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## RESEARCH ARTICLE

### HUMAN CHORIONIC GONADOTROPIN (HCG) AND ALPHAFETO-PROTEIN (AFP) IN SUDANESE PREGNANT WOMEN USING IMMUNORADIOMETRIC ASSAY

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#### ABSTRACT

In this study 672 pregnant Sudanese women were involved in order to determine the reference values of human chorionic gonadotropin (hCG) and alpha feto protein (AFP). Blood samples were collected from different maternity centers in Khartoum and Omdurman maternity. Sensitive immunoradiometric assay (IRMA), method was used for measuring HCG and AFP in maternal serum. The data collected reveals that, the behavior of both AFP and hCG resemble that of the international one, where the peak concentrations of hCG are reached at 7-9 weeks of pregnancy then decrease, then staying relatively constant during the second trimester and increasing slightly towards term. The maternal serum concentration of AFP increases during pregnancy, reaching its peak during the last trimester. The concentration of AFP and hCG in maternal serum with relative couples was also compared to that of irrelative couples. Relative couples showed significant increase in maternal AFP level in the 1<sup>st</sup> and 3<sup>rd</sup> trimesters ( $p= 0.001& 0.000$ ) respectively. The hCG concentration in both groups was not significantly different throughout the pregnancy ( $p >0.15$ ). It is recommended that each laboratory establishes its own normal values. Since Sudanese obstetrician depends previously on values from abroad, this study may help them to handle their patients depending on our own reference values.

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## INTRODUCTION

**Human chorionic gonadotropin (hCG):** Human chorionic gonadotropin (hCG) which consists of 237 amino acids and approximately 30% carbohydrates, belongs to a family of closely related glycoprotein hormones, including the lutenizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). hCG resembles LH structurally and exerts its action through the same receptor. Methods for the determination of hCG play an important role in the diagnosis of some of the most common problems in obstetric and gynecological practice. Lack of vaginal ultrasonography in Sudan where it is still developing leads to think in how to get benefit of the available reagents for the determination of serum hCG in early pregnancy detection.

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HCG is also an extremely sensitive marker for trophoblastic tumors. This is due to the combination of very low background levels in non-pregnant women and an extremely high specific production of hCG by trophoblasts, i.e.  $10^7$  choriocarcinoma cells produce enough hCG to increase the measured serum levels (Braunstein, 1973). Relapse of a trophoblastic tumor, which often indicates development of malignant trophoblastic disease can therefore be rapidly detected. In most cases, simultaneous determination of hCG and hCG $\beta$ , allows differentiation between benign and malignant disease at presentation. An hCG $\beta$ /hCG ratio above 5% (calculated on the basis of molar concentration) is a specific sign of chorionic cancer (Stenman, 1985; Ozturk, 1988; Ghazaeli, 1989).

**Physiology:** The syncytiotrophoblasts of the placenta produce hCG in large quantities. In pregnancy an increase in serum hCG can normally be detected 22-24 days after the last menstrual period (LMP) or 7-9 days after conception. This

corresponds to the time of implantation of the conceptus in the endometrium. Peak concentrations of hCG are reached at 7-9 weeks of pregnancy. After this the levels decrease, then staying relatively constant during the second trimester and increasing slightly towards term (Ulf-Hakan, 1994). After giving birth the hCG levels again drop below the limit of detection at a half-life of 24-36 hours.

At day 31, when the hCG level is about 700 IU/L, the gestational sac is large enough to be visible by vaginal sonography (Cacciatore, 1990). Values above normal distinctly indicate multiple pregnancies, while hCG values that are too low are observed in non-intact pregnancies (e.g. ectopic pregnancy, threatened abortion). Pathologically increased hCG concentrations occur in the presence of trophoblastic tumors and also in a large number of non-trophoblastic tumors. Thus the hCG test is suitable for clinical application in gynecology and oncology for the detection and control of the course of pregnancies and tumors. The urine concentration of hCG are similar to those in serum, However, the urine concentration are dependent on urine volume and in dilute urine the hCG concentration is reduced in proportion to the dilution factor. Even after correction for dilution, the results are not suitable for follow up of hCG level during pregnancy (Ulf-Hakan, 1994).

