Course Name :An Overview on Maternal Health Antenatal, Intranatal and Postnatal Care

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Invasive and non-invasive Prenatal Diagnostic test

Hello students. I hope you are all in good health and high spirits. Welcome you all to today's session for the NPTEL online certified course for the topic and overview on maternal health, the antenatal, intranatal and postnatal care. I am Dr. Barnali Ghosh, an obstetrician and gynecologist working as assistant professor at B.C.Roy Medical College and Medical Research Center, IIT, Kharagpur. So, today our topic of discussion is on the invasive as well as the non-invasive prenatal diagnostic tests.

In the previous class, we did have a detailed discussion regarding the first trimester and the second trimester screening test for aneuploidies which are to be performed in every pregnant woman irrespective of their age or other previous high risk histories, right. So, we need to perform the screening test which are generally non-invasive. There are different blood markers as well as certain USG parameters which are to be assessed in the first and second trimester in every woman irrespective of their gestational age. The next step are the diagnostic tests.

The mothers who screen positive for the prenatal screening test, right. So, they are at risk of aneuploid fetuses. So, we need to further evaluate them with the diagnostic test. So, the concepts covered in today's class are the invasive prenatal diagnostic tests which are chorionic villus sampling, amniocentesis, cordocentesis, right as well as there are the non-invasive prenatal diagnostic test which is known as NIPT. So, non-invasive test is known as NIPT, non-invasive prenatal test which is nothing, but cell free fetal DNA assessment in maternal serum.

Keywords are CVS, amniocentesis, cordocentesis, cell free fetal DNA and NIPT. So, coming to the previous class discussion we have dealt with the screening method of first trimester and second trimester, right. So, first trimester screening and second trimester screening. So, just a revision of previous class first trimester to be done between 11 to 14 weeks and there are two markers or two parameters number one is the dual test or the dual marker test in the maternal blood and number two is the USG-NT scan or NUCHAL translucency scan. Dual marker consists of two parameters number one is the maternal serum alpha-fetoprotein and number two

is the pregnancy associated plasma protein A, right.

These two will be assessed in the maternal blood and in USG the Nuchal translucency, right NUCHAL translucency will be measured. We have seen the criteria for measurement how it is measured you know in the widest plane from inner border to inner border and if this measurement you know if this measurement should always be less than 3 millimeter this is normal. If it is more than equal to 3 millimeter then it is abnormal and with the NT scan we have also seen the nasal bone presence or absence, the tricuspid regurgitation present or absent and number three is the ductus venosus flow pattern. So, this was the first trimester screening and these screening tests all taken together with the consideration of the maternal age, maternal weight, ethnicity as well as the period of gestation taking all these together we calculate the risk ratio for this particular pregnancy for aneuploidy screening and this risk ratio if it is it comes no more than the cutoff value then the female is at risk of anomalous baby and so she should be investigated or followed up further. Now coming to the second trimester screening this is also a screening method which we do between 15 to 22 weeks of gestation here also blood markers that is triple test and quadruple test.

So, triple test will be having three parameters in the maternal blood which are we have read it alpha-fetoprotein, unconjugated estriol and beta HCG and quadruple marker is triple marker test plus dimeric inhibin A right. So, these are all regarding aneuploidy screening and in Down syndrome in Down syndrome which is the most common anomalous aneuploidy present in today's scenario we come across this that is trisomy 21. It has high what are high what what parameters are high in Down syndrome H means beta HCG is high and I means inhibin A is high right. So, these two parameters are high rest all are low these two will be high rest all are decreased right. So, this was regarding the blood.

Now coming to the USG parameters yes there are some USG parameters called as the soft markers which are to be seen in the second trimester like nasal bone, echogenic foci, bowel echogenic focus or intracardiac echogenic focus or short femur, short humerus, saddle gap, semen crease, choroid plexus cyst all these markers will hint towards a anomalous baby and if they are present we need to now tell the counsel the couple and take the mother for the diagnostic tests. So, just in a nutshell regarding maternal serum alpha-fetoprotein of all the biochemical analytes we have discussed maternal serum alpha-fetoprotein this is raised right increased in amniotic fluid. So, when we go for amniotic fluid tapping in case of amniocentesis we mark the levels of alpha-fetoprotein and it is increased in cases of open neural tube defects in case of anterior abdominal wall defect right. So, this this is a neural tube. So, this neural tube defect means this is a meningocoele right.

