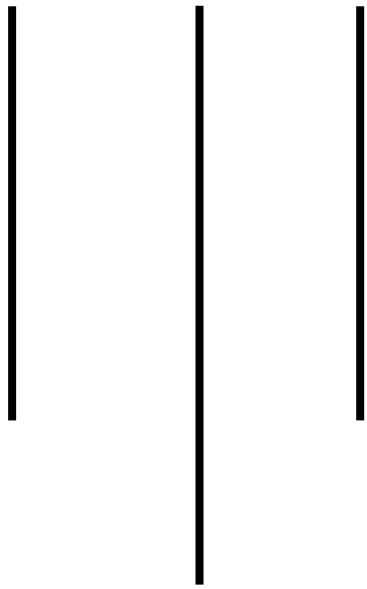
Title of the Research:

Screening of high-risk pregnancies for Down Syndrome using quadruple test at a tertiary care center of Nepal

RESEARCH REPORT



Submitted to

Government of Nepal

Nepal Health Research Council

Ramshah Path, P.O. Box: 7626, Kathmandu, Nepal

DECLARATION

I hereby declare that this research work on the title "**Screening of high-risk pregnancies for Down Syndrome using quadruple test at a tertiary care center of Nepal"** was done by me (Principal investigator) with the collaborating effort from my co-investigators. This research received the NHRC provincial research grant with the protocol registration number: 572/2021 P.

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Dr. Apeksha Niraula
Assistant Professor
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ETHICAL CLEARANCE FROM NEPAL HEALTH RESEARCH COUNCIL (NHRC)

Government of Nepal

Nepal Health Research Council (NHRC)

Estd. 1991

Ref. No.: 1839

23 January 2022

Dr. Apeksha Niraula

Principal Investigator

Institute of Medicine, Tribhuvan University Teaching Hospital

Kathmandu

Ref: Approval of research proposal

Dear Dr. Niraula,

This is to certify that the following protocol and related documents have been reviewed and granted approval through the expedite review process by the Expedited Review Sub-Committee meeting for it's implementation.

Protocol Registration No/ Submitted Date	572/2021 P 10 October 2021	**Sponsor Protocol No**	NA
Principal Investigator/s	Dr. Apeksha Niraula	**Sponsor Institution**	NHRC
Title	Screening of high-risk pregnancies for Down Syndrome using quadruple test at a tertiary care centre of Nepal		
Protocol Version No	NA	**Version Date**	NA
Other Documents	1. Data collection tools 2. Informed Consent Form 3. Support letter	**Risk Category**	Minimal risk
Co-Investigator/s	NA		
Study Site	Tribhuvan University Teaching Hospital, Kathmandu		

Type of Review	√	**Expedited**	**Duration of Approval** 23 January 2022 to 23 January 2023	**Frequency of continuing review** NA
		Full Board		
	Meeting Date: 21 January 2022			

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ABSTRACT

Title: Screening of high-risk pregnancies for Down Syndrome using quadruple test at the Tertiary Care Center of Nepal

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Background: Down syndrome (DS) is the most common cause of developmental delay and accounts for 15-30 percent of individuals with intellectual disabilities. No finite published data suggests the prevalence of DS in Nepalese children. There is no definitive protocol from the government or professional bodies regarding screening, though the practice for screening DS is increasing both in the public and private sectors.

Objectives: To screen high-risk pregnant women (Age ≥ 30 years) by quadruple biochemical test in the second trimester for Down syndrome and other chromosomal abnormalities. To evaluate the sensitivity and accuracy of the second-trimester screening (quadruple test with genetic sonogram) for trisomy 21 as compared to biochemical testing.

Methods: This prospective observational study was conducted in the Department of Clinical Biochemistry in collaboration with the Department of Obstetrics and Gynecology, Institute of Medicine, TUTH, Maharajgunj, Kathmandu, Nepal. Pregnant women at 15-21 weeks of gestation were enrolled. Quadruple test (Alpha-fetoprotein (AFP), beta-human gonadotrophin (hCG), unconjugated estriol (UE3), and Inhibin-A in the laboratory using the technology of

Chemiluminescence micro particle two-step immunoassay. Risk estimation using values of hormone levels and the resulting MoM was done using PRISCA 5.0.2.37.

Results: 256 high-risk pregnancies were screened for trisomy 21, trisomy 18, and neural tube defects. The mean age of the patient was 33.65 ± 3.71 years. Out of 256 patients, 24 (8%) patients were identified as high risk for trisomy 21, 6 (1.96%) patients for Trisomy 18, and 6 (1.96%) patients for neural tube defects. Multiples of Median (MOM) for AFP in high-risk pregnancy were (1.06 ± 0.57; Sensitivity: 43.8% and specificity: 47.2 %), B-HCG (1.27 ± 0.59; Sensitivity: 87.5% and specificity 71.9%), UE_3 (0.86 ± 0.45; Sensitivity: 31.3% and specificity 30.2%) and Inhibin (2.67 ± 1.16; Sensitivity: 81.3% and Specificity: 85.4%) respectively.

Conclusion: Second-trimester quadruple test provides an effective screening tool for Down syndrome in the Nepalese population. Quadruple tests combined with sonograms can lower the rates of unnecessary amniocentesis in high-risk populations.

Keywords: Biomarkers; Chromosome Disorders, Down Syndrome; Maternal age; Pregnancy; Prenatal diagnosis/methods

INTRODUCTION

Down syndrome (DS) is considered one of the prominent causes of intellectual disability in the world.[1] Globally, it accounts for 15-20% of the intellectual disability population.[2] In Western countries, the disease has a prevalence of 1:700 births in the general population.[3-4] The disorder leads to various medical and societal challenges for the affected person and their family members.[4] DS can be unfailingly diagnosed during pregnancy by chromosomal analysis of fetal cells obtained by amniocentesis. Amniocentesis is an expensive and risky procedure that can even lead to fetal loss in several instances.[4] Hence, maternal screening tests by the biomarkers are commonly being used for the identification of pregnant women with high risk for DS.[5] The most widely used screening test is dependent upon the measurement of three serum analytes during the second trimester. This test which is also called as "Quadruple test" detects 70 to 75 percent of DS cases by identifying 7 to 8 percent of the pregnancy population at high risk.[6] Prenatal diagnosis for major genetic disorders and congenital disabilities with a poor prognosis and discontinuation of the pregnancy if the fetus is affected, is an accepted strategy for reducing the burden of genetic disorders.[6-7] Several prenatal screening tests are now available for DS.6-8 Screening for DS is common in Western countries, with data of more than 2 million pregnant women being screened annually in the U.S. for DS.5 In India, the frequency of DS has been reported to be 1 in 916.9 No data has been reported yet regarding the prevalence of DS in the Nepalese population. There is no strict protocol for a pre-natal screening implemented in Nepal which could identify women's risk of carrying a fetus with a chromosomal disease beyond her age-related risk. The risk of a woman having a baby with DS increases in a gradual, linear fashion until age 30 years and thereafter increases exponentially.[10] Early attempts at incorporating maternal serum markers into screening for aneuploidies focused on the second trimester of pregnancy and demonstrated a substantial improvement in detection rates of DS, compared with screening by maternal age. At a false-positive rate of 5%, the detection rate improves from 30% in screening by maternal age alone to 60 to 65% by combining maternal age with serum AFP and free β-hCG (double test), 65 to 70% with the addition of uE3 (quadruple test) and 70 to 75% with the addition of inhibin A (quadruple test).[11-13]

