Overview and Integration of Cellular Metabolism

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Lecture 36: Metabolism of Phenylalanine and Associated Disorders

Hello everyone, we are now at a very important part of our lecture season over which we will be discussing the metabolism of an integration of cellular metabolism where we will be starting with metabolism of individual amino acids. Today's class we will be discussing metabolism of phenylalanine along with its associated disorders alright. So, we will be mainly discussing regarding the whole metabolism of phenylalanine, we will be focusing on the disease that is characterized by disorder of phenylalanine metabolism that is phenylketonuria, we will be discussing its etiology, the various variations of phenylketonuria, how it is inherited, what is the incidence of phenylketonuria, the various clinical features, diagnosis and treatment will be covering it all alright. So, here is a chart about all the essential and non-essential as well as conditionally essential amino acids, this is the total 20 in number alright. And among them we have chosen to start with this amino acid phenylalanine alright. Do you have any idea why you will get the answer very soon, but today's class we will be discussing phenylalanine metabolism.

Mind it this chart will be essential for you to consider all the essential, conditionally essential as well as non-essential amino acid for multiple choice question purposes. So, it is very important you at least know few example of essential, non-essential as well as conditionally essential amino acids ok. So, phenylalanine this is the structure of phenylalanine alright. We all know whenever we are discussing an amino acid there is a carbon skeleton right, there is a carbon central carbon atom and there is a COOH group attached to it, there is an amino group, there is an hydrogen and this is the part that varies which isolates each amino acid from another.

So, in case of phenylalanine it is a benzene ring along with a CH2 group that is attached to this carbon atom right. Remember the simplest amino acid was is glycine, but this is simply an hydrogen molecule. So, this is a aromatic amino acid alright because this is a presence of an aromatic benzene ring. Again essential amino acid means there is no synthesizing phenylalanine in our body, we must have phenylalanine from diet. Mind it whenever we are discussing an essential amino acid you do not need to read about

synthesis because they are not synthesized in our body.

Whenever we are reading a non essential amino acid synthesis of that amino acid is must right for your exam purposes. The metabolic fate this being both glucogenic as well as ketogenic, when we will read phenylalanine metabolism we will complete the degradation path of phenylalanine we will see that is converted to acetoacetyl coenzyme A as well as fumarate. This is the ketogenic part and this is the glucogenic part alright. However, the beauty of it is phenylalanine is first converted to an another amino acid that is tyrosine. Since phenylalanine is converted to tyrosine in our body of course, you have known you can sense it by now the tyrosine is not an essential amino acid.

Means if we have got enough amount of phenylalanine diet tyrosine will automatically be synthesized. And the reverse is also true means the amount of phenylalanine required in diet will be reduced if there is adequate amount of tyrosine in diet. This is known as pairing action of tyrosine on phenylalanine means if we have got adequate tyrosine in diet the amount of phenylalanine required is less and this is very important alright. So, as I told you predominant metabolism of phenylalanine occurs through tyrosine means first phenylalanine is converted to tyrosine and thereafter tyrosine is degraded alright. And not only it is degraded tyrosine from tyrosine multiple very important compounds are obtained in our body.

To name a few catecholamines that is epinephrine, norepinephrine, adrenaline, noradrenaline neurotransmitters like dopamine, thyroid hormones, the pigment melanin all are important products that are derived from tyrosine. So, indirectly they are derived from phenylalanine also right. So, when this is our metabolic goal right mind it whenever we complete any amino acid metabolism lecture series in this session we will be correlating our progress with this figure. In today this is where our goal lies phenylalanine it is converted to acetacetylcholine and here it is phenylalanine it is converted to fumarate alright mind it tyrosine also goes hand in hand with phenylalanine, but and this is the total metabolism of phenylalanine and tyrosine basically it is the same. The whole metabolism of phenylalanine tyrosine only varies in the first extra step where phenylalanine is converted to tyrosine.

And in today's class we will be focusing on tyrosine only on this step right. It is so our goal is clear phenylalanine is getting converted to tyrosine if there is a problem there will be disorder. So, this will be our today's discussion right. So, let us elaborate this single step in detail, but do not worry the entire step will be covered in future classes when tyrosine metabolism will be discussed alright. So, how exactly what is this reaction if we look into this reaction detail this is simply a hydroxylation.

Tyrosine is nothing, but para hydroxy phenylalanine hydrox an OH group is attached to the para position from your aromatic I mean organic chemistry knowledge you already know what is ortho what is methane what is para position right I am not going to detail. So, phenylalanine is hydroxylate the para position by an atom of oxygen to produce OH. So, this is already H is there right. So, an O atom will get inside right and what happens to another atom of the oxygen it is reduced to water. So, oxygen gets in and water comes out alright, but once initially it was thought that oxygen is simply going inside right.

