu Y, Na L, Sun C. Dietary manganese and type 2 diabetes mellitus: two prospective cohort studies in China. Diabetologia. 2018 Sep;61(9):1985-1995. doi: 10.1007/s00125-018-4674-3. Epub 2018 Jul 3. PMID: 29971528.
22. Tsuji M, Koriyama C, Ishihara Y, Isse T, Ishizuka T, Hasegawa W, Goto M, Tanaka R, Kakiuchi N, Hori H, Yatera K, Kunugita N, Yamamoto M, Sakuragi T, Yasumura Y, Kono M, Kuwamura M, Kitagawa K, Ueno S. Associations between welding fume exposure and neurological function in Japanese male welders and non-welders. J Occup Health. 2023 Jan;65(1):e12393. doi: 10.1002/1348-9585.12393. PMID: 36823734; PMCID: PMC9950351
23. Santamaria AB, Cushing CA, Antonini JM, Finley BL, Mowat FS. State-of-the-science review: Does manganese exposure during welding pose a neurological risk? J Toxicol Environ Health B Crit Rev. 2007 Nov-Dec;10(6):417-65. doi: 10.1080/15287390600975004. PMID: 17710609.
24. Kwakye GF, Paoliello MM, Mukhopadhyay S, Bowman AB, Aschner M. Manganese-Induced Parkinsonism and Parkinson's Disease: Shared and Distinguishable Features. Int J Environ Res Public Health. 2015 Jul 6;12(7):7519-40. doi: 10.3390/ijerph120707519. PMID: 26154659; PMCID: PMC4515672.
25. Jiang YM, Mo XA, Du FQ, Fu X, Zhu XY, Gao HY, Xie JL, Liao FL, Pira E, Zheng W. Effective treatment of manganese-induced occupational Parkinsonism with p-aminosalicylic acid: a case of 17-year follow-up study. J Occup Environ Med. 2006 Jun;48(6):644-9. doi: 10.1097/01.jom.0000204114.01893.3e. PMID: 16766929; PMCID: PMC4180660.
26. Pine M, Lee B, Dearth R, Hiney JK, Dees WL. Manganese acts centrally to stimulate luteinizing hormone secretion: a potential influence on female pubertal development. Toxicol Sci. 2005 Jun;85(2):880-5. doi: 10.1093/toxsci/kfi134. Epub 2005 Mar 2. PMID: 15746010.
27. Lee B, Pine M, Johnson L, Rettori V, Hiney JK, Dees WL. Manganese acts centrally to activate reproductive hormone secretion and pubertal development in male rats. Reprod Toxicol. 2006 Nov;22(4):580-5. doi: 10.1016/j.reprotox.2006.03.011. Epub 2006 May 12. PMID: 16697554.
28. Peng Gao, Yutian Tian, Qi Xie, Liang Zhang, Yongjian Yan, Dongmei Xu, Manganese exposure induces permeability in renal glomerular endothelial cells via the Smad2/3-Snail-VE-cadherin axis, Toxicology Research, 2020; 9, (5). pp 683-692, doi:10.1093/toxres/tfaa067

Author's contribution	
Samreen Memon	Conceptualization, Formal Analysis, Supervision, Writing Review & Editing
Pashmina Shaikh	Writing – Review & Editing, Project Administration
Kahaf Naz	Data Curation, Project Administration
Sana Kashif	Data Curation, Methodology
Umbreen Bano	Writing – Original Draft, Funding Acquisition, Validation
Amber	Data Curation, Methodology, Writing – Original Draft