The quadruple test is a screening test done in the blood. It is based on the use of four serum markers as modifiers of the maternal age-related risk of the fetus having DS syndrome. The markers used are Alphafetoprotein (AFP), human chorionic gonadotrophin (hCG) unconjugated Estriol (uE3), and Inhibin-A.[14-15] The test is performed at 15 to 20 weeks of

gestation.[16] The maternal serum levels of each of these proteins and of steroid hormones vary with the gestational age of the pregnancy. With trisomy 21, second-trimester maternal serum levels of AFP and unconjugated estriol are about 25 percent lower than normal levels and maternal serum hCG is approximately two times higher than the normal hCG level.[17]Also, the second-trimester serum inhibin level is raised in the serum of pregnant women carrying a fetus with DS.[18-19] Thus, the incorporation of inhibin A into maternal serum DS screening in the second trimester, along with AFP, hCG, and uE3, was named the quadruple test, and was first used in 1996.[20] Before this discovery, the only available screening test for DS involved asking a woman her age and for women 35 years or older at the time of delivery would be offered amniocentesis and fetal karyotyping.[18] Women, today are career oriented and have their own academic and career goals.[20] The tendency of late marriage and late conception is increasing[20] which is common even in Nepalese women.[21] Thus, the age-related risk for the occurrence of DS syndrome is higher in today's era. The burden of high-risk pregnancy for DS syndrome has not been reported in Nepal due to the lack of resources to undergo screening tests in most healthcare settings. At present, there is no nationwide consensus regarding the nature and timing of these prenatal screening protocols. Due to the absence of any definite guidelines and the additional lacunae in the awareness regarding the appropriate prenatal screening in the country, women with high-risk pregnancies are not been screened despite the relentless efforts by the treating obstetricians. Thus, this study aimed to screen high-risk pregnancy based on maternal age and previous history of genetic abnormalities using quadruple test screening in women attending the TUTH, the largest tertiary care center in Central Nepal.

OBJECTIVES

General objectives:

- To screen the high-risk pregnancies for DS using a quadruple test screening method in pregnant women≥ 30 years of age attending TUTH.

Specific objectives:

- To estimate serum levels of AFP, ß-HCG, UE3, and Inhibin-A in the second trimester in high-risk pregnancies.

- To determine the risk of DS in the second trimester between familial and non-familial marriages.

RESEARCH QUESTION

- What is the risk of DS in high-risk pregnancies in Nepalese women as determined by the quadruple test screening method?

RATIONALE AND JUSTIFICATION OF STUDY

DS is the most common cause of intellectual disability among live-born children and is amenable to prenatal detection. The clinical presentation is variable, but the phenotype is characteristic and is always accompanied by a certain amount of mental retardation. Its incidence in the population is 1 per 770 live births or 1.3 per 1,000 live births. The incidence increases gradually with maternal age up to the age of 30 and very quickly thereafter. A commonly practiced process for pregnant women aged 35 and older worldwide is amniocentesis to diagnose DS and other chromosome abnormalities. However, although the risk of giving birth to a child with DS is higher after the age of 35, most of the affected children are born of mothers under the age of 35, since there are fewer deliveries after this age. Furthermore, amniocentesis is an invasive procedure that carries a risk of complications, including the iatrogenic loss of an unaffected fetus. No study has been reported that has determined the risk of DS in high-risk pregnancies and the maternal age that could pose the risk of DS from Nepal. Further, the obstetricians who suspect any cases of high-risk pregnancy for DS (i.e., advanced maternal age ≧ 35 years of age or with a history of congenital anomalies in previous pregnancy or family history of congenital defect) request the patient to undergo this test in the neighboring country, India or abroad. Patients who cannot afford the investigations do not undergo the test due to economic constraints, though been advised, and endure fatal complications like IUFD, and infants with DS have medical morbidities. Thus, this test will determine the risk of DS in high-risk pregnancies in Nepalese women and the utility of the tests in our setting.

METHODOLOGY

Research method: Quantitative Method

Types of study: Observational (Descriptive study)

Study population/Sampling frame: All high-risk pregnant women undergoing antenatal checkup in the Department of Obstetrics and Gynecology at TUTH.

Study site and its justification: This descriptive cross-sectional study will be conducted in the Department of Clinical Biochemistry in collaboration with the Department of Obstetrics and Gynecology, TUTH. TUTH is the largest tertiary care center in Central Nepal. It has been providing exemplary patient services through its huge infrastructure, ample bed facility, and quality and experienced doctors and staff. There are around 12,000 deliveries conducted in the Department of Obstetrics and Gynecology. Thus, being an ideal place to conduct the research study focused on the pregnant women of Central Nepal.

Sampling method: Non-probability sampling (Purposive sampling)

Sample size: Based on a study reported by Bhandari et al,[21] the prevalence of congenital defects in the hilly region of Nepal was 56% in the hilly region and 58% in the Terai region. TUTH is the largest tertiary care center located in Maharajgunj, Kathmandu. It is a valley located in the Central Nepal. Hence, we took the prevalence of the hilly region for the sample size calculation.

Taking this into account, the sample size calculation is done by the Cochran formula:

$$n=z^2pq/d^2$$

Where,

n = Desired sample size

z = Standard normal deviation; usually set at 1.96 which corresponds to 95% confidence level.

p = Proportion in the target population estimated to have a particular characteristic

q = 1-p (Proportion in the target population not having the particular characteristics)

d= Degree of accuracy required (Taken as 10% of p for the present study)

The respective values for the variables are:

z =1.96

p = 0.56

q (1-p) = 0.44

d = 0.056

Hence, Keeping the values in the above formula,

$n = (1.96)^2 (0.56) (0.44)/ (0.056)^2$

$n = 3.8416 \text{ X } 0.2464/ (0.056)^2$

$n = 301.84 \approx 302$

Hence, the calculated sample size is 302.

But we could only include 256 pregnant women due to resource constraints (financial constraints).

Sampling technique: Purposive sampling technique

Control groups: No control groups will be included.

Probable duration of study: 1 year

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria:

- All pregnant women ≥ 30 years of age within the gestational age of 15-20 weeks
- History of congenital defects in the previous pregnancy irrespective of the maternal age.
- Family history of DS or any congenital defects irrespective of the maternal age.

Exclusion Criteria:

- Women ≤ 30 years of age without any history of congenital defects in the previous pregnancy.
- Pregnant women who are outside the range of gestational age of 15-20 weeks
- The patient is unwilling to participate.

STUDY VARIABLES:

Demographic Data: Age, parity, geographic location, ethnicity, education, smoking history, pre-pregnancy body mass index, History of any systemic disease, History of previous baby with any genetic abnormalities, family history of any systemic and genetic disease.

Anthropometric Measurement: Height (cms), Weight (kgs) and BMI

Clinical Investigations: Blood pressure (Systolic and Diastolic BP)

Biochemical Parameters:

5 ml of venous blood was collected from pregnant women at 15-20 weeks of gestation in a vial containing clot activator or serum separator (gold or red colored top vial) for the measurement of Alpha-fetoprotein (AFP), human chorionic gonadotrophin (HCG), unconjugated estriol (UE3) and Inhibin-A. Serum levels of AFP, HCG, UE3, and Inhibin-A were estimated by enhanced chemiluminescence immunoassay method. Serum AFP, B-HCG, and UE3 were estimated in Siemens Immulite 1000 Immunoassay system and Inhibin-A was estimated by paramagnetic particle, chemiluminescent immunoassay in Access 2 immunoassay analyzer (Beckman Coulter). Risk estimation using values of hormone levels and the resulting MoM was done using PRISCA 5.0.2.37.

Table 1: Cut-off for risk estimation for Neural Tube Defect, Trisomy 21 and Trisomy 18.

Disease	Inference
Neural Tube Defect	An alpha-fetoprotein (AFP) multiple of the median (MoM) <2.5 is reported as screen negative. AFP MoM ≥2.5 are reported as screen positive.
Trisomy 21	Calculated screen risks <1/250 are reported as screen negative, risks ≥1/250 are reported as screen positive.
TRISOMY 18	Calculated screen risks <1/100 are reported as screen negative, risks ≥1/100 are reported as screen positive.

