Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 48 : Prenatal diagnostics and NIPT

Hello students, Namaskar and welcome back. So, today we are continuing with module 10 of our course and today's lecture is on prenatal diagnostics and NIPT. So, what is NIPT? It is basically stands for Non-invasive Prenatal Testing all right. However, before going into non-invasive prenatal testing we should which is a newer emerging branch of prenatal testing right. But as a part of genetic testing we know there can be multiple aspects which we have discussed in detail in the previous two classes. However, specific to prenatal testing we should know what prenatal testing is and what are its indications right.

This might be a bit of recapitulating lesson from previous classes. We should know what are the prevalent traditional methods of prenatal testing which includes both screening as well as diagnostic tests and in the later part of the lecture we will be going into the NIPT that is non-invasive prenatal testing where we will be discussing what is the need of this test, what are the procedures, what are the uses, risks, demands etcetera right. So, let us start with prenatal diagnosis. So, what is prenatal testing or prenatal diagnosis right? Basically these are procedures undertaken to diagnose genetic abnormality all right or structural anomaly.

Often in the early embryo or in the fetus right even before the baby is born. So, during prenatal phase procedures that are done to diagnose these problems genetic abnormality or structural abnormality all right. Why so that the mother is prepared so that the healthy baby is delivered. So, the timely intervention is possible all right intervention might be in order to terminate the pregnancy or prevent the complication of a born child all right. So, mainly the main issue is in case of life threatening conditions or which proves the viability of the fetus will be in jeopardy it is much better and safe to terminate the pregnancy early all right.

Preventing a wastage of perinatal mortality when the baby is much more advanced right it is much more problematic both physically and emotionally for the mother to terminate the pregnancy all right. So, these diagnostic procedures are in place to prevent that

grievous outcome right. So, if we are to jot down these first one word number one timely medical treatment and condition on before birth all right. Mind it if there is some genetic abnormality which does not qualify for medical termination of pregnancy rather there are there are multiple disorders which can be diagnosed in utero. So, that they will prepare the family the mother so that early treatment can be done right.

So, not only termination, but also early treatment. Next helps to make the parent informed decision again told you in the present day whether to about the fetus with diagnosed condition right. As well as helping the parents to prepare psychologically socially financially and medically for the healthy baby right as well as for the babies who might have a potential health problem or disability basically determines the outcome of a pregnancy all right. So, what are the indications why do we do prenatal testing right mind it should be done as I told you in last class in primary care it should be every pregnancy should go some bit of prenatal screening to prevent any I mean to detect any complication or any genetic abnormality which might happen in all cases the chance might be minuscule, but it can happen. However, specific for prenatal diagnosis the importance is more when you already know advanced maternal age that is more than 35 previous child with chromosomal abnormality.

In pregnancy or more triplet pregnancy very important pregnant with multiples all right and other high risk family history right family history of any single gene disorder neural tube defect any chromosomal abnormality or other abnormalities that have been identified during pregnancy. For example, exposure to teratogenic drugs I mean viral infection maternal illness not only that positive history of previous miscarriages always arises high suspicion for prenatal testing. So, these all have been discussed in previous class right. So, these are we are all recapitulating. Now to get hold of the proper tools of prenatal testing they are they comprises of screening test, they comprises of diagnostic test.

Screening test gives us likelihood about what can happen what are the chances of having a disease all right. Screening tests are negative we can be not 100 percent, but almost sure that the baby the pregnancy will be uneventful right. However, as I told in the previous class no screening test is 100 percent sensitive and 100 percent specific. So, a healthy or negative screening test does not guarantee a healthy baby right. So, what will confirm the diagnosis these diagnostic tests right.

So, screening tests are ultrasonography magnetic resonance imaging. So, these are pretty non-invasive these are all purely non-invasive in which a probe is placed on the mother and these are maternal serum markers in which blood from the mother is taken right minimally invasive right. However, the diagnostic tests are very invasive procedures these are amniocentesis, cordocentesis, cholonic villus sampling, fetal tissue

biopsy, celosentesis we will be discussing all of them in brief in this very lecture right. So, I am not going into each of these details in right in this slide, but these are invasive procedures. So, we will start with the non-invasive ones you already must be might be aware if not already aware of ultrasonography where we visualize the baby the health condition right ultrasonography can diagnose many conditions inside abdomen and in any body cavity, but in case of prenatal diagnostics we try to visualize the baby the embryo what are its features and it can be done very early right from detection of pregnancy right to first second third trimester pregnancy to test multiple features.

