Effect of experimental phenylketonuria on the development of skin of prenatel and newborn fetuses

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Abstract

Phenylketonuria (PKU) is a genetic disorder that is characterized by an inability of the body to utilize the essential amino acid, phenylalanine. The disease results from a deficiency in phenylalanine hydroxylase, the enzyme catalyzing the conversion of phenylalanine to tyrosine. The present work studied the Effect of experimental phenylketonuria on the development of skin of prenatel and newborn fetuses.The disease was induced in pregnant rats by daily intragastric administration of 30 mg. DL-a-methylphenylalanine/kg body weight plus 60 mg/kg body weight at 12 h intervals throughout pregnancy till parturition. Treatment with alpha-methylphenylalanine/phenylalanine resulted in significant decrease of accumulated body weight gain during pregnancy as well as exhibited marked growth retardation of prenatal fetuses and delivered newborn. The growth retarded fetuses was manifested by decreased body weight, malformed both fore -& hind limb, oedematous skin & superficial hematomas widely spreads in different parts of the body. Vibrissae skin hair were characterized by curved free ends as well as reduction of their size and length. Cornification was retarded . The epidermis attained a considerable thinning with delayed differentiation of epidermal cell layers.The growing hair follicles lacked normal characteristic appearance. These results suggested that exposure of the fetus to high plasma concentrations of phenylalanine caused a delay in the biochemical maturation of the fetal rat.

Keywords: Phenylketonuria, Rats, Skin, Gestation, Newborn

1 Introduction

Phenylketonuria (PKU), a disease was first described in 1934 by a Norwegian doctor named Asbjorn Falling (1934), he concluded that the disorder of phenylalanine metabolism was the cause of the children's mental retardation and gave the name Phenylketonuria to this condition. Subsequent examinations of PKU patients excreting phenylpyruvic acid proved that phenylalanine was accumulated in their bodies .In Phenylketonuria, deficiency or malfunction of the liver enzyme phenylalanine hydroxylase(PAH), lead to accumulation phenylalanine in the blood which under normal conditions converts phenylalanine to L-tyrosine (Pietz, 1998). Consequently, individuals with PKU are low in L-tyrosine (Roberts *et al.*, 2001) which may contribute to behavior problems (Tam and Roth, 1997).Phenylalanine is one of the building of protein. However, when the phenylalanine hydroxylase enzyme is absent or deficient, phenylalanine abnormally accumulates in the blood. Phenylalanine during infancy and early childhood produces a variety of problems including mental retardation. Newborn screening can identify the genetic defect and these problems can be greatly reduced by placing the child on a special diet within the first few days of life (Medical Research Council Working Party on PKU 1993). There are two causes of PKU.The first common cause is a genetic defect in the gene for PAH, where most patients suffering from PKU have one or another of several possible mutations in this gene. A secondary cause is a defect in the generation of adequate amounts of the cofactor tetrahydrobiopterin (BH4). Tetrahydrobiopterin is an essential coenzyme not only for the hydroxylation of phenylalanine to tyrosine, but also for the hydroxylation of tyrosine to L-doba required for dopamine biosynthesis and for the hydroxylation of tryptophan to 5-hydroxytryptophan, the substrate for serotonin biosynthesis. This group appears to constitute 3% of all hyperphenylalaninemic patients (Kaufman *et. al*, 1983).

Genetic analysis, using recombinant DNA techniques, has established that the genetic locus for PKU is on

chromosome 12. DNA analysis has shown that the classical form of the disease is due not to deletion of the entire gene for PAH, but is instead due to mutations within the gene's sequence leading to amino acid substitutions; results in an enzyme that does not work properly and therefore the body cannot metabolize phenylalanine (Pietz, 1998). The gene for PAH was cloned in 1982 Robson et al. and by now more than 400 different mutations in the PAH gene have been identified, (Zschocke, 2003).

Excessively high or low levels of phenylalanine may occur during pregnancy, both of which may adversely affect the fetus (Brenton and Lilburn, 1996). Maternal PKU can lead to fetal malformations, including small head size (microcephaly), cardiac abnormalities, intrauterine growth retardation and mental retardation (Levy and Ghavami, 1996; Koch *et al.*, 2000). Adverse effects on the offspring can be reduced by family planning and by careful dietary control both prior to and during pregnancy (Rouse et al., 1997)in addition, low L-tyrosine levels in pregnant women with PKU may contribute to fetal damage (Rohr *et al.*, 1998). Untreated children with persistent severe hyperphenylalaninemia (Elevated plasma Phe concentration; HPA) show impaired brain development. Signs and symptoms include microcephaly, epilepsy, severe mental retardation, and behavior problems. The excretion of excessive phenylalanine and its metabolites can create a mousy body odor, sensitivity to sunlight and skin conditions such as eczema. The associated inhibition of tyrosinase is responsible for decreased skin and hair pigmentation, light skin. Affected individuals also have decreased myelin formation and dopamine, norepinephrine, and serotonin production. Further problems can emerge later in life and include exaggerated deep tendon reflexes and paraplegia or hemiplegia(Williams 1998). The present work aims to illustrate the adverse effect of Phenylketonuria on the skin of developmental stages, 14th and 16th day of gestation and on delivered newborn.