Expected time and duration of the study: 1 year

Tools and techniques for data collection: A structured proforma was used to record the data of the patients including the demographic and clinical profile, anthropometric and biochemical measurements. Patients were informed about the research process with the help of an information sheet and were recruited only after signing the informed consent.

Plan for data management and statistical analysis:

Data was entered in MS Excel 2010, and analyzed with Statistical Package for Social Sciences (SPSS version 22.0). The normality of the data was checked using the Kolmogorov-Smirnov test. Descriptive and inferential statistics were applied accordingly. For descriptive statistics, Mean, Standard Deviation, Percentage, and Range were calculated. Chi-Square test was used to analyze the categorical variables. For parametric variables, the student's "t" test and for non-parametric variables, the Mann-Whitney U test was used. Correlation between patient age, BMI, and serum AFP, HCG, and UE3 was determined using Spearman's correlation coefficient. Multiple regression analysis was used to evaluate the association of the maternal age, BMI, family history of genetic disease, and history of congenital defect in previous pregnancy with the quadruple screening test. ROC curve was applied for the diagnostic performance of the quadruple screening test; a p-value ≤ 0.05 was considered statistically significant.

RESULTS

We recruited a total of 256 pregnant women who were in the gestational age of 15-22 weeks. The findings from the present study describe that the mean age of the patient was 33.36 ± 3.71 years with the majority of the pregnant females being less than 35 years (73%). The majority of the patients were Brahmin/Chhetris (35%) followed by Janajatis (28%). BMI of the patient was 26.67 ± 4.00 kg/m^2, mean blood pressure (SBP) was 108.81 ± 11.92 and DBP was 70.45 ± 7.73 mm Hg respectively as depicted in **Table 2**. The clinical details of the patients depicted that the majority of the patients were multigravida (72.3%) and multiparous (56%). 2% of the patients were smokers and there was no significant history of consanguineous marriages (0.65%). Medical history of patients revealed that thyroid disorders were present before pregnancy in 6% (n=18) of the patients followed by Diabetes Mellitus (1.97%) and Hypertension (1.6%) respectively as shown in **Table 2**.

We determined the differences in serum levels and MoM of AFP, B-HCG, UE3, and Inhibin-A in high-risk and low-risk populations and found that B-HCG, UE3, and Inhibin-A were significantly different between high-risk and low-risk pregnant women ($p<0.05$) as shown in **Table 3 and 4**.

We found the MoM of quadruple screen markers i.e. for AFP was 1.10 ± 0.47 [Sensitivity=43.8%; specificity=47.2%], B-HCG was 0.73 ± 0.4 [Sensitivity=87.5%; specificity=71.9%]; UE3 was 1.10 ± 0.43[[Sensitivity=31.3%; specificity=30.2%] and Inhibin-A was 1.50 ± 0.71 [[Sensitivity=81.3%; specificity= 85.4%] respectively as represented in **Table 5.**

The risk of Neural tube defect, trisomy 21, and trisomy 18 as calculated by screen test data was 7.9%, 1.9%, and 1.9% respectively as shown in **Table 6.**

Multiple linear regression analysis for the prediction of trisomy 21 using quadruple markers depicted that age, AFP, B-HCG, and Inhibin-A were significantly associated with a high risk of Trisomy 21 ($p<0.05$), while no association was seen with UE_3 levels and risk of Trisomy 21 as shown in **Table 7.**

Receiver operating Characteristics (ROC) curve was plotted to determine the diagnostic accuracy for quadruple test markers (AFP, B-HCG, UE3, and Inhibin-A) compared with traditional triple markers (AFP, B-HCG, and UE3) which depicted that quadruple markers had the higher area under the curve (AUC) i.e., 0.879 versus Triple markers i.e., 0.775. Similarly,

the sensitivity and specificity of quadruple test markers were 82.4% and 87.7% whereas triple markers were 70.6% and 77% respectively as described in **Table 8.**

Table 2: Clinico-demographic profile of the study population (n=256)

VARIABLES	VALUES
AGE	33.36 ± 3.71
≤ 35 years	186 (73%)
≥ 35 years	73 (27%)
Ethnicity	
Brahmin/Chhetri (Hills/ Mountain)	91 (35%)
Brahmin/Chhetri (Terai Madhesh)	54 (21%)
Janajati (Mountain/Hill/Terai)	72 (28%)
Other ethnicities (Dalit, Muslim, Marwari)	39 (15%)
BMI (kg/m$^{2)}$	26.67 ± 4.00
SBP (mm Hg)	108.81 ± 11.92
DBP (mm Hg)	70.45 ± 7.73
Gestational age (weeks)	17.74 ± 2.15
GRAVIDA	
Primigravida	69 (27.8%)
Multigravida	187 (72.3%)
PARITY	
1	77 (30%)
2	143 (56%)
>2	36 (14%)
Personal History	
Smoker	5 (2%)
H/O consanguineous marriage	2 (0.65%)
Past History	
H/O Genetic disease in previous pregnancy	8 (3.3%)
H/O genetic disease in family members	2 (1%)
Medical History	
Hypertension	4 (1.6%)
Type 2 Diabetes Mellitus	5 (1.97)
Thyroid disorders	15 (6%)
Autoimmune Disease (SLE, RA)	2 (1%)
Organ Transplant	1 (0.4%)

Table 3: Biochemical parameters in the study population (n=256)

Parameter	High Risk	Low Risk	p value
AFP	24.89 ± 1.80	23.14 ± 5.78	0.21[a]
B-HCG	11847.82 ± 859.53	18390.04 ± 4597.51	0.003[a*]
UE3	1.25 ± 0.93	0.80 ± 0.62	0.01 [a*]
Inhibin-A	236.09 ± 99.53	435.04 ± 202.27	0.001 [a*]

a= Independent t test; *p value <0.05 is considered to be statistically significant

Table 4: Multiple of Median (MoM) of quadruple markers in high-risk and low-risk population

Quadruple parameters	High Risk (n=	Low Risk	p value
MoM AFP	1.06 ± 0.57	1.09 ± 0.42	0.83[a]
MoM B-HCG	1.27 ± 0.59	0.67 ± 0.33	0.001[a*]
MoM UE3	0.86 ± 0.45	1.12 ± 0.42	0.04[a*]
MoM Inhibin-A	2.67 ± 1.16	1.37 ± 0.51	0.001[a*]

a= Independent t-test; *p value <0.05 is considered to be statistically significant

Table 5: Sensitivity and Specificity of quadruple test parameters in the study population

Parameters	MoM	Sensitivity	Specificity
AFP	1.10 ± 0.47	43.8%	47.2%
B-HCG	0.73 ± 0.41	87.5%	71.9%
UE3	1.10 ± 0.43	31.3%	30.2%
Inhibin-A	1.50 ± 0.71	81.3%	85.4%

Table 6: Trisomy 21, Trisomy 18, and Neural Tube Defect risk in the study population

Risk	Proportion (n=256)
Trisomy 21	20 (7.9%)
Trisomy 18	5 (1.9%)
Neural Tube Defect	5 (1.9%)

Table 7: Multiple linear regression analysis for the prediction of trisomy 21 using quadruple markers

Variables	Coefficient	S.E.	t value	p value
Intercept	0.457	0.193	-	-
Age	0.15	0.005	2.31	0.02*
BMI	0.02	0.004	0.38	0.69
MoM AFP	6.33	2.20	8.27	0.05*
MoM B-HCG	4.51	1.53	8.63	0.009*
MoM UE3	3.90	1.63	5.69	0.30
MoM Inhibin-A	3.81	0.93	16.67	0.001*

Coefficient: Regression coefficient; SE: standard error; $*p$ value <0.05 is considered to be statistically significant

Figure 1: **Receiver Operating Characteristic (ROC) curve to determine the diagnostic utility of Quadruple markers as compared to the traditional triple markers**