So, these are some parameters see these are the whole branch specialty and super specialty of obstetrics and gynecology and radiology lies in this right. So, I please do not expect to learn everything related to ultrasound in prenatal diagnostics in one slide in one course not possible right, but however, there are some pointers that should be noted what are the things that we look for right. Nucleal translucency and nasal bone any deformity all right specially in fetal anomaly this is done in first trimester all right for Down syndrome right depressed nasal bridge and increased nuchal translucency. So, increased nuchal translucency and depressed nasal bone right are features of Down syndrome aneuploidy anomaly right, but they need to be done by certified sonographers the one who are actually trained to identify can only identify all right. Next there are multiple fetal anomalies of the whole body specially the heart that is tested right we go for something known as anomaly scan very very very important all right and this is non-invasive easy to done and does tell us a lot of information about fetal anomalies any abnormalities in development right.

However, it is not perfect definitely. So, a normal untral sound does not mean a healthy baby right there might be something that might go unnoticed and to help that if we are suspecting any anomaly right or if this is a high risk pregnancy, but the fetus appears to be normal we can definitely use a much costlier, but better imaging system again non-invasive that is MRI magnetic resonance imaging the short form is MRI again used for multiple investigations across the whole organ system of our whole body again we are using it for pre-neutral diagnostics coupled with USG it gives a much better visualization we can even reconstruct the whole thing in three dimension using MRI software based features right. So, USG and MRI non-invasive, but does give idea about any structural abnormality in the fetus and with the help of some science also give us some idea about genetic anomalies like aneuploidy. Next another minimally invasive that is maternal serum screening that is we already learnt in the previous class right in primary care this should be done in primary care respective of that let us discuss it again. So, it can be done in first trimester in second trimester it involves collection of blood from mother and testing for various analytes alright.

So, what is done in first trimester generally pregnancy associated plasma protein A very

important these are serum markers might be an beta HCG beta subunit of human chorionic gonadotropin. See all these are all proteins they are all serum markers that increases due to presence of neural tube defects or any trisomies. Why do they happen because these are actually gene products right. So, strictly speaking these are not genetic tests these are testing of proteins these are proteomic tests as I told you in very earlier stage testing for gene markers protein also consist of part of the diagnostics which comprises of the entire spectrum of genetic diagnosis alright. So, when we go to second trimester remember just remember these two parameters PAPPA and beta HCG.

When we come to second trimester there are multiple markers. So, triple test, quadruple test these are two options. So, in triple test we measure alpha-fetoprotein, unconjugated istriol and beta HC. So, three markers are measured in maternal blood alright and combined based on their high and low positivity rate based on multiple population data this is the detection rate of triple test or triple marker right it can be called triple test or triple marker test down syndrome trisomy 18 neural tube defect. So, the percentage is lying front of you definitely not 100 percent, but a huge positivity rate predictive value is high.

What happens in quadruple test another additional marker is done along with these three that we are done in triple test. So, that is inibin A also called dimeric inibin A or DIA whatever the short form you will see the same thing right. So, this inibin A plus these three markers make four markers. So, quadruple marker or quad test Q U A D simply refers to the same thing and the detection percentage increases right it has it is better. Hence it is much more preferred whenever available available either I mean the testing facilities available or whether the patient can afford it financially, but this covered by insurance there are multiple factors, but quadruple test always preferred over triple test right.

So, these are all minimal invasive or non-invasive marker or screening marker screening marker means this will give us a likelihood a probability that there might be a disease. So, what will give tell us definitely that the disease process is there these are the invasive procedures. So, invasive procedures I will be discussing them very briefly one is chorionic villus sampling. You have been hearing this term since the last class CVS this is basically sampling or extraction of tissue of chorionic villus is a part of a placenta right which under ultrasound guidance is aspirated using needle it is a very specialized technique right. The risk is written same as amniocentesis.

So, we should discuss amniocentesis right. So, amniocentesis actually is extracting the amniotic fluid with a needle alright. Here also mind it just like chorionic villus sampling we need an ultrasound probe it is done with the help of ultrasound guidance. So, ultrasound waves are being sent. So, we get a clear picture of where the amniotic fluid is

again we are drawing or aspirating the amniotic fluid.

Mind it chorionic villus sampling done in first trimester amniocentesis is done in late second or third trimester 15th to 20th week of pregnancy both carries a risk of miscarriage alright it increases the risk of miscarriage by more than 1 percent any invasive procedures increases the risk of miscarriage. So, it is a huge deal alright it is a huge concern that it is a risk it does not mean that miscarriages will happen, but it there is a chance that it might happen. So, these are only reserved for cases with high suspicion or higher risk related disorder positive screening test positive family history with positive screening test only then we are doing these ok. Next cordocentesis also known as percutaneous umbilical cord blood sampling or PUBS right. It is done in 18th week of pregnancy again high risk of miscarriage what we are doing we are aspirating cord blood right we are aspirating cord blood and we are analyzing the cord blood with the various genetic tests.