#### **Diagnosis of Pregnancy and Pregnancy-Related Disorders**

**Ectopic Pregnancy:** Diagnosis of pregnancy is the single most important application of hCG determination. For this purpose urine sample are generally used. A positive pregnancy test tells nothing about whether a pregnancy is normal or not, but a negative pregnancy test (with a cut-off of 25-50 IU/L) reliably excludes ectopic pregnancy (Stenman, 1983), which is the most common serious complication of early pregnancy. A suspicion of ectopic pregnancy is mostly evoked by symptom of lower abdominal pain and/or vaginal bleeding in a patient with a positive pregnancy test. The diagnosis can be confirmed by excluding an intrauterine pregnancy using ultrasound and quantitative determination of serum hCG. IF the level of hCG in serum exceeds 1000 IU/L, an intrauterine gestational sac should be visible by vaginal sonography in an intrauterine pregnancy, and its absence is a strong indication for ectopic pregnancy. Monitoring of the patient by sonohrophy and serial serum hCG determination enable the clinician to differentiate between spontaneous abortion, ectopic pregnancy and a normal pregnancy complicated by pelvic or abdominal disease (Cacciatore, 1990).

**Conservative treatment of ectopic pregnancy:** The frequency of ectopic pregnancies has increased mainly due to an increase in pelvic inflammatory disease and in the use of IUD's (5). Ectopic pregnancies are now diagnosed and treated earlier than before as a result of the combined use of ultrasound and sensitive pregnancy test. While this has greatly reduced the complications of a potentially life threatening disease, it has also led to unnecessary treatment. A considerable numbers of ectopic pregnancies abort spontaneously and consequently does not require treatment. Conservation treatment is now studied as an alternative to surgery, since if surgery can be avoided, the prospects for future fertility are improved. In patients with mild symptoms and a serum hCG level which is below 2000 IU/L and which is decreases during follow- up, conservative management is often successful.

Monitoring hCG at 2-3 days intervals is essential in conservative treatment (Korhonen, 1994). For this purpose quantitative assay for serum hCG with a large working range are most useful.

**Early detection of ectopic pregnancy in high risk patients:** Patients with a history of ectopic pregnancy carry a high risk of this complication in later pregnancies. Monitoring such patients during attempts to conceive facilitates early diagnosis. Recent studies have shown that this can be achieved by serial determination of hCG and sonography. Ectopic pregnancies are quite common in these patients and they can be detected reliably before symptoms appear. At this stage treatment is safe and complications threatening later fertility can be avoided (Cacciatore, 1994).

**Alpha fetoprotein (AFP):** AFP is a glycoprotein with a molecular weight of about 70000. It is formed during pregnancy in the fetal liver and the yolk sac. It is detectable in the embryonic serum from the sixth week of pregnancy onwards, rising to a maximum concentration between

the 13<sup>th</sup> and 15<sup>th</sup> weeks of pregnancy and then decreasing to lower values, at which levels it remains until birth. After birth the AFP concentration in the serum of the new born infant fall very rapidly and are usually within the range of the adult values when the infant is 4 to 6 weeks old. The pattern followed by the concentrations in the amniotic fluid is similar to that in the fetal serum (max. 14<sup>th</sup> to 16<sup>th</sup> week of pregnancy), but is lower by a factor of ~100. The fetal AFP passes into the maternal circulation from the amniotic fluid and is metabolized in the liver. The physiological significance of AFP is still not known for certain. It is thought that AFP may be a fetal albumin.

The maternal serum concentration of AFP increases during pregnancy, reaching its peak during the last trimester (34<sup>th</sup> – 35<sup>th</sup> week of pregnancy). Raised serum AFP concentrations are determined in patients with liver disorders such as hepatitis, cirrhosis of the liver and liver cell carcinoma. Fetal trisomy 21 Determination of hCG and AFP in maternal serum during second trimester are used for prenatal diagnosis of fetal trisomy 21 (Cacciatore, 1994). For all the above, the laboratory can now provide clinician, with appropriate tools for solving some of the most common problems associated with pregnancy. For diagnosis of pregnancy and pregnancy related disorders, most of the pregnancy tests, currently available are well suited. A detection limit of 25-50 IU/L is sufficient, and further lowering of this limit does not offer any practical advantage. In contrast, this increased the risk of false positive results in perimenopausal women.

#### **Objectives of the study**

- Establishment of the normal region for hCG in Sudanese pregnant women.
- Follow-up of pregnancy-related disorders (ectopic pregnancy, early fetal loss, and early detection of ectopic pregnancies in high-risk patients) using serial determinations for hCG in combination with ultrasonography.
- Prenatal diagnosis of fetal trisomy 21 through serial determinations for hCG and AFP during second trimester.
- Early detection of malignant and benign trophoblastic disease through the determination hCG.

