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Concentration of maternal biochemical markers: Complications during pregnancy and their effect on the detection of trisomy 21 in the first trimester, by combined test (study carried out at a private clinic in CDMX)

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ABSTRACT

Aneuploidies are alterations that affect the number of chromosomes. Historically, a combination of markers has been used that includes: maternal age, concentrations of biochemical markers and ultrasonographic markers during pregnancy, as well as screening in the first trimester in order to detect Trisomy 21 (T21).

Objective:

To identify if biochemical markers such as Pregnancy Associated Placental Protein A (PAPP-A) and Beta human Chorionic Gonadotropin (β-hCG) are altered by complications during pregnancy and if this leads to an increased risk of developing T21 in pregnant women with healthy babies (combined test).

Material and method:

Retrospective, observational and cross-sectional study, included 73 pregnant women. Anthropometric data from the mother and the newborn were collected. A combined test was performed in the 1st trimester and biochemical markers were recorded in maternal serum (β-hCG AND PAPP-A) and ultrasonographic markers [Nuchal Translucency (NT)]. In addition, the evaluation of Cell-Free Fetal DNA (cffDNA) in peripheral blood was performed to identify aneuploidies and a pathological study of the placenta.

Results:

In the group of patients with intermediate risk for T21, there was a higher prevalence of complications during pregnancy [fetal growth restriction (4.5%), preterm labor (4.5%), miscarriage (9.09%) and death (9.09%). In the same way, patients with intermediate risk presented a higher prevalence of placental alterations such as calcifications, atrophy, congested dilated vessels, hemorrhage, hematomas, Chorioangioma, extravasation of erythrocytes and villus infarcts.

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Conclusions:

The development of placental alterations and the presence of complications in pregnancy could modify the peripheral blood concentrations of β-hCG and PAPP-A, and alter the results of the combined test for T21.

KEYWORDS: Pregnancy, trisomy 21, biochemical markers, combined test, Cell-Free Fetal DNA.

MANUSCRIPT

Introduction

Aneuploidies are alterations in the number of chromosomes that can occur in 0.5% of live newborns, in the form of monosomies or trisomies. The best known monosomy is Turner Syndrome [monosomy 23 (M23), only affects women]. The most studied trisomies are: Patau syndrome [trisomy 13 (T13)], Edwards syndrome [trisomy 18 (T18)] and Down syndrome [trisomy 21 (T21)]. The prevalence of the latter varies according to the literature consulted. For T13 the prevalence is between 1/12,000 to 1/29,000, for T18 from 1/6,000 to 1/8,000; and for T21 in 1 in 1,000 live births [1, 2, 3, 4]. A risk factor that increases the prevalence of aneuploidy is maternal age, with a prevalence of 1 in 1,000, 1 in 2,500, and 1 in 8,000, in women aged 20 years at T21, T18, and T13 (week 12 of gestation) respectively. The risk factor increases in women over 35 years of age, with a prevalence of 1 in 250, 1 in 600 and 1 in 1800, respectively, reporting a prevalence of trisomy 21 up to 30% in women older than 35 years [5].

Historically, for the detection of T13, T18 and T21, a combination of markers has been used that take into account maternal age, concentrations of feto-placental biochemical markers in maternal serum, including Alpha-fetoprotein (AFP), Beta Human Chorionic Gonadotropin (β-hCG), Inhibin A, unconjugated estriol (E3), Pregnancy-Associated Plasma Protein A (PAPP-A), ultrasonographic markers such as Nuchal Translucency (NT), absence or hypoplasia of the nasal bone, reverse wave of the ductus venosus, and tricuspid regurgitation, among other "soft markers" that allow reporting rates of up to 96% with a false positive rate of 2.5% for T21 [4, 5, 6, 7, 8, 9, 10]. Table 1 shows the different screens focused on T21 detection, which use different combinations of the aforementioned markers and their detection rate.

EVALUATION METHOD	Detection rate (%)	False Positive Rate (%)
ME	30	5
First trimester		
MA, TN	75 - 80	5
MA, β-hCG and PAPP-A	60 - 70	5
MA, TN, β-hCG AND PAPP-A (combined test)	85 - 95	5
Combined test, nasal bone or tricuspid flow or venous duct	93 - 96	2.5

Table 1. Different methods for detection of trisomy 21.

MA: Maternal Age, NT: Nuchal Translucency, β-hCG: β-Human Chorionic Gonadotropin and PAPP-A: Plasma Protein A associated with pregnancy. Table modified from Kypros, 2011.