So, the CSF will leak out right because there is no covering in case of open neural tube defect and alpha-fetoprotein will be raised in amniotic fluid, amniotic fluid is all around in the amniotic

sac right. So, anterior abdominal wall defect also will cause increased alpha-fetoprotein in case of multiple gestation in case of renal anomalies. And, sometimes in case of underestimated gestational age. So, here it will increase and where it will decrease? Decreased alpha-fetoprotein in case of Down's syndrome, in case of molar pregnancy here it will decrease. So, IUFD intrauterine fetal death it will increase.

So, raised levels of alpha-fetoprotein will hint that there is some anomaly within the fetus and you need to look into the know the causes of increased alpha-fetoprotein you need to exclude the causes right. So, next the gist or the essence of today's class is first trimester screening is either equal or even superior to second trimester screening. So, every pregnant woman should undergo the first trimester screening at least and if it comes out to be normal if the risk ratio calculated after the first trimester screening is below the cutoff risk that is she belongs to the low risk category then she may be exempted from further testing. All these tests are costly they you know increase the cost burden on the patient on the on the patient's family. So, if you know if it is not required or if she asks whether she needs to go for further second trimester screening then you know coming from the result of the first trimester screening you can sometimes say that yes you are a low risk group you know there is less chance of anomalous baby and you can also you know may not go for further tests.

So, first trimester screening is more superior and if she screens positive then she must go for the diagnostic test at an early date so as to exclude the possibility. If it comes that the from the diagnostic test that the fetus is anomalous then you can give her an option of termination of pregnancy and if it is done early it is safe for the mother both from the psychological point of view as well as from the obstetric point of view right. And a targeted ultrasound, targeted ultrasound in the second trimester with a fetal echocardiography these two should be done in case the NT comes more than 3 millimeter 3 or more if the nuchal translucency is 3 or more you need to investigate further. Now coming to the screening result which I was telling that you get a risk ratio right. So, this risk ratio is calculated from the blood parameters from the USG parameters as well as taking into consideration the maternal age, the maternal weight, the maternal ethnicity as well as the period of gestation and you get a cut you know a risk ratio for that particular pregnancy.

For India in South Asian population this cut off is 1 is to 250 for Down syndrome and 1 is to 100 for you know Edward or Patau syndrome. So, if the risk ratio is more than 1 is to 1000 say the risk ratio comes to be 1 is to 150 for Down. So, that means, she is screen positive right. So, that was for India. Now the fetal medicine foundation people they have provided a cut off for them the cut off is 1 is to 1000 if it is less than 1 is to 1000 then it is low risk right no further testing is required, but if it is less than 1 sorry if it is more than if it is more than 1 is to 100 then it is high risk then you need to go for the invasive diagnostic testing which are amniotic fluid study from amniocentesis, chorionic villus sampling and choriocentesis and in between if it is in

between 1 is to 1000 to 1 is to 100.

So, this in between is the intermediate risk and for them you need to go for another secondary screening test this is also a screening test, but it is much more sensitive and much more specific which is called as the NIPT where we use the cell free fetal DNA circulating in the mother's blood. So, this was the assessment of screening results. There are different types of assessing these screening results number 1 is the integrated screening where all the female all the pregnant female will undergo first trimester as well as second trimester screen and then the final risk is given at the end of the second trimester screen right. So, you get the final risk calculation at the end of the second trimester screening. In case of sequential screening there are 2 types contingent sequential screening and stepwise sequential screening.

Contingent sequential screening is same as the sequential fetal medicine foundation people who have prescribed right. So, there the cutoff is 1 is to 100 if more than 1 is to 100 it is high risk go for invasive. If it is between 1 is to 100 to 1 is to 1000 then it is intermediate risk go for the secondary test that is also screening test that is the NIPT and if NIPT is positive NIPT is positive NIPT is showing that yes there may be you know there is a chance of anomaly in the fetus then you definitely have to go to invasive test right. And if NIPT is negative then you are you know assured that yes the fetus is normal and no further testing is required right. So, this was one scenario cutoff less than more than 1 is to 100 in between 1 is to 100 and 1 is to 1000 and if the cutoff is less than 1 is to 1000 then no problem it is low risk no further testing right.