2 Materials and Methods
Experimental animals:
Eighty fertile virgin female and fertile males of albino rats with an average body weight of 100 –110 grams (ratio of 1 male : 3 females) were obtained from Hellwan Animal Breeding Farm, Ministry of Health, Cairo, Egypt and used for experimentation. Rats were housed in cages in the animal House of Department of Zoology, Faculty of Science, Suez Canal University at ratio of four / cage. They were maintained in a temperature of 20 –25 C with 12 h light- 12 h dark cycle and stayed for acclimatization for one week before starting the experiments. They were fed on standard diet composed of 50 % grinding barley, 10% grinding yellow Maize, 20% milk and 10% vegetables was supplied. Water and food were available for consumption throughout the experimental period. Rats were observed daily and only healthy animals were used in these experiments.

Experimental work:
Mating was carried out by housing females' rat with fertile males in separate cages at a ratio of three females with one male for overnight between 8 hour pm. till 8 hour am. The presence of vaginal blugs or the presence of sperms in the vaginal smear determined the zero date of gestation. The pregnant mothers were divided into two main groups, thirty animals per each:

I- **Control**: Thirty pregnant mothers were divided into three subgroups ten animals in each; sacrificed at 14th and 16th day of gestation as well as at parturition.

II- **Experimental Phenylketonuria group**: Each selected pregnant females at the 6th day of gestation was intragastrically administered 30 mg. DL–α-methylphenylalanine/100 g. body weight (to inhibit maternal phenylalanine hydroxylase) plus 60 mg/g body weight L-phenylalanine (to raise fetal plasma phenyalanine) dissolved in milk, at 12 h intervals. The applied dose was selected according to Rech et al., (2002). The thirty pregnant mothers diseased with experimental PKU were divided into three subgroups ten animals in each; sacrificed at 14th and 16th day of gestation as well as at parturition.

Investigated parameters:
1- Percentage of mean body weight gain during pregnancy:
All of the control and experimental rats were weighted during pregnancy till parturition. The percentage of body weight gain was calculated at the 8th, 10th, 12th, 14th, 16th, 18th and 20th days of gestation as follows:

$$\frac{\text{final body weight} - \text{initial body weight}}{\text{initial body weight}} \times 100$$

Statistics were calculated with SPSS for windows version 13.0; the means value obtained in the different groups were compared by paired student's t-test. All results were expressed as mean values ± SE and significance was defined as $p < 0.05$ and highly significant when < 0.01 (Field, 2000).

2- Effects on mothers during utero life as well as at parturition:
The total numbers of prenatal fetuses at 14th and 16th day of gestation as well as delivered newborn for both control and experimental groups were recorded. The incidence rates of growth morphological abnormalities were recorded. Photographs for both control and experimental groups were taken. Dead specimens were discarded. Both fetuses and delivered newborn of both control and experimental groups were fixed immediately after separation in 10% formol saline for 24 hours.

3- Body weight, size and crown-rump length:
The average body weight (g.), size (ml³) and crown-rump length (mm) of prenatal fetuses aging 14 and 16 days as well as newly born of both control and experimental groups were determined and recorded.

4- Light microscopic study of the skin of fetuses at 14 and 16 days and newborn tissue.

At parturition, ten mothers of both control and experimental PKU were sacrificed and rapidly dissected. Skin were removed and immediately fixed in formol saline for 24 hours.To study histogenesis of the skin of 14 , 16 days and newborn of both control and experimental group were separated and immediately fixed in 10% formol saline.The skin specimens washed several times in tap water, dehydrated in ascending grades of ethyl alcohol, cleared in terpineol for two days, then washed in benzene for 10 minutes and embedded in three changes of molten paraplast 58-62 C. Serial 6 μ thick histological sections were cut and stained in haematoxylin and eosine(Drury and Wallington, 1980) examined carefully under bright field light microscopy and photographed.

5- Scanning microscopic study:
Skin of fetuses at 16 days and newborn of both control and experimental PKU mothers at parturition were separated and fixed in 2. 5% glutaraldehyde followed by washing in phosphate buffer and keeping in ethyl alcohol. The specimens dehydrated in ascending grades of ethyl alcohol and dried through carbon dioxide, followed by fixing to a stub with scotch double-stick tap. Finally all the specimens were sputtered coating with gold in a sputter coater and examined under scanning electron microscope.

3 Results

Morphometric observation:

In experimentally induced PKU pregnant mother, the growth of prenatal fetuses aging 14 and 16 days as well as delivered newborn were markedly retarded and not matched with the control. Their body size, weight and crown-rump length exhibited a considerable significant reduction (Table 1, Fig1).Prenatal fetuses at 14 and 16 Aso ,they possessed various pattern of morphological abnormalities including superficial hematomas in both auditory and cephalic region, kyphosis, microcephaly, reduced neck region, kinky tail, cyanosed skin and malformed limb region. The mentioned abnormalities were detected at higher incidence rates in treated offspring's (Tab.2, plate 1).