Nowadays, cffDNA is added to screening tools (it is a screen or predictor of T13, T18 and T21) in the first trimester with a high detection rate. Being a Non-Invasive Prenatal Test (NIPT), it has further reduced the use of invasive methods such as: chorionic villus biopsy or amniocentesis. At the same time, the associated risk of fetal loss (0.6 to 2%) with these tests is decreased. It has been observed that cffDNA increases sensitivity for T13, T18 and T21 of 93.8, 97.4 and 99.3%, with specificity for T13, T18 and T21 of 99.98, 99.98 and 99.96% respectively, and in all cases a false positive rate of <1% [11, 12, 13, 14, 15, 16, 17, 18, 19]. Finally, chorionic villus biopsy or amniocentesis used to confirm the results of these two non-invasive tests (combined test and cffDNA) continues in indicated cases.

On the other hand, the concentrations of maternal biomarkers can be altered by different degrees of placental dysfunction that result in perinatal complications in patients without chromosomal abnormalities or with neural tube defects [20]. This is because maternal biochemical markers may reflect fetal placental function, as well as endocrine, immunologic, and endothelial dysfunction. In this sense, altered levels of β-hCG and PAPP-A in peripheral blood can be associated with maternal vascular malperfusion, Fetal Growth Restriction (FGR), Placental Abruption (PA), Premature Detachment of

Membranes (PDM), preeclampsia, hypertension, Preterm Birth (PB) and Pregnancy Loss (PL) [21, 22, 23].

Therefore, the objective of this work is to analyze whether the calculated risk, in the first trimester for T21 by the Combined Test, is influenced by the presence of placental alterations that modify the concentrations of maternal biomarkers and their association with adverse perinatal outcomes such as FGR, PA, PDM, preeclampsia, hypertension, PB and PL.

Material and Method

Retrospective, observational and cross-sectional study, which included 73 pregnant women who underwent prenatal care at the PRONATAL Clinic (Mexico City), between 2016-2021. The included patients were scheduled between weeks 11 and 13+6 of gestation to undergo first-trimester screening (Combined Test), which allowed them to classify and identify their risk of having a child with T21. Maternal characteristics, clinical history, obstetric history, biochemical markers in maternal serum (β-hCG AND PAPP-A) and ultrasonographic markers (NT) were recorded by the specialist in maternal-fetal medicine from the Fetal Medicine Bité Clinic (Hospital Bité Médica, Mexico City). The data obtained were added to The Fetal Medicine Foundation software, for the calculation of risks in the first trimester. With the above, 3 groups were formed: 1) LR-T21: Low risk of having a child with T21, 2) IR-T21 Intermediate risk of having a child with T21 and 3) HR-T21: High risk of having a child with T21.

Regarding cffDNA to detect aneuploidies, it was indicated when the results obtained in the combined test resulted in intermediate or high risk for T21, or when patients with low risk for T21 requested it. cffDNA was evaluated in peripheral blood by a private laboratory [Target DNA-based technology: (DANSRTM, FORTE), DANSRTM analysis fragments from the specific chromosomes and SNP analysis distinguishes maternal from fetal DNA and quantifies the fetal DNA].

Baseline anthropometric data of the mother were collected at each consultation by the nursing team, taking into account data such as: age, weight, height and Body Mass Index (BMI), as well as vital signs (blood pressure in each arm, heart rate, etc.). The same as for newborns in the Labor, Delivery and Recovery (LDR) unit (Hospital Bité Médica, Mexico City), taking into account weeks of gestation (WoG), weight, height, APGAR score 1m and APGAR score 5m.

The histopathological study of each placenta was carried out in the Bité Médica Department of Pathology using the formalin fixation technique, paraffin process and hematoxylin/eosin staining.

Informed consent was recorded for each test performed on each patient and personalized genetic counseling was offered.

Inclusion criteria:

- Pregnant between 11 and 13+6 weeks of gestation.
- Had complications during pregnancy (FGR, DPP, PDM, preeclampsia, hypertension, PL and GL)
- Patients with:
  - Complete files.
  - Combined test.
  - cffDNA study.
  - Placental pathology at birth.