**Table 1. The HCG and AFP levels in relative and irrelative couples**

trimester	Mean log hCG		p	Mean log AFP		p
	relative	Irrelative		relative	Irrelative	
1 <sup>st</sup>	4.66	4.63	0.168	1.06	0.99	0.001
2 <sup>nd</sup>	4.25	4.26	0.86	1.86	1.85	0.22
3 <sup>rd</sup>	4.231	4.233	0.56	2.10	1.97	0.000

**Table 2. AFP in Sudanese (SUD) and French (FR) maternal serum**

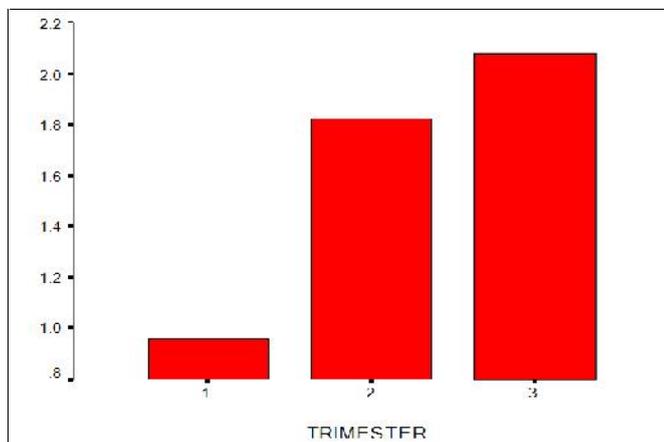
Gestational age (in weeks)	SUD Range (ng/ml)	FR range (ng/ml)	SUD Median	FR median
15	3.2-53	16.3-70.7	25.2	27.2
16	5.4-143	21.2-92	41	35.4
17	21-132	24.7-105	38	40.3
18	24-95	27.7-120	46.5	46.2
19	24-114	34.5-149	39	57.5
20	34-257	39-170	49	65.5
21	27-183	-	68	-

**Table 3.**

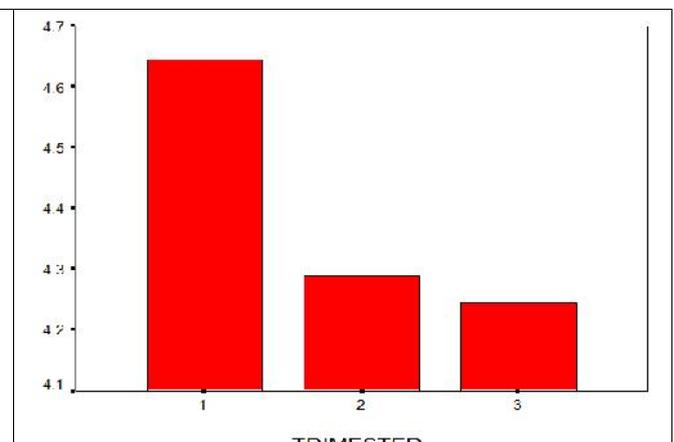
Gestational age (in weeks)	Mean (ng/ml)	SD	Mean ± 1SD	number
14-15	27.6	21.2	6.4 - 48.8	51
16-17	48.1	42	6.0 - 90.0	46
18-19	54.2	45	9.0 - 99.0	62
20-21	98.1	92.4	15.0 - 190.4	32
22-23	121	79.9	41.0 - 201.0	55
24-25	135.6	104	32.0 - 240.0	44

**Table 4.**

Gestational age (in weeks)	Mean (mIU/mL)	SD	Mean±1SD	number
8-9	90494	66164	24330-156658	44
10-11	85233	57010	28223-142243	38
12-13	53802	41803	11999-95605	30
14-15	50723	31682	19041-82405	51
16-17	26365	14415	11950-40780	46
18-19	22353	11875	10478-34228	62
20-21	19129	9975	9154-29104	32
22-23	15916	7876	8049-23783	55



**Fig. 1. Human chorionic gonadotropin in different trimesters**



**Fig. 2. Alpha feto protein in different trimesters**

**Subjects, materials and methods**

For the determination of the normal values, samples were taken between 5<sup>th</sup> and 40 current week of gestation. Approximately 40 patients for each week were investigated. An appropriate amount of blood was taken from the patient (3ml), and the serum was separated by centrifugation after 30-60 minutes and used directly in the assay, or stored for up to 24 hrs at 2-8 C°, or for longer period below -20 C°. Control samples from healthy non-pregnant women were included in this study (approximately 100 samples).