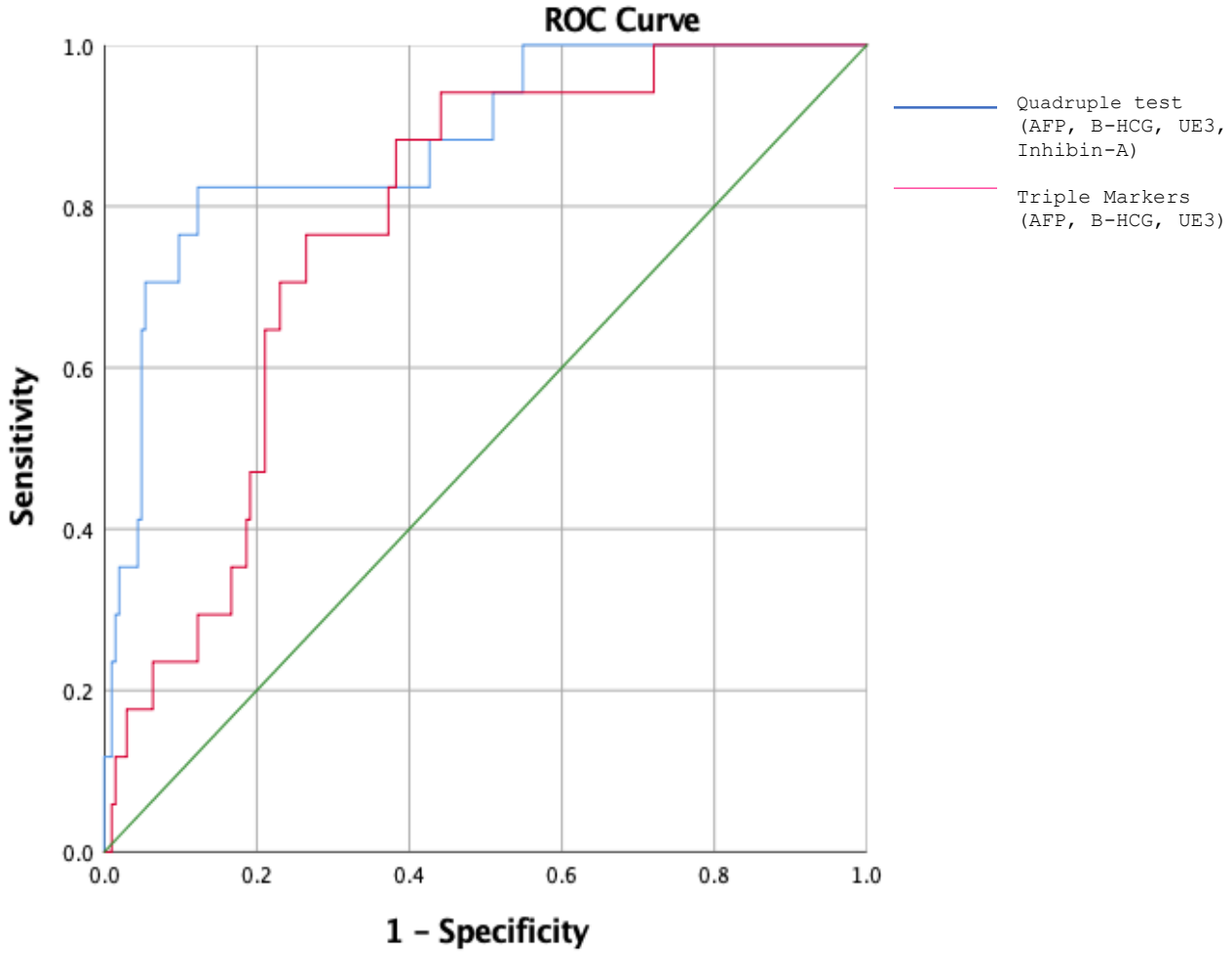

Table 8: Sensitivity and specificity of Quadruple and Triple markers for prediction of Trisomy 21

Variables	Area Under Curve (95% CI)	Sensitivity	Specificity	PPV	NPV
Quadruple Test Markers (AFP, B-HCG, UE3 and Inhibin-A)	0.879	82.4%	87.7%	74.8%	98.7%
Triple Markers (AFP, B-HCG and UE3)	0.775	70.6%	77%	67.46%	77.14%

DISCUSSION

We report the results of second-trimester quadruple screening for DS in the Nepalese population. Our detection rate was 87.7%, which is comparable to most of the quadruple test studies in the world.[22-26]

Any pregnancy can be affected by chromosomal abnormalities in the developing fetus. These disorders could also lead to death before or shortly after birth and/or lifelong infirmities. Early diagnosis of any fetal disorder can aid the parents in deciding whether to continue or terminate the pregnancy.[28-29] Prenatal diagnosis is meant to provide accurate information on the short- and long-term prognosis, risk of recurrence, and potential treatments for the affected fetus.[30]

However, screening tests for fetal abnormalities detection are not 100% accurate and can be problematic. Also, the accuracy of the tests may vary depending on the maternal age and other existing characteristics.[30]

Our study reports that the majority of pregnant women (73%) who underwent quadruple screening tests were ≤ 35 years and 27% were ≥ 35 years. The findings were slightly higher compared to the study reported by Zournatzi V et al[31] (12.1%) and Patne et al[32] (12%) for women ≥ 35 years old.

The overall incidence of DS is reportedly one in 1361[33] to one in 6924 live births and is the most common genetic cause of developmental delay. The risk of DS increases gradually up to the age of 33 years and subsequently increases exponentially till the age of 45 years after which it plateaus.[34] In the United Kingdom (UK), the National Down's Syndrome Cytogenetic Register indicated that without improved screening tools between 1989 and 2008, the continuous rise in maternal age would have caused a 48 percent increase in live births with Down's syndrome.[35] Apart from maternal age, biochemical and ultrasonographic markers introduced since the early 1980s have markedly increased the sensitivity of screening programs.[36] Next-generation sequencing is the latest non-invasive screening tool for the detection of aneuploidies.[37]A pregnancy screened positive for DS on quadruple test, risk re-assessment (in which quadruple test is combined with the genetic sonogram) gives a detection rate of 80 percent with a three percent FPR (ISPD 2015).[38] Nevertheless, in both situations, CVS/amniocentesis remains the gold standard diagnostic test for the detection of DS. The risk of iatrogenic fetal loss with these invasive tests is approximately 0.7-1 percent.[39]

A proper screening modality has not yet been incorporated as national antenatal screening programs in countries like Nepal[40] and India.[41] In the Western world, the screening for chromosomal abnormalities begins as early as the first trimester which is done as a dual test

combined with the nuchal translucency/nasal bone scan. Second-trimester screening begins at 15 weeks in which a quadruple test is done and at 18 weeks a genetic sonogram is done to assess the risk of having a DS child.[36] The results of the genetic sonogram and the quadruple test are combined with the first-trimester screening results. This is called sequential screening for DS and has a detection rate of 95 percent, [36] a method that needs to be adopted universally. In India, in many government and private settings, screening generally begins in the second trimester and the triple test which was introduced in 1988,[42] is still being increasingly used as a second-trimester biochemical screening tool for DS.[40]

The combined test (first and second-trimester test) has a detection rate of 93.8% with a 1.9% false positive rate has not been adopted in many southeast Asian countries like Nepal and India.[43] The reliability of the quadruple test as compared to the triple test in the screening of DS is well established.[12] SURUSS study,[12] 2003 found 77 percent detection rate for triple test against an 84 percent DR for quadruple test keeping the false positive rate of five percent. This study concluded that the quadruple test is a better screening tool but because the only commercially available assay for inhibin A was not suitable for use in a routine laboratory (insufficiently stable and intra-batch assay variation was excessive, 17%), it could not be used in national screening protocols.[12] However, by 2007, the UK National Screening Committee had incorporated the quadruple test in its screening program.[44]