In reality what happens the hydrogen atom that is shaded in pink is transferred directly from C 3 to C 4 ok by carbon levelling it was shown by the National Institute of Health and Hence this shift is known as NIR shift the hydrogen which is originally over here actually shifts over here ok. And one hydrogen from what from the adjacent site actually goes to the fourth position anyway. So, that is not our detail organic chemistry we do not need to go into that much detail what we need to know the enzyme that is causing is known as phenylalanine hydroxylase and that enzyme requires a coenzyme right which is biopterin. Biopterin in the form of tetrahydrobiopterin this is very much similar in structure to folic acid ok the it is a vitamin folic acid is a vitamin and this tetrahydrobiopterin is very much similar in structure to folic acid. So, this is the enzyme phenylalanine hydroxylase and this is the coenzyme tetrahydrobiopterin right.

What happens when the reaction actually happens the active form the of tetrahydrobiopterin it is oxidized to dihydrobiopterin which is basically in quinoid form. Thereafter dihydrobiopterin is acted upon by reductase and the tetrahydrobiopterin is regenerated ok. So, you can see over here is it is oxidized and then it is again reduced right and this phenylalanine hydroxylase enzyme is present in liver. Well there is also another intermediate if we want to really want to learn in detail this is the actual form where there is a carbenolamine derivative that is also formed right. So, in reality in order to regenerate the tetrahydrobiopterin two enzymes are playing role one is carbenolamine dehydratase and one is dihydroteridine reductase right.

Mind it the name of the enzyme is dihydroteridine is dihydroteridine reductase often termed as dihydro biopterin reductase it goes hand in hand more or less the same thing right. So, phenylalanine hydroxylase is the enzyme tetrahydrobiopterin is the coenzyme this is the active form it gets reduced and ultimately it again gets regenerated and these are the intermediary enzymes that are all acting together to catalyze this one single step of conversion of phenylalanine to tyrosine all right. So, now whenever we are discussing so, our one step that is goal of today is done. So, what we will be discussing now on is what if this step is hampered all right. So, in general whenever we are dealing with any inborn error of metabolism whenever there is loss in enzyme due to either deficiency or loss in genetic mutation whatever be the reason what happens generally enzyme reacts

on substrates in the presence of cofactor in forms product.

Whenever enzyme or in some case even the coenzymes are having a problem this step will not be happening which will lead to excess accumulation of substrate and naturally there will be depletion of the product. This is a phenomena of every enzyme deficiency all right and we have already read a few regarding ammonia metabolism right. So, enzyme deficiency of ureocycle the same concept holds there also any enzyme defect the substrate accumulated the product deficient right. So, the disease that happens when this enzyme phenylalanine hydroxylase is deficient is known as phenylketonuria which is also abbreviated as PKU ok. So, this is the most common disease of amino acid metabolism and that is the reason why we are studying why this course has been designed in such a way.

So, that you need to know the worst disease at first this is the most common amino acid metabolisms. Hence we have chosen phenylalanine metabolism to start with now you know the answer. This deficiency to phenylalanine hydroxylase enzyme multiple choice question where what is the gene that codes for this enzyme PAH gene located in the long arm of chromosome 2012 and locus 22 another MCQ question very important you should note it down in your separate notebook if you are if you do have a habit of making your own notes. Inheritance via autosomal recessive mode and what is the result the substrate will accumulate phenylalanine cannot be converted tyrosine phenylalaninemia hyper phenylalaninemia alright. So, when phenylalanine hydroxylase is deficient phenylalanine will be accumulated there is no problem in understanding this, but even if phenylalanine hydroxylase is fine, but somehow the coenzyme is having a problem the level of coenzyme is not functioning or this not present in active form then also this reaction will not happen and phenylalanine will be accumulated alright.

I hope there is no problem in understanding that anywhere problem in either enzyme or coenzyme will lead to disease process and we have already read that these are the intermediary enzyme that actually help in regeneration of active form of tetrahydrobiopterin right. So, this is dihydroterridin there is also another enzyme if this dihydroterridin or dihydrobiopterin synthetase DHBS even if that enzyme is deficient that enzyme is deficient it indirectly leads to accumulation of phenylalanine because all these three enzymes if deficient this leads to depletion of coenzyme and hence this reaction cannot proceed right. So, based on all of these etiologies the phenyl ketonuria can be classified into classical phenyl ketonuria which is the most common most variant variety and it is characterized by deficiency of the enzyme phenylalanine hydroxylase. However, there are atypical causes of hyper phenylalaninemia right due to deficiency of the coenzyme that is tetrahydrobiopterin and these are the intermediary enzymes any ones deficiency may lead to a atypical hyper phenylalaninemia. Mind it whenever we are

referring to phenyl ketonuria for all practical purpose it refers to the deficiency of phenylalanine hydroxylase and classical phenyl ketonuria these are all atypical and often these are not termed as phenyl ketonuria ok.