Light microscopic observations:

In control 14 days' fetuses, the vibrissae skin is differentiated into two main layers; epidermis and dermis. The stratum germinativum is composed of 2: 3 layers of columnar or cuboidal cells. The nuclei occupied almost the size of the cell and possessed one or two nucleoli. The stratum spinosum is formed of 4 layers of cuboidal cells. Many of them showed oval nuclei with one or two nucleoli. The superficial cell layer showed either shrunken or missing nuclei. The superficial cell layer of the epidermis is stratum granulosum and made their first appearance during 14 days fetuses. The hair follicles are formed of 3-4 cell layers (Plate2:A)

In 14 days fetuses of maternally induced PKU mothers, the epidermal thickness appeared comparatively reduced. The epidermis formed of 4: 5 cell layers thick including the stratum germinativum and stratum spinosum. The stratum germinativum was formed of irregularly arranged cuboidal cells with marked pyknotic nuclei. Numerous necrotic patches were detected in between the stratum germinativum disrupting their regular integrity. The stratum granulosum appeared missing. In the dermis, the dermal cells lacked their regular arrangement.(Plate2:B).

In control sixteen days rat fetuses, the epidermis attained more differentiation. Newly formed stratum corneum was added and formed two layers thick. The stratum granulosum exhibited more abundance of keratinocytes. The dermal tissue exhibited densely arranged fibroblast cells. Numerous growing forms of hair follicles emerged from the epidermis and traversed the dermis. (Plate 3: A).

In sixteen days rat fetuses of PKU mothers, the cornification of epidermis was markedly delayed. The epidermis appeared markedly thinner with marked degeneration of different epidermal cell layers. Many terms of deformed hair follicles was detected and lacked differentiation of internal compartments as well as delayed and not matched with the growth of the control (plate3 :B and C).

In control delivered newborn, the stratum germinativum attained marked growth and differentiated into three cell layers. The innermost cell layer is called stratum basal appeared irregular with deep invagination in the dermal layer. The epidermal - dermal junction appeared well developed. The stratum spinosum is formed of 3-4 cell layers with characteristic pattern structures as mentioned in fourteen-day fetuses. The stratum granulosum is composed of 2-3 cell layers of keratohyaline-containing cells. Many keratinocytes appeared regularly distributed in this layer.. (Plate 3: D and E).

In delivered newborn of maternally induced Phenylketonuria (PKU), the epidermal cell layers were markedly reduced. Many of the stratum germinativum and stratum spinosum cells appeared swollen and degenerated. The stratum granulosum showed numerous irregularly shaped keratohyaline granules. Many keratinocytes appeared lysed or degenerated. The cornification layer was comparatively reduced. Many of the growing hair follicles were malformed. They emerged from the stratum germinativum and irregularly distributed in the dermis. The hair follicle regions appeared compact. Numerous degenerative phases were observed in both cortical zone and hair bulb (plate 3 F and G).

Scanning microscopic observations

Scanning electron microscopic of Skin (Vibrissae Skin): preservation in sixteen-day fetuses of control mothers, the vibrissae skin showed numerous folds regularly arranged in rows in the lateral position of mouth opening. The peridermal cells were appeared polygonal and covered the skin surface. At regular intervals of each skin fold, numerous hairs of varying sizes have piercing the conical-shaped outgrowths encircled the base of each hair, the periderm surface exhibited early signs of cornification manifested by the presence of thin sheets of cornified cells spreading over the ingrowing hair follicles as leaflet-structures as well as throughout the skin surface over the peridermal layer, stratum granulosum(plate 4: A and B).

Table 1:Effect of phynylketon urea on body size , body weight and crown rump) of prenatal fetuses at 14and16 day of gestation and delivery newborn of both control and PKU mother

1: body size (mp) of prenatal fetuses at 14and16 day of gestation and delivery newborn of both control and PkU mother

	Prenatal at 14th day of gestation		Prenatal at 16th day of gestation		Delivered Newborn	
	Control	PKU	Control	PKU	Control	PKU
Mean	1.61	0.71	2.56	1.82	5.39	4.24 I
±S.D.	0.12	0.17	0.15	0.17	0.18	0.34 I
±S.E.	.032	.044	.040	.045	.049	.089
T. test		24.96		13.24		10.47
P. Value		.000		.000		.000
Significance		S		S		S

S. D = Standard deviation. P = Probability.S. E. 'Standard error.   S = highly significant (P<O.O1).

2: body weight (gm) of prenatal fetuses at 14and16 day of   gestation and delivery newborn of both control and PkU mother

	Prenatal at 14th day of gestation		Prenatal at 16th day of gestation		Delivered Newborn	
	Control	PKU	Control	PKU	Control	PKU
Mean	1.71	0.74	2.67	1.74	5.73	4.07
± s. D.	0.09	0.10	0.11	0.23	031	0.59
± S. E.	0.02	0.02	0.02	0.06	0.08	0.15
T. Test		24.38		15.58		10.51
P. Value		.000		.000		.000
Significance		S		S		S

S. D = Standard deviation. P = Probability. S. E. = Standard error. S = highly significant (p<O.OI).

3: Crown rump length (cm)of prenatal fetuses at 14and16 day of   gestation and delivery newborn of both control and PkU mother