Exclusion criteria:

- *Patients who did not accept their inclusion in the study.*
- *cffDNA study with "no detected" result.*

Statistical analysis: Maternal data: age, weight, height and body mass index (BMI) and newborn (weeks of the pregnant, weight, height, APGAR m1 and APGAR m5), are reported with mean ± standard deviation (SD) and were evaluated using a Student's T. For its part, the prevalence of: 1) T21 by Combined Test (CT) (low, intermediate and high risk), 2) T21 by cffDNA and 3) placental pathology, is reported in Percentage and number of total individuals [% (n/N)], evaluated using a Chi2, being statistically significant in all cases a difference ≤0.05 for "p". The statistical package SPSS version 25 was used.

Results

This work includes 73 patients aged 36.3 ± 3.8 years, who were divided according to the result of the CT in the first trimester of pregnancy, which includes Low Risk (LR-T21), Intermediate (IR-T21) and High for T21 (HR-T21).

This classification was defined in part by first-trimester ultrasonographic and biochemical markers included for the combined test, reporting an increase in nuchal translucency thickness and peripheral blood concentrations of β-hCG. In addition, a decrease in PAPP-A concentrations in peripheral blood, placing 6.4% (12/73) of pregnancies in HR-T21 and 30.1% (22/73) in IR-T21, surpassed by 53.4% (39 /73) that resulted with LR-T21 (Table 2). Only 25% (3/12) of HR-T21 (combined test) were positive for T21 in the cffDNA

study [100% (3/3)], confirmed by performing karyotype in these 3 cases [100% (3/3)] (Table 2).

After classification, no statistically significant difference was observed between groups made up of the following parameters evaluated, but a numerical difference was observed, showing that HR-T21 were younger compared to IR-T21 and LR-T21 (34.9±3.9 vs 36.7±3.4 and 37±4.3); IR-T21 presented lower weight compared to HR-T21 and LR-T21 (57.6±7.6 vs 61.2±11.6 and 61.5±7.8) and LR-T21 presented greater height (1.64±0.06 vs 1.61±0.06 and 1.61±0.07) and BMI (23.6±3.1 vs 22.03±0.8 and 22.6±3.7) compared to IR-T21 and HR-T21 (Table 2). For its part, the highest prevalence of nulliparity was presented by LR-T21, followed by IR-T21 and ending with HR-T21 (35.8, 22.7 and 8.3%, respectively) (Table 2).