A Taiwanese study[16] assessed quadruple tests in 21,481 women and found a detection rate of 81.1 percent with a 4.4 percent false positive rate. The false positive rate of the triple test in the present study was 21.6 percent whereas the false positive rate for the quadruple test was 17.8 percent and for the quadruple test + genetic sonogram was 8.9 percent.[16]

In a study reported from Gujarat, India,[45] 2111 women were investigated by triple marker screening between 14 and 20 weeks of gestation, of whom 224 women were found to be screen positive for trisomy 21, and further, on karyotyping of 105 of the screen-positive cases, eight had trisomy 21 and one had mosaic trisomy 21 quoting the DR and FPR of that study.[44]

A two-year data of a referral institute from northern India,[46] reported that in four out of 68 women (4.4%) with triple-test positivity for DS, amniotic fluid karyotyping was found to show trisomy 21.[46]

We report a higher concentration of AFP, hCG, uE3, and inhibin A on average than the concentrations established for the Caucasian population.[47] Ethnic differences have been noted in comparisons of black, Caucasian, and Asian populations in Europe and the USA.[47-49] It is known that Asian women have the highest levels of AFP, hCG, and uE3[48-50] and our study was in line with the previous studies. Also, Inhibin-A has been reported to be highest among the

Asian women.[51] However, some studies have reported that black women showed higher levels of Inhibin-A than Caucasian women[52] while no reports have been published comparing the levels between Asian and Caucasian women.

MoM of HCG in Nepalese women who were at high risk for trisomy 21 was 1.27 ± 0.59 which was lower compared to studies reported in Chinese,[49] Korean,[50,] and Caucasian Populations.[52] Though our study has been restricted to a smaller group of the population like other studies [48-50,52], the median MoM of each serum marker in high-risk pregnant women with Down syndrome may reflect racial differences.

In addition, numerous factors contribute to the performance of Down syndrome screening which includes the use of ultrasound scans to estimate gestational age, maternal weight, insulin-dependent diabetes, and smoking.[13, 53-57] We had no patients with insulin-dependent diabetics, while there were only 2 women who were smokers hence, this had a negligible effect on the results.

The findings from our study provide evidence that quadruple test screening is effective where more than 82% of the fetuses with Down syndrome could be prenatally diagnosed. To the best of our knowledge, this is the first-ever study reported from the Nepalese population concerning the median MoM of quadruple and triple test markers. Moreover, nowadays non-invasive prenatal testing (NIPT) seems to be the most attractive and effective test,[58-59] it is impossible to implement it free of charge in developing countries like Nepal for all pregnant women where the quadruple test seems to be a feasible option. However, the exploration of cost-effectiveness and incorporation of quadruple test screening into the national policy program is yet to be established. In addition, we found that the quadruple test seems to be relatively simple in terms of management in large-scale use in developing countries like ours where the use of integrated tests and sequential serum biomarker screening (both stepwise and contingent screens) are complicated and unrealistic. Reports have suggested that pregnant women coming for antenatal visits are common after the first trimester. [60] Thus, this also makes the quadruple test more practical and superior to other prenatal testing in developing nations like Nepal.

CONCLUSION

Quadruple test is highly effective in terms of its sensitivity and specificity in our population compared to the triple test markers. Further, the quadruple test is advantageous in terms of simplicity, feasibility, and relatively lower cost when compared to NIPT and other genetic testing.

Biases: Since, the study was conducted in one tertiary care center, i.e., TUTH, so the results could not be generalized to the whole population.

Limitations of the study: This cross-sectional study recruited pregnant women attending the outpatient department of the Obstetrics and Gynecology department of TUTH, Maharajgunj only. Hence, comprising only a small group of the population. The results therefore cannot be generalized for the other parts of the country.

Safety considerations: All patients were informed about the research purpose and their contribution to this research. After obtaining the informed consent patients were asked for 5 ml of blood for biochemical investigation. Blood was obtained by a trained phlebotomist under aseptic conditions. All the details of the patients were kept confidential.

Plan for supervision and monitoring: The principal investigator along with the respective co-authors was responsible for supervision. Investigators were involved in patient recruitment, data entry, monitoring data entry, and analysis.

Expected outcome of the research:

Primary (main outcome):

- Assessment of risk of DS in high-risk pregnancies in women of Central Nepal.

Secondary Outcome:

- The usefulness of second-trimester quadruple screening test for risk assessment of DS in pregnant women of Central Nepal.
- Estimation of risk of DS in the second trimester between familial and non-familial marriages.

Plan for dissemination of research results: The findings of the research will be presented in the national/international forum and the results will be published in a reputed indexed journal.

Plan for utilization of the research finding: The research finding was provided to the treating obstetricians and the concerned patient. Needful intervention was carried out by the concerned doctor. Prenatal screening practice in Nepal needs to be strengthened and reinforced all over the country. This study was a pilot study for future studies to be undertaken in DS and other genetic disorders and will help to develop an elemental pre-natal screening practice in the country. In addition, we believe that this study will form a basis for the formulation of policymaking in the field of prenatal screening for DS.

Potential Impact of the Project:

Pregnancies affected by fetal DS show an increased risk of intrauterine fetal demise (IUFD). There is an estimated report of loss rates between 5 to 16% after 20 weeks of gestation in pregnancies with fetal DS. There is a high risk of perinatal morbidity and mortality associated with DS. In addition, children with DS demonstrate high rates of numerous morbidities like cognitive disability, congenital heart defects, respiratory and hearing problems, Alzheimer's disease, childhood leukemia, and thyroid disorders compared to the reference population. Diagnosis of DS by screening test (quadruple test) in high-risk pregnant women will determine the likelihood of a baby having this condition or not. As per the sustainable development Goal 3 (SDG 3), healthy lives and well-being for all at all ages should be ensured. Our study fulfills the second priority of SDG 3 which states to end the preventable deaths of newborns and children under 5 years of age by 2030. This is a novel study in the context of our country which intended to screen high-risk pregnant women for DS by quadruple screening tests for early detection of fetuses with DS which can be further managed with cautious medical advice.

Impact at local and National levels: To the best of our knowledge, no study has been reported that has determined the risk of DS in high-risk pregnancies and the maternal age that could pose the risk of DS from our country. Further, the obstetricians who suspect any cases of high-risk pregnancy for DS (i.e., advanced maternal age ≧ 35 years of age or with a history of congenital anomalies in previous pregnancy or family history of congenital defect) request the

patient to undergo this test in the neighboring country, India or abroad. Patients who cannot afford the cost do not undergo the investigations, though been advised, and endure fatal complications like intrauterine fetal death (IUFD) and infants with DS having medical morbidities. Thus, this study aimed to determine the risk of Down syndrome in pregnant women of the Nepalese population and evaluate the quadruple test sensitivity and specificity in our setting. Prenatal screening practice in Nepal needs to be strengthened and reinforced all over the country. Thus, we believe that this study will form a basis for the formulation of policymaking in the field of prenatal screening for DS.

International level: The current scenario of DS in high-risk pregnancies in Nepal is unknown. There is a non-governmental organization working on patients with DS. The screening test is available in a few private laboratories in the capital city with no laboratories offering the test in the periphery. The usefulness of the quadruple test in our setting is still a question until proven by any scientific research. Thus, this research will be a breakthrough for determining the risk of DS in high-risk pregnancies using the quadruple test screening and disseminate the findings to the international platform as well.