	Prenatal at 14th day of gestation		Prenatal at 16th day of gestation		Delivered Newborn	
	Control	PKU	Control	PKU	Control	PKU
Mean	3.43	2.35	3.94	3.30	5.60	3.98
±S.D.	0.07	0.27	0.09	0.16	0.10	0.15
±S. E.	0.01	0.07	0.02	0.04	0.02	0.04
T. Test		14.03		15.02		21.52
P. Value		.000		.000		.000
Significance		S		S		S

S. D = Standard deviation.   S. E. = Standard .   P = Probability error.   S = Highly significant (p<O. 0 I).

Table (2): abnormalities of prenatal fetuses at 1 day postnatal

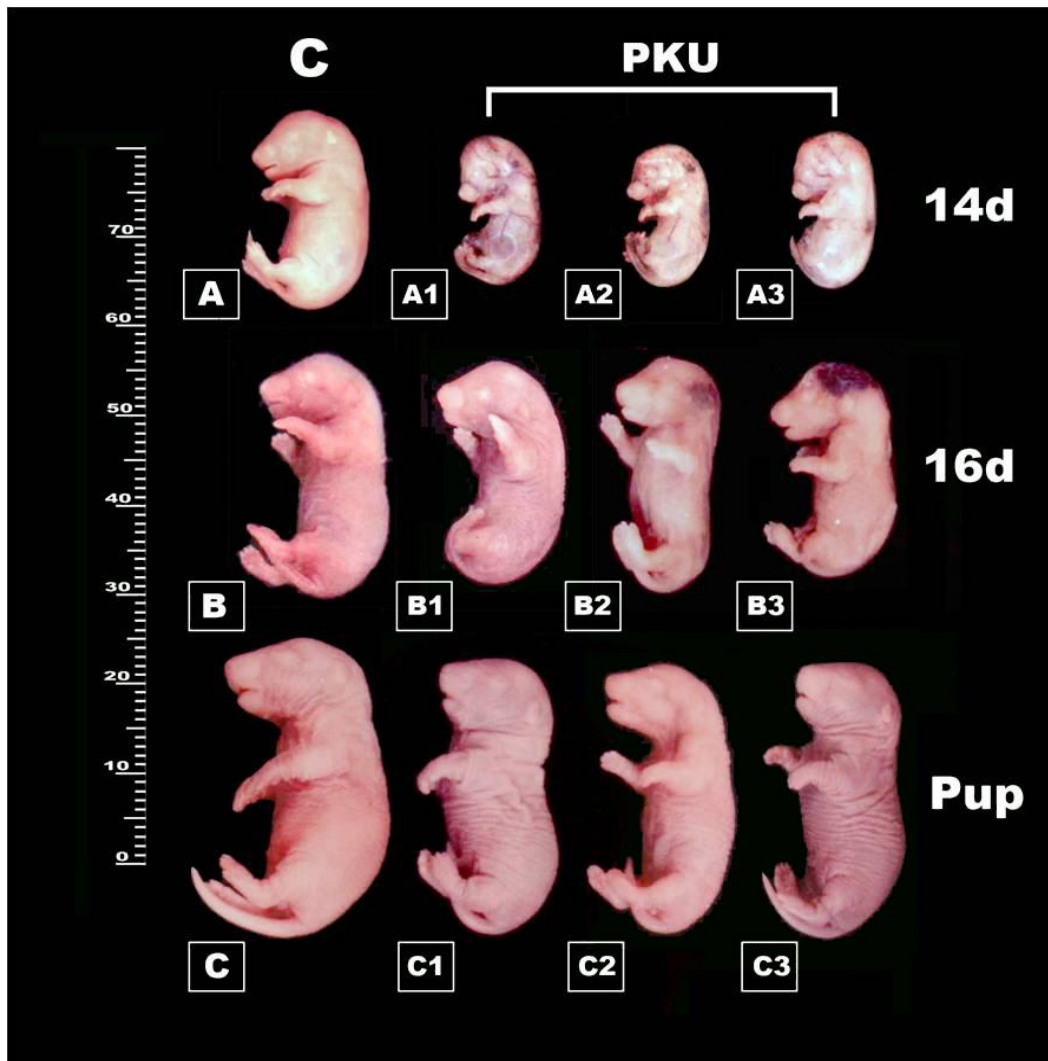| Abnormalities	1 day postnatal PKU
Kyphotic body	15 (48.38 %)
Microcephaly	6 (19.35 %)
Reduced neck	8 (25.80 %)
Kinky tail	7 (22.58%)
Abnormal skin appearance	9 (29.03 %)
General edema	6(19.35 %)
Abnormal fore limb	1 day postnatal PKU
Unilateral	5 (16.12 %)
Bilateral	6(19.35 %)
Abnormal hind limb	1 day postnatal PKU
Unilateral	1 (3.33 %)
Bilateral	3(9.67%)