We all know what are the genetic test we have been reading them in module 4, 5, 6, 7 right the techniques we have learnt we can implement all those techniques these are the samples alright and we look for specific genetic markers we look for aneuploidy we look for the chromosomal abnormality right. So, whatever is our suspicion we can look for it by the genetic test to confirm right, but again invasive procedures it is done percutaneous right the needle is inserted from the abdomen right we insert a needle in mother's belly. Next fetal tissue biopsy much more invasive procedure alright. So, what is done here a vaginal transducer I mean the probe is placed in the vagina right and a needle is inserted to through the vaginal tract to aspirate tissues from the placenta alright which will give a definite diagnosis as it is an invasive procedure we get tissue sample isolate genetic material from the tissue sample go for the diagnostics.

So, again invasive procedure. Amniocentesis just like amniocentesis, but done very very very early. So, basically is the what a coelomic space is it is a space between the amniotic membrane and the uterine cavity also known as extra embryonic coelom is an anatomic term extra embryonic coelom it is a space where the there is a fluid and we can aspirate that fluid. However, it has been found that the risk is lower than that of amniocentesis. However, the general rule of thumb is any invasive procedure is risky and it increase the risk of miscarriage compared to non invasive procedures. However, as you have been seeing non invasive procedures they help in screening and the invasive procedures they confirm the diagnosis and is only reserved for the cases with high risk of genetic disorder and with positive screening test right.

It may also happen that the screening tests are negative, but there are some suspicions symptom wise there have been previous history of eventful pregnancy with such disorders. Then also there is a it is a decision that has to be taken up by the team in

consultation genetic expert whether one should go for invasive test or not. However, mind it even if the risk is high the invasive test is much more worth it compared to a misdiagnosed pregnancy with genetic disorder of fetus with genetic disorder if the mother has to continue with the pregnancy it will be very ill fated event for both the mother and the baby alright. So looking at all these we definitely need a new test the medical science needed a new test mainly because of the fact that non invasive methods they are not genetic tests right. Truly speaking we are not testing genetic material we are testing the maternal blood right and some protein markers alright and they provide us a likelihood of diagnostic condition rather than accurate diagnosis right that is for sure you have seen the percentages.

Invasive methods they increase the risk of miscarriage. So this new test this new phenomena comes in the form of cell free fetal DNA alright also abbreviated as CFF DNA or cell free DNA in some cases also known as CF DNA alright. However in this case for prenatal diagnosis you have already learnt about liquid biopsy right when you are being taught about cancers or diagnostics in cancer right. However know this in this case the same concept of liquid biopsy can be exploited in maternal blood because fragments of fetal DNA they do cross the placenta they do enter the maternal blood. So we are targeting this fragment of fetal DNA it is the you can see the maternal DNA is the majority alright fragments of fetal DNA are very less it may be as low as less than 10 percent compared to the 90 percent that is present in maternal blood.

However they can be isolated and identified. But as early as 7 weeks of gestation and naturally as the pregnancy progresses the amount of cell free fetal DNA do increase. So what is done this testing of cell free fetal DNA is actually NIPT non invasive prenatal this is a new term NIPT this is the way of examining fetal DNA by taking sample of maternal blood from a pregnant woman. From here the cell free fetal DNA is isolated and can be examined for specific chromosomal markers specific genetic markers anything that is done that can be done with the samples of invasive test can be done here alright. Now you see the what is the procedure maternal blood we take extract the fetal DNA we analyze the fetal DNA signal based on various test and we give a personalized risk score mind it this is a screening test this is a screening test.

So, it also gives us a likelihood ok this is going to be important. So, at this point you might be questioning so the screening test gives us only percentages right. So, we definitely need diagnostic test to confirm that is true that is true for all screening test still this is good let us see why. So, what are the uses primarily used to detect aneuploidy that is trisomy down syndrome, edwards syndrome, patao syndrome alright. Also sex chromosome aneuploidy for example, Turner syndrome, Kleinfelter syndrome can be detected accurate termination of sex because we are directly looking at the chromosomes from fetal DNA we can diagnose the gestational age much earlier than ultrasound mind

this is illegal in India as per the PCPNDT act which stands for preconception prenatal diagnostic techniques right and the act itself in the name says prohibition of sex selection right.

So, sex determination not allowed illegal in India. However, in other countries western countries it is very much allowed and this NIPT has got huge role in early determination of sex ok this was revised in 2003. So, continuing with the uses of NIPT see not only chromosomal aneuploidy and sex chromosome related aneuploidy with the process can now also detect single genetic disorder single gene disorder for example, cystic fibrosis apart syndrome these examples have been taken from western countries because this is a newer test it is being done in western countries mainly we will definitely adapt this in routine screening it is being done at higher centers, but at primary level far from it. However, mind it in these cases this is very important does not need to be confirmed by invasive tests in these cases single genetic variation and this panel is increasing right. So, this is actually the term for that that case is used as non-invasive prenatal diagnosis right.