So, this is the whole picture in case of stepwise what we do we in the first trimester we go for the risk assessment by screening and if it is more than the cutoff risk more than the cutoff risk then directly you go for NIPT or in invasive test right. And if it is less than the cutoff risk less than cutoff risk then go for the second trimester screen. So, this is sequential and then after the second trimester screen again you calculate the cutoff risk and depending upon that you say that yes she is a case of low risk or high risk right. So, you know this way you get a sequential screening. So, you know you can segregate all the pregnant females need not go for NIPT all the pregnant females need not go for invasive test only screening will be sufficient to screen the high risk and low risk and depending upon that risk ratio the low risk will be no separated they will go for normal scan and normal pregnancy no follow up, but the high risk needs some further diagnostic test to say that yes the fetus is anomalous and if proven that the fetus is anomalous there is some anomaly then you can go for termination of pregnancy.

So, this was regarding the prenatal screening test. Best screening procedure as per ACOG ACOG American Committee of Obstetrics and Gynecology they have opined that the best screening procedure will be combined first and second trimester screen. So, they are telling that yes all pregnant females should go for first trimester screen also should go for second trimester screen and combined risk ratio should be calculated for every pregnant woman, but this I have

told that yes these are costly and that will increase the cost burden for the couple. So, that you have to know at the back of your mind because you know expenditure is also a important issue. So, that was the screening test now coming to the pre-natal diagnostic test.

Diagnostic test meaning these can be invasive or non-invasive. Non-invasive is NIPT which is actually a screening test, but it has very high specificity. If it is negative then you can surely say that yes the fetus is not anomalous, but if it is positive then you should again go for further testing with the invasive test to know determine or to confidently say that yes the fetus is anomalous. In case of invasive test we have three procedures number one is the chorionic villus sampling, then the amniocentesis and this is the cordocentesis which is also called as percutaneous umbilical vein blood sampling. So, fetal blood sampling is sample is taken from the umbilical vein percutaneously right.

So, coming to one by one chorionic villus sampling it is know can be done by two routes number one is the trans-cervical and then is the trans-abdominal right. So, first you need to know when it should be done it is done from 9 to 13 weeks of gestation. So, beginning at 9 weeks it can be done from 9 weeks up to 13 weeks of gestation and as the name suggest chorionic villus so that means, fetal tissue from the chorion chorion frondosum which is the part of the placenta originating from the fetus. So, chorionic frondosum part of the placenta there we take the fetal trophoblastic tissue and we examine the genetic material within the fetal cells. It can be done trans-cervically what we do in the trans-cervical method this is the trans-cervical method where you are inserting this is a malleable rubber catheter right.

This is a malleable rubber catheter and just at the beginning within the long malleable rubber catheter you have sorry rubber polyethylene. So, long malleable polyethylene catheter you have a metal obturator inside and you introduce inside the cervix you go inside the uterine cavity in the extra ovular space you can see here and you reach the chorion frondosum site. So, this is the chorion frondosum the fetal part of the placenta. Now, after reaching you pull out the metal obturator you keep the catheter you attach a 20 ml syringe and with negative suction you will be aspirating the fetal trophoblastic cells approximately 15 to 25 milligram of fetal trophoblastic cells are collected and they are you know transferred to a tissues culture media right which is present inside the syringe. Now, after collection we will go for the different genetic tests to evaluate the genetic makeup of the fetus.

So, these are actually the genetic material collected from the fetal trophoblastic cells and this is always done under ultrasonic guidance right and this is the trans abdominal route where with a spinal needle we pierce through the maternal anterior abdomen inside and under ultrasonic guidance we will go at the chorion frondosum plate of the placenta, but will be away from the site of cord insertion we will collect in the same way by negative pressure in the syringe you know a part of the fetal trophoblastic cells. So, trans abdomen in the spinal needle size used is

18 to 20 gauge. So, this was the fetal chorionic villa sampling and to note that what chorionic villa sampling provides earlier diagnosis than amniotic fluid study which are done late in the second trimester starting from 15 weeks. So, you know from 9 weeks onwards we can go for chorionic villa sampling and earlier the anomaly is detected earlier we can go for termination of pregnancy which is good for the mother. Only 15 to 25 milligram of villi are aspirated in a 20 ml syringe creating a negative pressure right.