Plate 1: Lateral view photomacrograph of gross morphology of prenatal fetuses at 14,16,and 1 day postnatal PKU . C – control fetuses   PKU – treated fetuses .
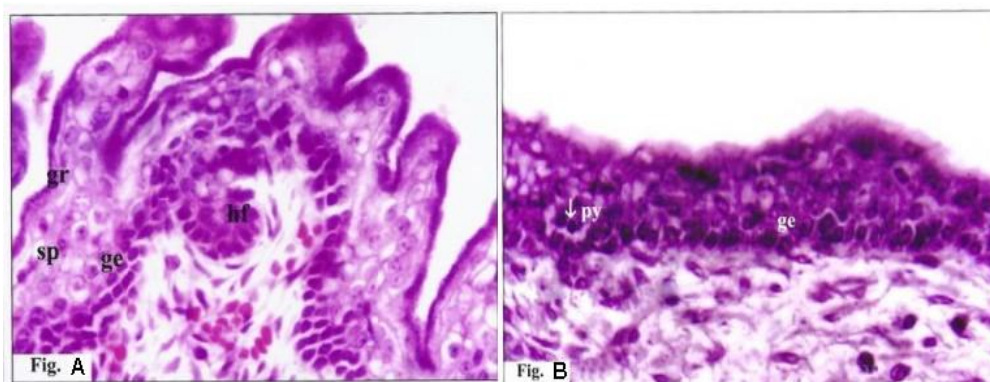

Plate 2   Photomicrograph of vertical histological section of vibrissae skin region of 14-days fetuses
Fig. A- Control   showing normal pattern of the epidermal layers; stratum germinativum (ge), stratum spinosum (sp), stratum granulosum (gr) and normal growing hair follicles (hf).   (x40) .
Fig. B experimentally induced PKU mother showing reduced folding and thickness of epidermis. The stratum germinativum (ge) was formed of irregularly arranged cuboidal cells with marked pyknotic nuclei (py). The stratum granulosum appeared missing. In the dermis, the dermal cells lacked their regular arrangement. (x400).
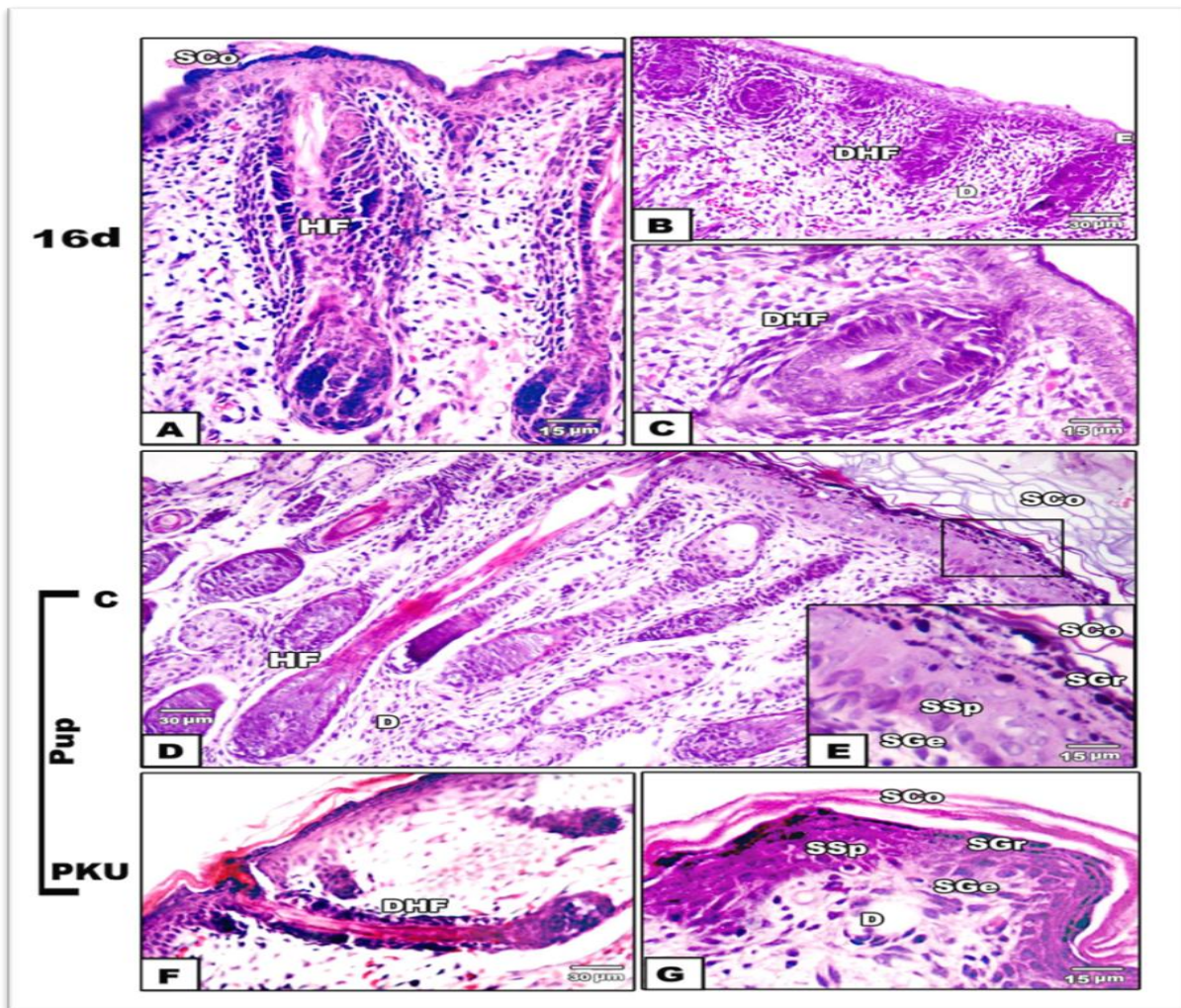
Plate 3: Photomicrograph of vertical histological section of vibrissae skin region of 16-days and offspring rat fetus

A: control 16-days rat fetus showing normal pattern of the epidermal layers which attained more differentiation. Newly formed stratum corneum (SCo) was added. Numerous growing forms of hair follicles (HF) emerged from the epidermis and traversed the dermis.   (x 400).