**Assay method:** A wide variety of methods can be used for quantitative determination of hCG. The radioimmunoassay initially used have largely been replaced by sandwich type immunometric assay based on two monoclonal antibodies. Very sensitive immunoradiometric methods have been described (Odell, 1989; Marcillac, 1992). Immunoradiometric assays can be optimized to determine the concentration of hCG during the third trimester without dilutions of the sample. This is an advantage when hCG determinations are used for diagnosis of Down's syndrome. RIA-Gnost® hCG reagents from CIS bio international (France), permits the in vitro

determination of intact, biologically active chorionic gonadotropin (hCG) in serum. Sera with hCG values out of the measuring range were diluted. The kit contains an assay buffer, which makes it possible, in addition to its use as the incubation medium, to prepare the dilutions. The measuring range is (0-1000 mIU hCG/ml).

## RESULTS

The number of samples used in this study was 672 obtained from Sudanese pregnant women. The data obtained from the AFP and HCG measurements were processed using SPSS. Since, the number of subjects was very big, we normalize its distribution by converting to log.

**Maternal serum HCG and AFP:** Figure 1 and 2 showed the levels of AFP and HCG in each trimester. It is obvious that the level reach maximum in trimester 3 and 1 for AFP and HCG respectively. HCG and AFP in pregnant with relative and irrelative couples. The comparison between the levels of HCG and AFP in relative and irrelative couples reveals that; there was no significant difference between the relative and irrelative couples in the level of hCG in different trimester ( $p=0.16, 0.86$  and  $0.56$ ) for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimesters respectively as shown in Table (1). However, the level of AFP was significantly different between them in the 1<sup>st</sup> and the 3<sup>rd</sup> trimester ( $p=0.001$  &  $0.000$ ) as shown in table 1 and 3. In the 2<sup>nd</sup> trimester there was no significant difference concerning the level of AFP ( $p=0.22$ ). The maximum and minimum concentrations for AFP and HCG by gestational age. It is recommended that each laboratory establishes its own normal values. The following values of AFP obtained in healthy females donors and values obtained from France through Immunotech (Table 2) for comparison.

**Reference range for AFP & HCG in Sudanese maternal serum:** The reference range for both AFP and HCG are calculated as the mean  $\pm$  1SD 3.4.1 AFP

## DISCUSSION

In this study 672 pregnant women in different gestational ages were involved. Serum hCG and AFP were measured. The data obtained was extremely scattered. So, the analysis through SPSS statistical package was done after its normalization. Determination of hCG plays an important role in the diagnosis of some of the most common problems in obstetric and gynecological practice. This can not be achieved unless we determine the reference range for our population. The data obtained in figure (1) have the same behavior of the international data shown by (Ulf-Hakan et al, 1994 (Ulf-Hakan, 1994). The physiological significance of AFP is still not known for certain. It is thought that AFP may be a fetal albumin. The maternal serum concentration of AFP increases during pregnancy, reaching its peak during the last trimester (34<sup>th</sup>- 35<sup>th</sup> week of pregnancy). Figure (2) shows the same behavior as it reaches the peak in the last trimester. Table (2) shows the values of maternal serum AFP among Sudanese and it is obvious that the level is increasing towards term. The values are comparable to the values obtained from France through immunotech, Table (2).

Measuring the concentration of both AFP and hCG in pregnant with relative and irrelative couples reveals some amazing results. The level of hCG was not significantly

different among both groups, whereas the pregnant with relative couples shows significant increasing in AFP levels in the 1<sup>st</sup> and the 3<sup>rd</sup> trimester (Table 1). For explanation of this result, we need to do further studies. In Sudan Screening for different diseases is not a mature activity and it is still a new trend. Few years ago we start to screen neonates for congenital hypothyroidism. At the beginning 2001 we started this work in collaboration with some obstetrician for monitoring early pregnancy as well as for detecting some abnormal pregnancies. These results may assist obstetrician to monitor and to follow abnormal pregnancies. As of 1972 an increase of AFP in the maternal serum has permitted the prenatal diagnosis of anencephaly and spina bifida. An AFP concentration below normal, when associated with other markers suggest the possibility of trisomy 21 (Down's syndrome) is work will be extended to determine the risk for Down's syndrome.

## Conclusion

- Local Normal Range of HCG and AFP for Sudanese pregnant women has been done, and it can be replaced the international one which can be used before this study.
- Using HCG for early detection of pregnancy (1 week before expected cycle) is very important for the health of both mother and fetus.
- The use of AFP & HCG can detect also early congenital abnormalities and this can save a lot of women life and help gynecologists to take suitable decision for such a cases.

## Recommendation

Although the high cost of the test, I strongly recommended measurement of AFP & HCG to be as routine investigation for detection as well as follow up of pregnancy. So that I ask the responsible authorities to make this investigation available and cheap (in the government hospital) even for the poor women as the rich women never feel such a problems.

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