BUDGET SHEET

SN	Particulars	Remarks	Total cost (Rs.)
A	**Special Task Based Remuneration** (≤10%)		
	Proposal preparation	*Printing / Binding*	2500
	Tools development	*Data Sheets Printing*	1000
	Progress report preparation	*Printing / Binding*	2000
	Data analysis	*Consultation Charges*	--
	Research article manuscript preparation	--	--
	Special experiment/task (specify)	--	
B	**Wet Laboratory Costs**		
	Equipment and Instruments (specify)	*Existing Facility*	0
	Special Reagents/Kits (specify)	*AFP, ß-HCG, UE3 and Inhibin-A*	3,00,000
	Chemicals	*Control Solutions*	15000
	Consumables (Gloves/Test Tubes/Vacutainers/Cotton/Swabs/Syringes etc.)		5000
	Service and Repair cost	*Reserve*	2500
	Testing service cost	*Existing Facility*	0
	Other (specify)		
C	**Field Costs**		
	Travel cost	*Hospital Based*	0
	Daily allowance (Assistants, Sample Collection)	*Lab Technicians*	15000
	Survey cost (hiring, subjects compensation, refreshment, special need)	Renumeration for Subjects	9000
	Travel gears	Hospital Based	0
	Other (specify)	--	0
D	**Office Costs**		
	Office equipment	--	0
	Personal computer and software	--	0
	Office supplies	Printing Paper / Cartridge	2500
	Telephone and internet cost		2000
E	**Consultant Services**		
	Special Professional Service	Study Design	2500
	Data Analysis	Statistician	
F	**Student Support**		
	Thesis Proposal Preparation Cost	Masters' X 1	2500
	Thesis Preparation Cost	Masters' X 1	10,000
G	**Facilities and Administrative Cost**		
	Institutional Overhead Cost (10%)		15000
	Administrative Travel Cost		5000
	Documentation and Publication Cost		0
H	Software package for Prenatal Down Syndrome Risk Calculation		1,00,000
I	Miscellaneous (<5%)		7500
		Subtotal	4,99,000
		Fund from other source [if identified, specify]	--
		Fund from other source (not yet identified)	--

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ANNEXURES

ETHICAL CLEARANCE FROM INSTITUTE OF MEDICINE

त्रिभुवन विश्वविद्यालय
चिकित्सा शास्त्र अध्ययन संस्थान
डीनको कार्यालय, महाराजगंज
पो.ब.नं.: १५२४, काठमाडौं, नेपाल ।
फोन नं. ४४१०९११, ४४१२०४०, ४४१३७२९, ४४१८१८७

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पत्र संख्या / Ref.:- 159(6-11)E2 078/079

मिति / Date:-

October 04, 2021

Research Department

Dr. Apeksha Niraula
Lecturer
Dept. of Clinical Biochemistry
MMC, IOM

Ref: **Approval of Research Proposal**

Dear Dr. Niraula

Thank you for the submission of your research proposal,entitled **"Screening of high-risk pregnancies for down syndrome using quadruple test at a tertiary care centre of Nepal."**

I am pleased to inform you that after careful evaluation, the above mentioned research proposal has been approved by Institutional Review Committee (IRC) of Institute of Medicine (IOM), Tribhuvan University on October 03, 2021.

As per our rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in title, objectives, problem statement, research questions or hypothesis, methodology, implementation procedures, data management and budget may be made so and implemented only after prior approval from IRC. Thus, it is compulsory to submit the details of such changes intended with justifications prior to actual change in the protocol.

Please note that you can start recruiting the research participants only after getting approval letter from the IRC. You are also requested to follow the ethical guidelines of IRC of IOM.

After completion of your study, you must submit a copy of final draft of your research to the Research Department.

If you have any further queries, please do not hesitate to contact us.

.....
Prof. Dr. Mohan Raj Sharma
Member Secretary and Research Director
Institutional Review Committee

Fax No. 4418186, E-mail: iomdean@iom.edu.np / website: www.iom.edu.np

PROFORMA SHEET

Title: Screening of high-risk pregnancies for Down Syndrome using the quadruple test at a tertiary care center in Nepal

_____

Signature of Researcher/Date

Name: **Address:** **State:**	**DOB/ Age:**	**GA at Screening Date:** **Estimated Birth Date:** **LMP:**
Pregnancy Type: **Singleton:** **Multiple** Obstetric History: Gravida: Parity: Living: Abortion:	**Undergoing IVF:** **Yes** **No** **Surrogacy: Yes** **No** **Egg Donor: Yes** **No**	**Radiological Findings:** Ultrasound Date: Nuchal Translucency (mm): Crown Rump Length:
Clinical Indication for Testing:		
Family History: H/O Hypertension: H/O Genetic Diseases: **Past History:** Any systemic disease:		**Gynecologist:**
Anthropometric Measurements: Height (Cm): Weight (Kg) at 1st antenatal checkup: Blood Pressure:	**Pregnancy-related details:** Smoking: Diabetes: Ovulation Induction: Donor/Frozen Ova: Twins Pregnancy:	
Biochemical parameters: **Blood/ Serum parameters:** AFP (ng/ml): B-HCG (mIU/ml): Unconjugated Estriol (ng/ml): Inhibin-A (pg/ml)		

Note: These tests do not reveal the gender of the fetus.

Patient Consent: I have been explained the risks, benefits, limitations, and implications of the quadruple test and by signing, I am indicating my consent to take this test and I understand that no therapeutic decisions should be made based on the screening results. I also give permission for my sample data to be used in a research study being conducted at the Institute of Medicine, TUTH to improve quadruple tests for other patients and for publications, if appropriate.

Date: Patients Signature:

Providers Statement: I acknowledge the risks, benefits, and limitations of the quadruple test. I have discussed the test(s) with the patient/guardian and have answered his/her questions regarding the tests in a language she/they understand. I have also provided the patient with the purpose, limitations, and possible risks of the tests.

Date: Medical Provider's Signature:

PARTICIPANT INFORMATION SHEET

Title of the Research: **Screening of high-risk pregnancies for Down Syndrome using quadruple test at a tertiary care centre of Nepal**

I am Dr. Apeksha Niraula, Lecturer in the Department of Clinical Biochemistry at Institute of Medicine, TUTH, Maharajgunj. I am going to conduct a research on Screening of high-risk pregnancies for Down Syndrome using quadruple test at a tertiary care centre of Nepal.

I am going to give you information and invite you to be the part of this research. You do not have to decide instantly whether or not you will participate in the research. You can have a talk with anyone about the research and take your decision. Please feel free to ask any words that you could not understand in this information, so that I may explain more clearly taking few more times. You can also have your queries with me, the study doctor or the staff.

The importance of this research is to determine the risk of Down Syndrome in high-risk pregnancies using quadruple test (Alpha fetoprotein, beta-human chorionic gonadotrophin, Unconjugated Estriol and Inhibin-A) in Nepalese women. This test will be done in the second trimester and will evaluate the risk of Down syndrome if present in the fetus.

Participant selection: We will be enrolling all the patients ≥ 30 years of age, patient with the history of congenital defects in the previous pregnancy and patients with family history of Down syndrome or any congenital defects.

Voluntary Participation: Decision for participation in this research should be taken by yourself. It will have no influence in the study and outcome whether you participate in the study or not. If you don't like to participate, you are free to withdraw at any time during the study.

Expected duration of the subject: Study will be completed in 1 year.

Procedures and Protocol: A detail history including obstetric history, h/o any systemic disease or previous pregnancy with any genetic disease will be noted. Biochemical parameters will be estimated by Enhanced Chemiluminescence Immunoassay in Vitros 3600 (Ortho Clinical Diagnostics).

Maintenance of Confidentiality: Confidentiality of each participant in the study will be considered strictly. Information about you that will be collected during the research will be put away and no one will be able to see it except the researchers.

Any information about you will be coded with a number instead of your name. Only the researchers will know your code number and all the information about you will be locked. It will not be shared with or given to anyone except those working within the Department of Biochemistry. Freedom of individual to participate and to withdraw from research at any time without penalty or loss of benefits to which the subject would otherwise be entitled.