But nevertheless these are all varial ketonuria variants they are atypical they are they cause hyper phenylalaninemia. So, regarding the altered metabolism of phenyl ketonuria now we are all in the same page because either if the enzyme is deficient or if the coenzyme is deficient phenylalanine will be accumulated and there will be no production of the product with that is tyrosine I hope you are fine with this right. So, in absence of phenylalanine or at the either the enzyme or the coenzyme cannot be converted to tyrosine. So, what is the problem? In the beginning I told you it is from tyrosine where multiple important products are formed. So, one by one we will be seeing what are the we will be naming here what are the products that are formed and that will be a problem in case of phenyl ketonuria because the downstream metabolism of tyrosine is fully blocked the downstream synthesis of important product from tyrosine is blocked and one of them is melanin.

What melanin does? Melanin helps to impart a colour blackish or brownish or reddish colour to our skin it protects us from sun ok. The harmful UV rays if melanin is deficient that will lead to conditions like fair skin, light skin, albinism, vitiligo there are multiple such disorders which will be discussed in detail when we are discussing melanin synthesis from tyrosine. But for now the common sense as you can rightly see tyrosine is not formed hence melanin level is much less that leads to light skin in phenyl ketonuria patient. Moreover the enzyme the rate limiting enzyme one of the most important enzyme in melanin synthesis tyrosinase alright. Tyrosinase is inhibited whenever phenylalanine concentration is high.

So, all in all melanin synthesis hampered and that leads to light skin in case of patients of phenyl ketonuria. Not only melanin what happens from tyrosine? Synthesis of catecholamine happens. So, if catecholamine means adrenaline not adrenaline they are very important neurotransmitters ok the very important mediators of multiple reaction. Not only that if the coenzyme is at fault it the coenzyme tetrahydrobiopterin is also involved in tryptophan metabolism. Again it will be taught to in detail where tryptophan gives rise to a very important neurotransmitter that is serotonin.

So, you see either the enzyme deficiency whenever tyrosine is not formed or even if there is deficiency in the coenzyme tetrahydrobiopterin it will lead to a situation where important neurotransmitters like catecholamine, serotonin are absent are produced in much less than required in our body and which will lead to problems right. Next see phenylalanine what happens if it is if it cannot be converted to tyrosine right. So,

naturally phenylalanine level is very high ok in blood these are essential amino acid. So, brain has got and they have got important requirement in brain for development in normal cases. So, phenylalanine what it does it is a large neutral amino acid and it is normally transported into the brain through the blood brain barrier with the help of large neutral amino acid transporter ok.

So, which it is fine, but if phenylalanine is excess in blood it will saturate the transporter. So, whatever transporter is there all will be attached to phenylalanine because it is much excess all will be binding to that ligand. And what will happen other neutral amino acid other long neutral amino acid cannot get inside the brain. Those long neutral amino acid are extremely necessary for proteins and other neurotransmitters synthesis in brain and that will be hampered if excess phenylalanine is present in blood. Which ultimately results in hampering of brain development specially in the growing period because mind it if all these deficiencies are congenital right these are all in born error of metabolism.

So, when there is an increased requirement of essential amino acid from brain development those will not be found and hence hampering in proper brain development will lead to intellectual disabilities right. So, this is one thing. So, phenylalanine is not getting converted to tyrosine. So, what is actually happening? Phenylalanine will always search to be metabolized in some way once something is being accumulated. So, in our body phenylalanine is mediated through alternate routes these are conversion to phenyl pyruvate, phenyl acetate and phenyl lactate.

So, be it transamination be it decarboxylation ultimately it leads to conversion of phenylalanine because it is present in very high into unwanted compounds which are normally produced in very less or negligible amount, but in this case in case of phenylketonuria these are produced in excess and all of these have got characteristic properties and this are also active agents if accumulated they also have been found to be culprit in brain damage right. So, with all these altered metabolisms if we now dissect the symptoms of phenylketonuria it will be very clear for you to guess all right. So, first CNS symptoms central nervous system symptoms mental retardation hyperactivity jerky movement failure to walk talk acidity abnormal movement like acid apoptosis seizure why because there is a problem in neurotransmitter essential amino acid etcetera ok. So, all of that leads to poor brain development intellectual disability. So, all of them culminates into CNS symptoms which is the major dreaded sequence of phenylketonuria if it is left undiagnosed ok.