So, that was chorionic villa sampling I have told that every invasive procedure is you know associated with certain complications what are the complications in CVS there can be episode of vaginal bleeding. In case of trans cervical approach there can be episodes of vaginal bleeding there can be also certain cases of fetal loss or miscarriage after the procedure. It can also so happen that there may be oromandibular limb defects right. So, in case certain cells of the inner cell mass are affected or you know they are mishandled man handled. So, there can be some defects in the you know up growing fetus right.

So, you need to be very careful expertise is a very important thing for these invasive tests. Coming to limb reduction deformity in CVS if CVS is done in less than 9 weeks of gestation it can cause limb reduction deformity. So, ideally CVS should be done after 9 weeks between 9 to 13 weeks of gestation. What are the contraindications of trans cervical root of chorionic villa sampling of chorionic villa sampling it may so happen that if there is a case of cervical myoma we cannot reach the placental site through the cervix. In case of hyper angulated uterus right there is a chance of uterine perforation.

In case of in case of say aneurysm of the anomaly right malformation uterine malformation, septation or say any type of malformation didelphys there is a always a chance of rupture because through the trans cervical root we will be going with the metal obturator and will be going inside the uterine cavity and in case of anomaly or malformation it can lead to rupture and bleeding right. Also in cases of vaginal bleeding you cannot go for the trans cervical root in case of infections like genital herpes right in case of cervicitis all in these cases trans cervical root is a contraindication for that case you need to go for trans abdominal CVS right. And lastly in case of Rh negative mother always you need to give anti D immunoglobulin IGG 50 microgram IM right because there is always a chance of fetomaternal hemorrhage. So, completed the CVS now coming to amniocentesis amniocentesis is also taking the fetal desquamated cells or actually desquamated, desquamated cells or fetal fibroblast right. So, these cells from the fetal skin these fibroblast they will come into the amniotic fluid and they keep floating inside the amniotic fluid and when you pierce under ultrasound guidance always under USG guidance you go through the anterior abdominal wall go into the amniotic cavity with a spinal needle right 20 gauge, 20 gauge spinal needle and you are then suck you suck the amniotic fluid by the syringe with negative pressure right under negative pressure and you collect the amniotic fluid and you will get the fetal fibroblast cells right in the amniotic fluid and then from these fibroblast cells you

will go for the genetic screening or testing.

When is the time it is done 15 weeks after 15 weeks 15 to 18 weeks of gestation technique yes I have told under ultrasound guidance with spinal needle of size 80 into 20 gauge and with a syringe you know it is collected by negative pressure right needle size is 20 gauge. Now coming to the competency of the operator the person or the obstetrician or the fetal medicine expert who is doing the invasive test he or she needs to do at least 30 procedures more than 30 procedures per year right really an expert an expert is one who is doing more than 100 procedures per year in case there is no audit which shows that there is 4 cases of fetal loss 4 cases of fetal loss per 1000 procedures. So, that requires an audit to note the competency of the operator. So, this was the assessment of the operator competency for doing the test. Now in case it is HIV positive woman what to do we will always start ART therapy and decrease the viral load right and after the viral load is minimum we will then go for the invasive test right that is CVS or amniocentesis ok.

Now coming to the complications of amniocentesis as such fetal loss or miscarriage is a complication and what happens in amniocentesis if it is less than you know say you know just background risk is 1 percent right this is background risk and you know this happens even if it was this invasive test was not done. So, that is called as background risk and 0.9 percent risk is for the procedural risk. So, together it is coming out to be 1.9 or approximately 2 percent risk of miscarriage following an amniocentesis procedure.

Other complications occurs in case of early amniocentesis right that means, less than 15 weeks of gestation when it is done it can lead to early fetal loss, it can lead to talpus. So, that is a deformity right it can lead to respiratory morbidity. So, all these are complications associated with amniocentesis. Now, what are the importance or the inferences that we get from the invasive procedure we collect the fibroblast cells from the amniotic fluid, we collect the fetal trophoblastic cells from the CVS, we collect the fetal blood cells from cordocentesis. So, all these fetal cells will provide with the genetic material that constitute the fetus and when these genetic materials are analyzed we can go for cytogenetic diagnosis that is chromosomal abnormality any type this can be detected by PCR and followed by fluorescent in situ hybridization right.