Amount of Sample taken: 5 ml of venous blood and sample will be taken from each participant.

Cost and source of investigations: The cost and sources of investigation will be applied for Grant and elsewhere by the candidate herself.

Sharing the Results: The report of the blood chemistry will be given to the participants. The knowledge that is gained from this research will be published in a scientific research journal and disseminated in scientific forums.

Whom to Contact:
If you have any queries you may ask me now or later, even after the study has been started. If you wish to ask questions later, you may contact:
Dr. Apeksha Niraula
Department of Clinical Biochemistry, IOM, TUTH, Maharajgunj, Kathmandu, Nepal.
Contact No: +9779852029730.
Email: apeksha.niraula@iom.edu.np

जानकारी पत्र

शिर्षक : डाउन सिन्ड्रोमको निर्धारणका लागि गर्भवती अवस्थामा रगतको परिक्षण

म, डा. अपेक्षा निरौला, चिकित्साशास्त्र अध्ययन संस्थान, त्रि.वि. शिक्षण अस्पताल, महाराजगंजको जीवरसायन विभागमा उप-प्राध्यापकको रुपमा कार्यरत छु । माथि उल्लेखित शिर्षकमा अनुसन्धान गर्न चाहन्छु र हजुरहरुलाई पनि यस अनुसन्धानमा सहभागी हुनका लागि बिनम्र अनुरोध गर्न चाहन्छु ।

यस अनुसन्धानमा डाउन सिन्ड्रोमको निर्धारण लागि गर्भवती अवस्थामा रगतको परिक्षण गरिने छ। । यसको लागि रगतमा तीनवटा जांच गरिने छ । मंजुरिनामा स्वेच्छाले हस्ताक्षर गरेर सहभागी हुने चाहना भएका व्यक्तिहरुलाई मात्र अध्ययनमा समावेश गरिने छ ।

यस अनुसन्धानमा हजुरको सम्पूर्ण परिक्षण निःशुल्क हुनुको साथै आवश्यक सावधानी अपनाइने छ । हजुरले दिनुभएको जानकारी गोप्य राखिने छ । प्रयोगशाला परिक्षणबाट आएको नतिजाको बारेमा र त्यसको असरहरु बारे अवगत गराइने छ । यस अनुसन्धानात्मक अध्ययनमा यहाँको सहभागिता स्वागतयोग्य रहने छ । यहाँको सहभागिता पूर्णरुपले स्वेच्छिक रहने छ र कुनै पनि बेला यो अध्ययनबाट बाहिरिन सक्नु हुन्छ। साथै यसबाट स्वास्थ्यमा कुनै हानी वा नकारात्मक असर नहुने जानकारी पनि गराइनेछ।।

सम्पर्क:

डा. अपेक्षा निरौला
उप-प्राध्यापक
जीवरसायन विभाग
चिकित्साशास्त्र अध्ययन संस्थान
त्रि.वि. शिक्षण अस्पताल
महाराजगंज
मोबाइल नम्बर: ९८५२०२९७३०
इमेल- apeksha.niraula@iom.edu.np

INFORMED CONSENT FORM

Quadruple Test को लागि रगत परीक्षणको लागि सूचित सहमति फारम

▪ Quadruple testing भनेको के हो?

◊ गर्भवती महिलाहरूको लागि Quadruple screening (गर्भावस्थाको 14 देखि 22 हप्तासम्म) मा 4 क्वाड मार्कर (Alpha fetoprotein, beta-human chorionic gonadotrophin, Unconjugated Estriol र Inhibin-A) रगतमा परीक्षण गरिन्छ |

◊ यस परीक्षणले क्रोमोसोमल असामान्यताहरू (डाउन सिन्ड्रोम-ट्राइसोमी 21, एडवर्ड सिन्ड्रोम-ट्राइसोमी- 18) र न्यूरल ट्यूब दोषहरू को लागि उच्च-जोखिम वा कम-जोखिमको रूपमा वर्गीकृत गर्न यो परीक्षण ले मद्दत गर्दछ| यसले बच्चा प्रभावित हुने जोखिम पहिचान गर्दछ। यो परीक्षण गर्भावस्थाको १४ देखि २२ हप्ताको बीचमा गरिन्छ। यद्यपि, यो परीक्षणको लागि उत्तम समय गर्भावस्थाको 15 र 20 हप्ताको बीचमा हो। यस परीक्षणको साथ अनुमानित पत्ता लगाउने दर 75% छ र 5% को गलत सकारात्मक दर रहेको छ|

▪ क्वाड्रपल स्क्रीनिंग टेस्ट द्वारा प्रदान गरिएको जानकारी:

◊ क्वाड्रपल स्क्रिन टेस्ट भनेको गर्भावस्थामा बच्चामा क्रोमोसोमल र न्युरल ट्युब डिफेक्टको जोखिम छ कि छैन भनी निर्धारण गर्न गरिने रगत परीक्षण हो।

◊ नकारात्मक परीक्षणले रोगको उपस्थितिलाई अस्वीकार गर्दैन किनकि परीक्षणको संवेदनशीलता 75%मात्र रहेको छ।

◊ क्वाड्रपल स्क्रिन टेस्टले मेरो बच्चा स्वस्थ छ वा सम्बन्धित रोगबाट मुक्त छ भन्ने ग्यारेन्टी गर्दैन।

◊ परिणाम व्याख्या साहित्य र वैज्ञानिक डाटाबेस मा हाल उपलब्ध जानकारी मा आधारित छ।

▪ <u>क्वाड्रपल स्क्रीनिंग टेस्टमा जाँच गर्न प्यारामिटरहरु:</u>

▪ यो परीक्षणले रगतमा चार मार्कर AFP, Unconjugated Estriol, β-HCG र Inhibin A को स्तर नाप्छ, जसलाई क्वाड मार्कर परीक्षण भनिन्छ।

1. AFP एक प्रोटिन हो जुन भ्रूण द्वारा उत्पादन गरिन्छ। यदि AFP को स्तर उच्च छ भने, यसले भ्रूणमा न्यूरल ट्यूब दोषहरू देखाउँछ वा भ्रूणको पेट अपूर्ण बन्द हुन सक्छ भन्ने संकेत गर्दछ। कम AFP स्तरको अर्थ डाउन सिन्ड्रोम भएको बच्चा जन्माउने उच्च जोखिम हुन सक्छ भन्ने जनाउछ |

2. HCG हर्मोन प्लेसेन्टा कोशिकाहरु द्वारा उत्पादन गरिन्छ। यदि एचसीजीको स्तर कम छ भने, यसले गर्भपात वा एक्टोपीक गर्भावस्थालाई संकेत गर्दछ। सामान्य भन्दा बढी B-HCGको स्तरले डाउन सिन्ड्रोम भएको बच्चा जन्माउने जोखिम बढ्न सक्छ भन्ने जनाउछ |

3. Estriol एस्ट्रोजन हर्मोनको एक रूप हो जुन भ्रूण र प्लेसेन्टा दुबैमा हुन्छ। यदि एस्ट्रियोलको स्तर कम छ भने, यसले डाउन सिन्ड्रोमको साथ बच्चा जन्माउने जोखिमलाई संकेत गर्दछ विशेष गरी जब AFPको स्तर कम हुन्छ HCG उच्च हुन्छ।

4. Inhibin-A एक हार्मोन हो जुन प्लेसेन्टा द्वारा उत्पादन गरिन्छ। यो एक डाइमर हो जसको मतलब यसको दुई भागहरू छन्। यसलाई कहिलेकाहीँ DIA वा dimeric Inhibin A पनि भनिन्छ। गर्भावस्था (गर्भावस्था) को 14 देखि 17 हप्तामा, मातृ रगतमा Inhibin-A को स्तर थोरै घट्छ र फेरि बढ्छ। Inhibin-A को बढेको स्तरले डाउन सिन्ड्रोम भएको भ्रूणलाई संकेत गर्छ। Inhibin-A ले डाउन सिन्ड्रोमका केसहरू सही रूपमा पहिचान गर्नको लागि संवेदनशीलता र विशिष्टता बढाउँछ |