Next hypopigmentation we already discussed because of decreased amount of melanin and mousy musty odor of phenylketonuria patient the urine the secretion the body fluids

have got a typical odor musty odor or a mousy odor ok that is actually found from surface of rodents why that is found because of presence of excess phenyl lactate phenyl acetate and phenyl pyruvate those alternative compounds that are produced in minor pathways from excess phenylalanine gets secreted into the body fluids breath skin and urine that leads to a mousy smell right. So, how this phenylketonuria is inherited? Phenylketonuria now we are discussing about the heredity as I have already discussed this mutation in PA gene is an autosomal recessive condition means both parent can be. So, the gene needs to be homozygous in order to express right. So, both copies need to be homozygous. So, if there is only single copy a man or woman will be a carrier.

So, carrier dad and mom if they conceive you know these are simple Mendel's law one of them that is 25 percent of each pregnancy will be affected if both are carriers all right and one of the babies will be non-carrier all right and 50 percent will be carriers this simple Mendelian genetics ok. So, how can we diagnose phenylketonuria? This diagnosis of PK is extremely important because if we cannot if we fail to diagnose there will be dreaded consequence of brain damage and intellectual disability which nobody should deserve right. So, naturally prenatal diagnosis even before the baby is born we can diagnose phenylketonuria prenatally how we will have an idea that the baby can have if the there is history of carry if the maternal or any of the parent is a carrier preferably both are carriers then the situation is almost dreaded it can happen right. So, prenatal diagnosis in fetus by detection of gene mutation pH gene mutation if it is mutated then there is a chance that the baby or there is a 100 percent chance that the baby will be phenylketonuria, but mind it as I always discussed previously a mutation may lead to total or partial loss of the enzyme. So, the how the symptom happens is varies from individual to individual.

Next after the baby is born neonatal diagnosis how it is done by assessing blood phenylalanine level normally phenylalanine level is about 1 mg per dl it is very low, but in case of phenylketonuria it is above 30 mg per dl it has to be above 30 mg per dl, but in milder cases it has it has also been found from 6 as low as 6 I mean cases of phenylketonuria with pH gene mutation 6 mg per dl 6 to 30 those are milder condition, but generally if there is a defect it is usually found to be above 30 mg per dl. So, it is a huge difference between the normal level and the raised level. So, once the level is raised there is nosecond thought in detection or diagnosing that this is the case of phenylketonuria. So, how a sample is collected in those small babies because these are all in border of metabolism right a neonate small babies are the patients from which samples need to be collected. So, basically a few drops of blood is obtained by heel prick all right and these are soaked on cards the all I mean customized cards are available which can actually diagnose or collect we can either collect blood in blotting paper or cards are available in which the bloods are soaked and then these cards are actually sent

to the lab for a newborn screening.

Because generally there have been cases where the symptoms overlap and once single mutation has been found multiple mutation are all can also in found. So, a child may be affected with multiple amino acid in border of metabolism. So, in general there is a newborn screening is done where one by one all the amino acid deficiencies or all the amino acid enzyme deficiencies are checked. So, this is the way how sample is collected I mean blood sample is collected by heel prick card soaked on card and sent for easy identification and early identification and implementation of prompt implementation of treatment right. So, what is the method of choice? So, nowadays all in border of metabolism if you get the MCQ this option you should always stick it that is tandem mass spectrometry or MS MS.

Any intermediate substrate or product deficiency or metabolite needs to be diagnosed in this case we are trying to diagnose the phenylalanine NPTEL is done by MS MS or tandem mass spectrometry. This is the this is the schematic diagram of mass spectrometry and this is beyond the scope of this class to discuss how mass spectrometry happens, but you can always enroll into other NPTEL biophysics classes to get your concepts clear about mass spectrometry all right. So, now we discuss about some test that are very convenient now to perform, but more nowadays we are not using them. So, these in near future will have historical importance, but nevertheless these are very important to know. So, what is Guthrie test right this was one of the earliest test that was devised for phenylketonate can be done in simple laboratory setting.