B- C: 16-days rat fetus of experimentally induced PKU mother showing reduced folding and thickness of epidermis with marked degeneration of different epidermal cell layers, the cornification of epidermis was markedly delayed. Ln the dermis, many forms of deformed hair follicles (DHF)were detected and lacked differentiation of internal compartments.   (x400).

D- E: control offspring showing column pattern organization of the epidermal layers; stratum germinativum (SGe), stratum spinosum (sp), stratum granulosum (SGr) and stratum corneum (SCo). Different forms of growing hair follicles (HF) emerged from the epidermis and traversed the dermis.   [D(x160) and E(x250)]

F and G: offspring of experimentally induced PKU mother showing retarded differentiation of epidermal cell layers, many of the stratum germinativum (SGeF) and stratum spinosum cells (sp) appeared swollen and degenerated. The cornification layer (co) was reduced. Malformed growing hair follicle (hf) with observed numerous degeneration in both cortical zone and hair bulb   [F(x200) and G(x400)].
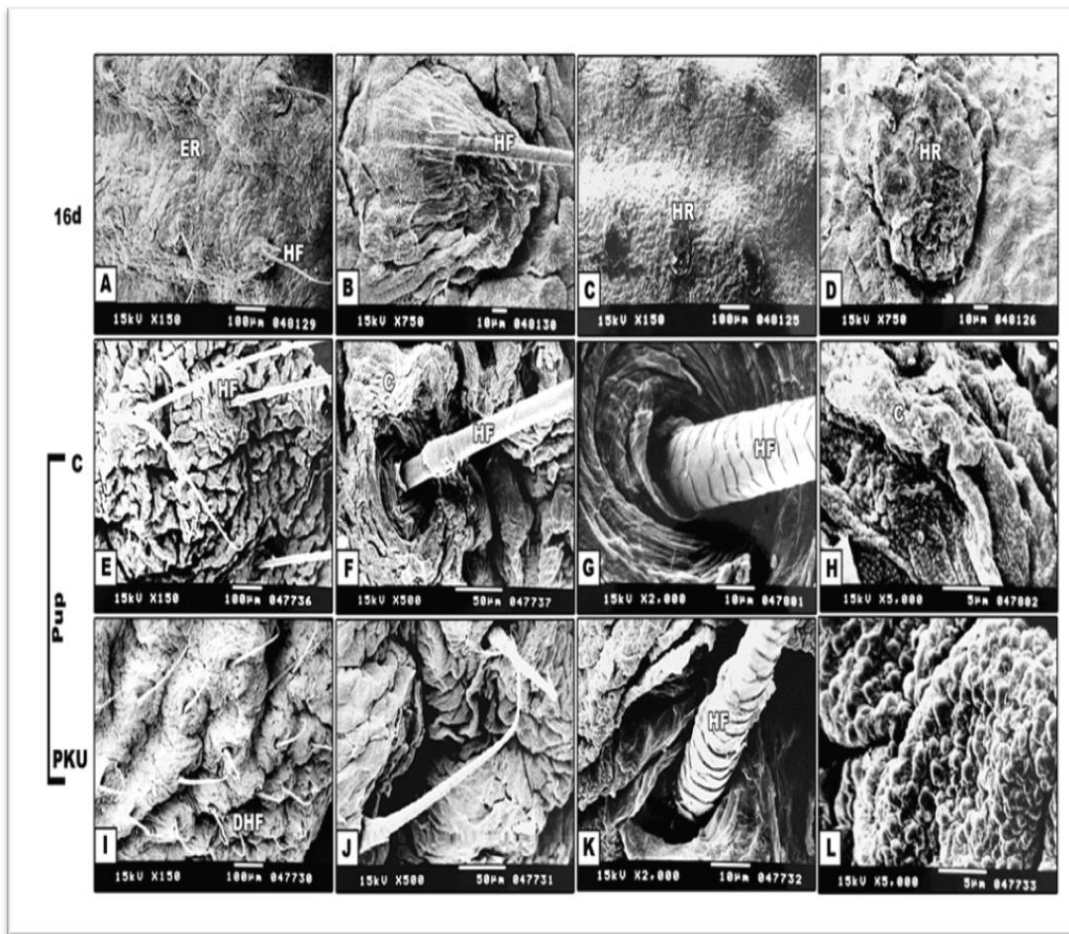
Plate 4: Scanning electron micrograph of vibrissae skin surface region

A-B: control 16-days rat fetus showing, numerous folds regulafly arranged in rows. The peridermal cells (pc) were appeared polygonal and covered the skin surface. At regular intervals of each skin fold, numerous hairs (ha) of varying sizes have piercing the conical-shaped outgrowths encircled the base of each hair. The periderm surface exhibited early signs of comification manifested by the presence of thin sheets of comified cells spreading over the in growing hair follicles. (AX 150 - BX 750).