<u>नमूना</u>

◊ खाली पेट अनिवार्य छैन।

◊ ५ एमएल (३ एमएल न्यूनतम) बाट सीरम |

◊ 14-22 हप्ता बीच मान्य । (उत्तम:15-20 हप्ता)

▪ क्वाड्रपल स्क्रीनिंग टेस्ट परीक्षणको सीमाहरू:

◊ क्वाड्रपल टेस्ट दोस्रो-ट्रिमेस्टर स्क्रीनिंगको महत्त्वपूर्ण भाग हो |

◊ परीक्षणहरूले सधैं निश्चित जवाफ प्रदान गर्दैन।

◊ कतिपय अवस्थामा, यदि रोग अवस्थित छ भने पनि परीक्षणले रोगको पहिचान नहुन सक्छ |

◊ यो वर्तमान चिकित्सा ज्ञान वा परीक्षण प्रविधि मा सीमितता को कारण हुन सक्छ। परीक्षण परिणामहरू क्लिनिकल निष्कर्षहरू, पारिवारिक इतिहास र अन्य प्रयोगशाला डेटाको सन्दर्भमा व्याख्या गरिन्छ।

▪ अस्वीकरण (Disclaimer):

◊ क्वाड्रपल स्क्रीनिंग टेस्टले तपाईंको बच्चालाई डाउन सिन्ड्रोम छ कि छैन भनी बताउन यी परीक्षणहरूले पुष्टि गर्दैनन्।

◊ पत्ता लगाउने दरहरू तपाईंको उमेर अनुसार भिन्न हुन्छन्।

◊ गलत सकारात्मक नतिजा हुने दरहरू अर्थात् तपाईलाई डाउन सिन्ड्रोम भएको बच्चा जन्माउने उच्च जोखिम छ भनिएको र त्यसपछि तपाईको बच्चालाई डाउन सिन्ड्रोम नभएको थाहा पाउनु उमेर अनुसार फरक-फरक हुन्छ।

▪ बिरामीको सहमति:

◊ **मैले सूचित सहमति कागजात पढेको छु र म अनुसन्धानकर्तालाई वर्णन गरिए अनुसार क्वाड्रपल स्क्रीनिंग टेस्टमा परीक्षण गर्न अनुमति दिन्छु। म अन्य बिरामीहरूको लागि परीक्षण विधि सुधार गर्न अनुसन्धान अध्ययनमा मेरो रगतको नमूना प्रयोग गर्ने अनुमति पनि दिन्छु ।**

Date: Patient's Name and Signature

SAMPLE REPORTS

Tel : 01-5352421

Government of Nepal
Ministry of Health & Population
Department of Health Services
National Public Health Laboratory
Teku, Kathmandu

Tel : 01-5352421
E-mail : nphl@nphl.gov.np

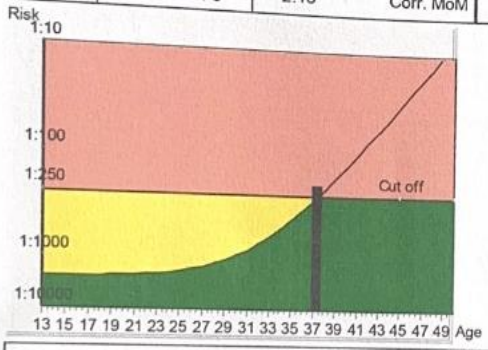
National Public Health Laboratory, Teku, Kathmandu

Result Down's syndrome screening

Name	GAIRE	Sample ID	Q036	diabetes	no
Patient ID	SRIJANA	D.O.B.	4/29/1986	Fetuses	1
Day of serum taking	RC-36	Age at delivery	37.3	Smoker	no
Date of report:	3/8/2023	Weight [kg]	65 kg	IVF	no
Previous trisomy 21 pregnancies	3/14/2023			Ethnic origin	Asian
	unknown				

Corrected MoM's and calculated risks

AFP	13.3	IU/mL	0.58	Corr. MoM	Gestational age at sample date	15 + 1
uE3	0.43	ng/mL	1.13	Corr. MoM	determination method	Scan
HCG	22303	mIU/mL	0.65	Corr. MoM	Physician	Dr. Bishal Khaniya
Inh-A	359.7	pg/ml	2.18	Corr. MoM		



Tr.21 risk
at term
1:196

Age risk
at term
1:252

Down's Syndrome Risk

The calculated risk for Trisomy 21 is above the cut off which represents an increased risk.
After the result of the Trisomy 21 test it is expected that among 196 women with the same data, there is one woman with a trisomy 21 pregnancy and 195 women with not affected pregnancies.
The calculated risk by PRISCA depends on the accuracy of the information provided by the referring physician.
Please note that risk calculations are statistical approaches and have no diagnostic value!

Neural tube defects risk	Risk for trisomy 18
The corrected MoM AFP (0.58) is located in the low risk area for neural tube defects.	**The calculated risk for trisomy 18 is < 1:10000, which indicates a low risk.**

below cut off | Below Cut Off, but above Age Risk | above cut off | Prisca 5.2.0.15 SP 2

Government of Nepal
Ministry of Health & Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

Tel : 01-5352421
E-mail : nphl@nphl.gov.np

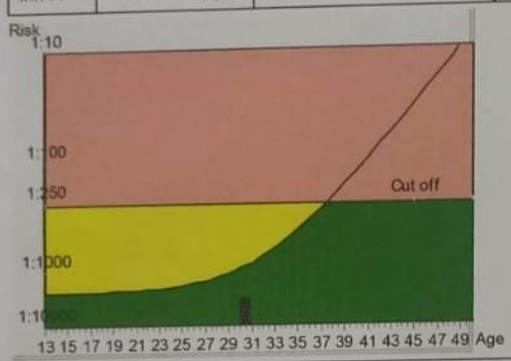
National Public Health Laboratory, Teku, Kathmandu

Result Down's syndrome screening

Name	KALIKOTE KABITA	Sample ID	Q116	diabetes	no
		D.O.B.	29/06/93	Fetuses	1
Patient ID	RC-116	Age at delivery	30.4	Smoker	no
Day of serum taking	03/08/23	Weight [kg]	70.15 kg	IVF	no
Date of report:	09/08/23			Ethnic origin	Asian
Previous trisomy 21 pregnancies	unknown				

Corrected MoM's and calculated risks

AFP	48.3	IU/mL	0.76	Corr. MoM	Gestational age at sample date: 21 + 5
uE3	2.42	ng/mL	1.17	Corr. MoM	determination method: Scan
HCG	10439	mIU/mL	0.62	Corr. MoM	Physician: Dr. Hima Rijal
Inh-A	222.4	pg/ml	1.21	Corr. MoM	



Tr.21 risk
at term
1:4060

Age risk
at term
1:915

Down's Syndrome Risk

The calculated risk for Trisomy 21 is below the cut off which represents a low risk.
After the result of the Trisomy 21 test it is expected that among 4060 women with the same data, there is one woman with a trisomy 21 pregnancy and 4059 women with not affected pregnancies.
The calculated risk by PRISCA depends on the accuracy of the information provided by the referring physician.
Please note that risk calculations are statistical approaches and have no diagnostic value!

Neural tube defects risk	Risk for trisomy 18
The corrected MoM AFP (0.76) is located in the low risk area for neural tube defects.	**The calculated risk for trisomy 18 is < 1:10000, which indicates a low risk.**

below cut off | Below Cut Off, but above Age Risk | above cut off | Prisca 5.2.0.15 SP 2