So, we are telling that the disease is there right need not be confirmed by confirmative tests. If however, there is always a choice of the physicians of the parents of the mothers right of the couple to confirm it via confirmatory invasive tests right. However, the likelihood of a NIPT being positive for these mentioned genetic disorders is very very very high right the sensitivity is very high. Again one use detect RH compatibility. So, everything every possibility that is being told by invasive test are being told by these.

So, this will also be the indications or the uses of any invasive tests right mind it and in case of non-invasive they mainly detect the trisomy and the neural tube defects. So, RH compatibility or RH incompatibility between the fetus and the mother as well as many other hemoglobin disorders can be detected by NIPT. It is possible to sequence the whole genome from the cell free DNA alright cell free fetal DNA. However, currently it is too time consuming and costly to the routine even in western standards. However, just because it is too time consuming and costly to do means there is a much room for improvement and definitely this is one of the future directions where we will see research and development being catered to.

Now comparing the percentages. So, cell free DNA fetal DNA versus conventional prenatal screening. So, first trimester screening, um, nuchal translucency with the markers pregnant associated by protein A plasma protein A and beta HCG. They have got their sensitivity rate as well as false positivity rate right integrated prenatal screening including the USG as well as both the markers ok. Inclusion of third and fourth I mean triple and quadruple markers possible or integrated prenatal testing where we can use all the markers alright right from first trimester second see integrated prenatal testing means testing one first trimester marker along with second trimester marker right. However,

maternal I mean and this you already know what is done in either first or second.

So, we are combining both of them right and this means we are combining multiple markers across multiple pregnancies. So, these are all combination permutation that we can do to increase the sensitivity, but none can match the sensitivity of NIPT this is NIPT alright not only that the false positive rate. False positive rate means the screening test is positive right we are. So, it goes both ways just as told you a healthy USG or healthy positive negative screening test does not guarantee healthy baby similarly a negative test does not guarantee a I mean the other way the positive test does not guarantee a sick baby and the negative test does not guarantee healthy baby right. So, false positive rate is always there in case of these traditional markers traditional screening markers we are talking about screening only not diagnostics right.

However, so the positive predictive value for down syndrome as per these literatures are around 4 percent of these tests. However if cell free DNA or NIPT diagnose the trisomy you can see the statistical values that is why it is much much much more important and preferred right. However, we should know that it is not the holy grail right I means NIPT positive means the disease is there again no right there is something called placental mosaicism etcetera. So, we are not going into those details, but just know it for high risk population this is a screening test right this is non invasive this is being covered by insurance. However in order to confirm the test right it should be done by the invasive procedures all right.

This is a high probability more than 99 percent false positive rate is 0.4 percent, but before taking any action irrevocable action. So, before we are going to terminate the pregnancy in today's date all right it is still preferred to confirm the diagnostic using an invasive procedure. Maybe in future they will be near accurate and that will totally free us from all the invasive procedures all right. So, there are algorithms there are AI and ML that are working into all these things and we will be discussing them in the last week last module right how AI artificial intelligence and machine learning is improving molecular diagnostics right.

And few risks and facts about NIPT is there is no risk to the fetus whatsoever this is a very common question whenever this test is offered to any lady right. So, NIPT is being done routinely in NHS national NHS is the national health scheme in UK right and also in western country. So, one of the common questions might be what is the risk to the fetus no nothing the risk is same as taking blood from anti cubital in any disorder right. So, there is no specific risk to the fetus right. Of course, there might be complication related to venipuncture infection and that may indirectly to the fetal health jeopardy, but this is very minimal with proper aseptic procedures right.

Again no risk of miscarriage compared to invasive diagnostic procedures. So, we have got a high accuracy very low false positivity, but completely avoiding the complication of invasive procedure and one thing you need to know that not everything is perfect. So, this is not reliable not much accurate in women with multiple who are pregnant with multiples that is 2 N triplets or more. Mainly because of the fact that there are more than one set of fetal DNA is involved right and there are multiple other factors I am this beyond the scope of this class to discuss them in details alright. So, just know this if cell free DNA is a method of choice over many things it will not be the method of choice whenever there are multiple twins alright.

So, that is it for today's class to summarize we have discussed what are the prenatal methods what are the indications of prenatal testing, we have discussed the prevalent methods of screening and diagnostics, we have discussed the noninvasive and invasive methods and we have also discussed the concept of cell free DNA right. How it actually helps in detection of multiple disorders without in I mean going into the risk of invasive procedures and with a high sensitivity and very high predictive and very low false positive tests. We have discussed how it is done, we have discussed the multiple uses user case scenario have also discussed the risks and clinical demands of NIPT. So, these are the references for today's class and I thank you all for your patient hearing. Thank you.