C -D: 16-days rat fetus of experimentally induced PKU mother showing regular arrangement of skin folds but the peridermal cells lacked normal characteristic pattern structures with sign of disruption and degeneration. At regular intervals, primordial hair follicle (ht) structures appeared as deformed cone-shaped structures with flattened surface. The peridermal cell surface (pc) showed marked degeneration with sign of disappearance of cornification. C X 150 – D X 750).

E–F–G-H: control offspring showing, much more proceeded wrinkles of the vibrissae skin, numerous cylindrical-shaped hairs (ha) with thickened base and tapered ends. Each hair appeared covered with flattened cell layers overlapping on each other. Comification of the skin is highly developed. Numerous sheets of comified peripheral layers appeared. shedding from the surface as well as aligned near the base of the hair canal.   E X 150- F X 500- G X 2000-H X 5000).

I-J–k-l: offspring of experimentally induced PKU mother showing, varying degrees of deformity of the hair shafts (ha) manifested by reduction of their size, length and curved free ends, the base of the hair appeared loosely attached to the peridermal layer and comification less developed.   (IX 150 , J X 500 , K X 2000 and L X 5000).

In sixteen-days fetuses of experimentally induced PKU mothers: the peridermal cells lacked normal characteristic pattern structures with sign of disruption and degeneration. At regular intervals, primordial hair follicle structures appeared as deformed cone-shaped structures with flattened surface. Many of the peridermal cells were markedly degenerated with sign of disappearance of cornification (plate 4: C and D). In delivered newborn control rat, wrinkles of the vibrissae skin are much more preceded. Numerous hairs of varying sizes are observed over the skin surface. Each hair appeared as cylindrical-shaped structures with thickened base and tapered ends. Each hair appeared covered with flattened cell layers overlapping on each other. Cornification of the skin is highly developed. Numerous sheets of cornified peripheral layers appeared shedding from the surface as well as aligned near the base of the hair canal (plate 4:E, F ,G and H).

In delivered newborn rat fetuses of experimentally induced PKU mothers, the hair shafts showed varying degrees of deformity manifested by reduction of their size; length and less strengthen curved free ends in comparison with the control. The base of the hair appeared loosely attached to the peridermal layers and cornification was less developed (plate 4:I ,J ,K and L).

4 Discussion

Phenylketonuria (PKU) is an inherited metabolic disease, carried through a "recessive" gene. Phenylalanine hydroxylase deficiency is caused by mutations in the PAH gene resulting in a primary deficiency of the liver enzyme phenylalanine hydroxylase (PAH), (Jervis et al., 1940; Jervis, 1953; Scriver and Kaufman, 2001), and consequently interrupt the conversion of amino acid phenylalanine to another amino acid, tyrosine that results in excessive accumulation of the amino acid phenylalanine and reduced levels of the amino acid L-tyrosine in the blood (Diamond, 1996). Thus phenylalanine accumulates in the blood to concentration sufficiently high to activate an alternatively pathway of degeneration (Wurtman and Wurtman, 1979; Mission- Tsagaraki et al., 1988; Gazit *et al*., 2003).

Attempts to reveal the adverse effects of PKU the growth of optic region during prenatal life at 14 days of gestation and at parturition. understand the underlying cause for the abnormalities associated with maternal PKU have led to the use of animal model in which the histopathological investigations were encountered to clarify the disease pattern, exposure to the mother's metabolic abnormalities affects the fetus during the entire pregnancy. The abnormalities produced by the PKU mothers are not genetic but Intrauterine Environmental." Our results and those of others confirm this hypothesis.Jervis 1939 stated that the damage to the children of PKU mothers is not a genetic consequence (although the children will be at least heterozygous, as PKU is an autosomal recessive disorder). This can be proven by the fact that PKU fathers produce normal offspring (Fisch *et. al.* 1991) and also that well controlled pregnant PKU mothers can produce children without obvious handicap.Above symptoms result from high Phe concentrations in the blood of the fetus. It is a consequence of intrauterine Phe excess resulting from the positive transplacental Phe gradient. To keep fetal blood Phe below 500 µmol/l maternal concentration should be below 300 µmol/l (Gardiner, 1990 and Schoonheyt, *et al.* 1994).

The present findings revealed that the accumulated body weight gain of PKU mothers during gestation was markedly retarded reached the highest peak of depleted body weight gain at 20 day of gestation comparing with the control, similar findings had been achieved in PKU Dutch patients (Verkerk *et al*., 1994) and on experimentally induced animal (Gadallahet *et al.* 2004).the marked loss of maternal body weight gain during pregnancy may be related to the disturbance of phenylalanine metabolism and reflected on massive loss of weight during prenatal growth and at parturition.The present findings supported the work of Zaffanello *et al*., (2003) on offspring's among offspring of untreated PKU mothers, on the other hand, the growth of fetuses and delivered newborn of PKU mother was markedly retarded assessed by a significant decrease of body size, weight and crown-rump length.In addition , The present findings supported the work of Buh rd el *et al.* (1997) on infants of untreated, PKU patients.