So, you will get to know regarding the fetal aneuploidies that is trisomy 21, trisomy 13, monosomy X or called as Turner syndrome. DNA analysis by you know array based tests there are different tests to assess these fetal genetic defects and this can go for single gene disorder sorry. So, single gene disorder single gene point mutation can be assessed right how there occurs in point mutation in cystic fibrosis in Tay sach's disease in BRCA 1 or 2 mutation right in all these diseases point mutations can be analyzed from these diagnostic tests. So, and lastly biochemical how to go for biochemical the alpha-fetoprotein from the amniotic fluid can be

assessed and I have told that if amniotic fluid alpha-fetoprotein level is increased then there is a chance of anomalous babies. Last invasive test is the cordocentesis or percutaneous umbilical blood sampling right.

So, what to do here it is from the umbilical vein what we do we also this is a percutaneous procedure and this is also under ultrasound guidance you go through the anterior abdominal wall and you go into the cord right it is approximately 2 centimeter away from the cord insertion from at the cord insertion in the placenta. So, from that point you should be always away at least 2 centimeter away and with the needle which is approximately 13 centimeter in length this needle you push it inside through the anterior abdominal wall into the fetal you know the cord right the umbilical cord and you pierce the umbilical vein. Why umbilical vein because umbilical vein is larger in diameter there is less chance of hemorrhage there is less chance of fetal bradycardia and you collect approximately 1.5 to 2 ml of fetal blood right. So, with this fetal blood you will next go to the different diagnostic tests and what are the risks yes there is a risk of cord hematoma there is a risk of fetal, fetal hemorrhage, fetal maternal hemorrhage, risk of infection like chorioamnionitis right there is risk of preterm labor there is risk of premature rupture of membranes right all these risks are associated with it.

So, as there is a risk of fetal maternal hemorrhage we should always give 100 microgram of anti-d'immunoglobulin to Rh negative mothers after cordocentesis right. So, what are the important features we have already discussed the invasive procedure, the cytogenetic analysis, the DNA point mutations and the biochemical assay extra what we get from cordocentesis is hematological in case of fetal anemia, in case of fetal you know thrombocytopenia. Now causes of thrombocytopenia even autoimmune thrombocytopenia can be diagnosed right hemoglobinopathies right hemoglobinopathies, hemoglobinopathies thalassemia all these can be diagnosed from cordocentesis. Fetal infections viral infection toxoplasmosis can be diagnosed. Fetal blood gas and acid based status right in case of IUGR in case of growth retardation or asphyxiated baby there is a base deficit which can be elicited from the cordocentesis.

Fetal therapy sometimes they it needs blood transfusion in case of severe fetal anemia inside the uterus we can go for blood transfusion through this amyloidal vein. We can also go for drug or medical therapy in cases of fetal conditions right. So, hyperthyroid fetus may need propylthiouracil different drug therapy can be given. So, this was total regarding the 3 types of invasive procedure. Now in a nutshell in this table you can see these 3 procedures and CVS is done most early between 10 to 13 or 9 to 13 weeks this is the earliest right.

So, from that point it is associated with least risk it is safe right. There is no the effects of termination of pregnancy on the mother is very little because it is done at a very early stage of pregnancy and here we study the trophoblastic cell genetic material right. Direct preparation result comes within 24 hours culture needs 10 to 14 days right. Chance of fetal loss is less in

chorionic villus sampling. Now as you go further you know amniocentesis it is after 18 weeks, cordocentesis is after 18 to 20 weeks, amniocentesis around 15 weeks right.

In amniocentesis we study the fetal fibroblast in the amniotic fluid and also study the biochemical alpha-fetoprotein and here it as it is done in the second trimester it is risky and if it requires pregnancy termination it is more traumatic for the mother both physically and psychologically. In cordocentesis also it is done late so, it is risky and it is more traumatic, but both are more accurate than CVS right and in cordocentesis we can see the fetal blood cells in an any type of infection and other biochemical you know abnormality of the fetal blood can be detected in cordocentesis. So, that was regarding the invasive tests of the prenatal genetic diagnosis and next part is the non-invasive methods which we will be discussing in the next class. Thank you, keep reading and keep taking notes.