So, what happens blood the sample is placed is plated on an agar plate all right agar is a culture media and it contains a special type of bacteria that needs phenylalanine that is essential amino acid to grow. So, normally the media is devised in such a way that there is no phenylalanine or very less phenylalanine for the bacteria to survive and grow, but if the patients blood have got I mean if the patient is of phenylketonuria his blood level of phenylalanine will be so high that the bacteria will find it very conducive to grow. So, if there is bacterial growth phenylketonuria is positive. So, basically let us see what is happening in a figure. So, this is the culture plate where there are positive blood.

So, all the four types are usually placed. So, blood is placed in samples in dot fashion. So, you can see there are positive controls where increasing phenylalanine concentration are provided to see how much the bacteria is growing. So, more phenylalanine means more bacteria will grow and there will be a hallow around the spot the haze it signifies bacterial growth and if there is no negative I mean the negative control means there is no phenylalanine. So, we have got something to compare to right. In case of the sample you see over here it is showing a hallow which can be compared to the positive control right.

So, this case this is a patient where this patient has shown a positive phenylketonuria mind it these are all samples for individual patients right. So, in this case all of them have showed negative growth, but this has been showed a positive growth and we can surely say that the phenylalanine level is very high and the case is strongly suspected to be a phenylketonuria all right. Next there is another inexpensive test that is ferric chloride test it is generally done in urine. So, what it does how the test is performed freshly collected urine sample is diluted with ethanol and few drops of freshly prepared ferric chloride is added to it, fresh urine sample fresh ferric chloride. So, what will happen phenylpyruvate being a phenylketone actually gives an intense blue green color and it indicates a positive test.

So, if there is phenylalanine the ultimate it will be converted to phenylpyruvate ok alternative paths of metabolism and that will lead to phenylketonuria positive test. So, negative if the test is negative it will show brownish or yellowish color of ferric chloride ok. So, positive test for ketone bodies is I mean test for ketone bodies is positive in phenylketone it is a very important trivia. Remember when you are dealing with practical exams generally we have discussed when ketone bodies are present ketone bodies are present during prolonged starvation diabetes mellitus we have all read ketone body metabolism in our lecture series and we are discussed that rothira test is positive in that case. However, rothira test is also positive in presence of this disease phenylketoneuria because phenyl ketone gives a positive rothira test ok.

Now, let us come to the incidence of phenylketoneuria if we sort it by country Turkey this is a 2017 data Turkey has been shown to have the highest incidence of phenylketoneuria and if we compare it with the with India we are somehow in between we are not at the top ok you can see the incidence is very high in Turkey the incidence of America is also given in this study. So, what are the precautions if we are to if we diagnose a case of phenylketoneuria generally and if the patient has grown into adulthood there are ways by which it can be done we should always ask them to avoid artificial sweetener you know sugar free natura all those brand names that are available in India and multiple other brand names that are available in other countries. So, that is actually derived from phenylalanine and aspartic acid and that artificial sweetener will be a problem because we do not want to increase the phenylalanine load of the body because phenylalanine cannot be metabolized in case of phenylketoneuria. And very important if a woman with phenylketoneuria mind it she is suffering from the disease and she is in a childbearing age she should closely adhere to diets that are low in phenylalanine if excess phenylalanine is produced that will lead to again formation of those toxic compound and that will increase the result of miscarriage mental retardation of the fetus microcephalic and other congenital heart disease. So, all every time the

blood throughout the pregnancy the blood level of phenylalanine needs to be strictly monitored.

So, what is the treatment of phenylketoneuria? Sadly there is no cure there is no definitive treatment for phenylketoneuria. So, generally strictly controlled or phenylalanine free diet up to the age of 14 years all right. Permanent monitoring of blood phenylalanine level extremely essential. After this age generally after 14 years have passed brain development is not that much prominent. So, extent of phenylalanine or phenylalanine then can be relaxed some relaxation can be given in the diet and brain is not affected by the results of high phenylalanine in the body because the transporter even if it is saturated it will not hamper in brain development.

However, we should note that phenylalanine itself is an essential amino acid. So, small doses must be supplied right because there are products from which I mean phenylalanine itself is accumulated in multiple structural proteins as it is an essential amino acid. So, small amount should be provided why because insufficient if it is totally lack of phenylalanine diet again it will cause mental physical sluggishness loss of appetite anemia rashes and diarrhea all right. So, to conclude this lecture has covered the importance of phenylalanine as well as the metabolic fate of phenylalanine how phenylalanine metabolism is altered in phenylketonuria what are the etiology what is the main etiology and variations in phenylketonuria how it is inherited what is the incidence of phenylketonuria across countries what are the clinical features and symptoms of phenylketonuria what is the how we can diagnose phenylketonuria and how phenylketonuria is treated all right. So, these are my references and I thank you for your patient hearing. Thank you.