Moreover , The present findings supported the work of Saugstad (1972), Levy and Waisbren (1983), Spero and Yu (1983), Cho and McDonald (2001) whom reported a marked loss of body weight of girls and boys of women with untreated PKU.In accordance with Gadallah (2004) and MacDonald, Lilburn *et al.* (2004), both PKU patient and experimentally induced PKU animals failed to consume their protein substitute as a result of impairing conversion of phenylalanine to tyrosine impairing protein synthesis.

Meamwhile , When glutathione is decreased, cells enter a low-antioxidant mode and oxidative damage caused by free radicals (Halliwell and Chirico (1993). Animal studies carried out by Castillo *et al.,* (1988) revealed that induced experimental hyper phenyl alaninemia in 5-day old chick embryo by dietary treatment with phenylalanine and a-methyl phenylalanine led to a disturbance of phenylalanine l- tyrosine metabolism which interfered with the depletion of body weight, Similar pattern of growth malformations were reported by Lee *et al*., (2005) and Fisch and Stassart (2004), Matalon *et al*., (2003) in neonatal of PKU mother . Both syndromes possesses similar pathological manifestations concerning transplacental passage of both Phe and alcohol penetrating to fetus such as Phenylpyruvate - a byproduct of Phe metabolism and acetaldehyde - a byproduct of alcohol metabolism which are potent inhibitors of pyruvate dehydrogenase .   It is known that phenylalanine and tyrosine compete for the same transporter across the blood barrier. The phenylalanine had more affinity for transporting more than tyrosine leading to increase phenylalanine level as well as

their metabolites and consequently disturbed the metabolism (Aragon *et al*.,1982, Krause. *et al*., 1986). the fetal and neonatal growth defects of fetuses and newborn may be attributed to the marked decline of hepatic phenylalanine hydroxylase activity within 12 h of inducing hyperphenylalaninemia in developing mice reached to a decrease of 65- 70% of the enzyme activity (Binek *et al*., 1981) or to complete inhibition of the enzyme activity during intrauterine life (McGee *et al*., 1972) .

From the present work on the Skin of fetuses and newborn: At the light microscopic level, the epidermis attained a considerable thinning with delayed differentiation of epidermal cell layers as well as impairing the development of stratum granulosum in 14 days fetuses of maternally PKU mothers. There were a widespread of malformed differentiation of both dermal tissue and hair follicles. Retarding differentiation of skin was proceeded in delivered newborn and restricted mainly in reducing the stratum comeum. Many of the keratinocytes appeared lysed or degenerated. The growing hair follicles lacked normal characteristic appearance with peculiar degeneration of their cell layers.

The present findings supported the work of Bechelli *et al*. (1978) whom observed marked reduction of keratinocytes in skin of patients, Keratinocytes represent the main elementary structure of the stratum granulosum which is responsible for the formation of both soft and hard keratin of the stratum corneum and imparment formation or degeneration was clearly responsible for retarding cornification the effects of PKU may be attributed to the increased intracellular concentration of phenylalanine in the skin of PKU patients (Fisch *et al,* 1981) induce oxidative stress impairing the differentiation of both epidermal and dermal cell layers, similar, At scanning electron microscopic observations of fourteen-days fetuses and delivered newborn of PKU mothers revealed marked disruption and deformations of peridermal cell layers especially at 14 days fetuses where the primordial hair follicle structures appeared necrotic with abnormal flattened surface. There were no signs of cornification. In delivered newborn, Although advancement of skin development and differentiation proceeds, the hair shaft possessed abnormal characteristic appearance characterized by curved free ends as well as reduction of their size and length . The base of the hair loosely attached from the peridermal layers cornification was retarded.

Findings of skin diseases were reported by many authors after subjecting to PKU disease, Reticulosarcoma-like skin lesions were found to describe in a boy with Phenylketonuria. Association of the lesions with PKU was indicated by their dependence on the severity of the latter and their complete healing during treatment with low phenylalanine diet (Exss and Weber, 1975).Bechelli *et al* (1978) observed widespread of skin pigmentation in 3 cases of PKU Following ultrastructural study. The epidermal basal layer showed higher proportion of melanocytes. Keratinocytes contained a lower proportion of melanin. The author attributed the disturbance of skin disorder to the higher blood level of Phe and to the higher proportion of non function melanocytes that do not produce the melanosomes, and lower percentage of keratinocytes with melanin.

Belloso and Lowitt (2003) reported that a man with Phenylketonuria who was reported to have lichen sclerosus et atrophicus at age 16 years with large confluent areas of atrophy. Although sclerodermatous changes have been described in children with Phenylketonuria, this case offers a longitudinal view of the progression of skin lesions in a middle-aged man with Phenylketonuria, Finally, the present study led to the conclusion that adequate nutrition is very important to pregnant women with PKU, to be controlled, the pregnant need careful supply of adequate diet for maintaining fetal growth and to restrict phenylalanine supplementation throughout pregnancy to avoid PKU, effects and protect the growing fetus. Women with PKU need early identification, education, regard the importance of dietary compliance, careful monitoring, and emotional support to increase the better outcomes.

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