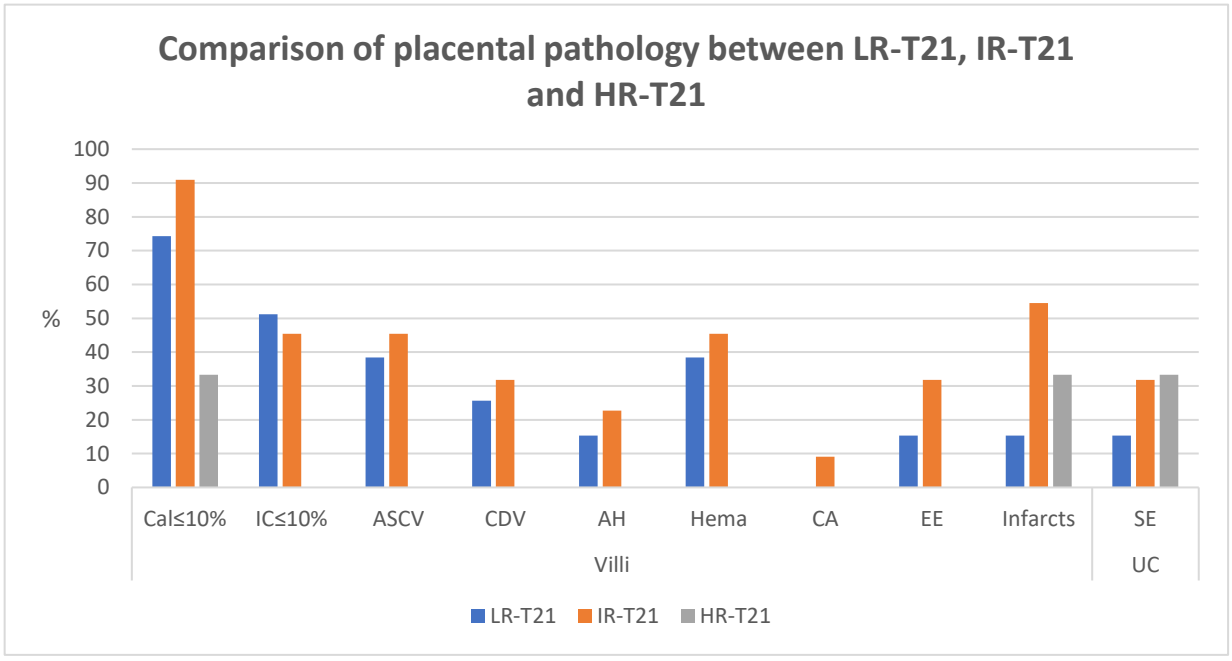
Patient and gestational characteristics in the entire cohort and low, intermediate and high-risk groups					
N		73			
Age (years, Mean ± SD)		36.3 ± 3.8			
		Low	**Intermediate**	**High**	**p**
Trisomy 21 (Risk in combinated test)		53.4% (39/73)	30.1% (22/73)	16.4% (12/73)*	≤0.05
Trisomy 21 (Positive in cffDNA)		0% (0/39)	0% (0/22)	25% (3/12)	NA
Mean cffDNA fetal fraction (Mean±SD)		11.3 ± 3.3	10.8 ± 4.4	8.3 ± 2.5	>0.05
Confirmation by karyotype		-	-	100% (3/3)	NA
Maternal and gestational characteristics					
Age (years, Mean±SD)		36.7 ± 3.4	37 ± 4.3	34.9 ± 3.9	>0.05
Weight (kg, Mean±SD)		61.2 ± 11.6	57.6 ± 7.6	61.5 ± 7.8	>0.05
Height (m, Mean±SD)		1.64 ± 0.06	1.61 ± 0.06	1.61 ± 0.07	>0.05
Median BMI (kg/m^2)		23.6 ± 3.1	22.03 ± 0.8	22.6 ± 3.7	>0.05
Nulliparity		35.8% (14/39)	22.7% (5/22)	8.3% (1/12)	>0.05
Combined test (Gestation Week, Mean±SD)		12.8 ± 2.4	13.2 ± 1.9	13.4 ± 1.3	>0.05
Prenatal screening and testing	**NT (mm)**	1.7 ± 0.5	1.6 ± 0.52	2.2 ± 1.4**	≤0.05
	β-hCG (MoM)	1.7 ± 1.5	1.8 ± 1.1	2.1 ± 1.3	>0.05
	PAPP-A (MoM)	0.8 ± 0.6	0.7 ± 0.5	0.6 ± 0.4	>0.05
Pregnancy and delivery outcome					
Preeclampsia		-	-	8.3% (1/12)	NA
FGR		5.1% (2/39)	4.5% (1/22)	-	>0.05
Gestational age		38.5 ± 1.5	38.5 ± 0.8	37.2 ± 2.1***	≤0.05
Live birth		100% (39/39)	81.8% (18/22)	75% (9/12)	>0.05
PB<34 wk		-	4.5% (1/22)	0	NA
Induced abortion		-	-	25% (3/12)	NA
PL		-	9.09% (2/22)	-	NA
Obito		-	9.09% (2/22)	-	NA
Birth weight		3010.1 ± 358.3	2972.8 ± 318.3	2853 ± 749.3****	≤0.05
Birth size		48.8 ± 1.9	48.8 ± 2.1	47.3 ± 2.2	>0.05
APGAR 1m		8.8 ± 0.4	9	8.6 ± 0.5	>0.05
APGAR 5m		9.5 ± 0.5	9.5 ± 0.5	9.6 ± 0.5	>0.05

Table 2. Patient and gestational characteristics in the entire cohort and low, intermediate and high-risk groups

* Significant difference when comparing HR-T21 vs LR-T21 and IR-T21 (p≤0.05, Student's T), **Significant difference when comparing HR-T21 vs LR-T21 and IR-T21 (p≤0.05, T of Student), *** Significant difference when comparing HR-T21 vs LR-T21 and IR-T21 (p≤0.05, Student's T), **** Significant difference when comparing HR-T21 vs LR-T21 and IR-T21 (p≤0.05, Student's T).

Among the alterations that occurred during pregnancy, only one patient in HR-T21 developed preeclampsia (8.3%), one with IR-T21 presented PB (4.5%), two patients with LR-T21 (5.1%) and one patient with IR-T21 (4.5%) presented FGR, two patients with IR-T21 had PL (9.09%) and two with IR-T21 ended in death (9.09%) (Table 2).

In contrast, the prevalence of live births was higher in patients with LR-T21 [100% (39/39)], followed by patients with IR-T21 [81.8% (18/22)] and finally, by patients with HR-T21 [75% (9/12)], remembering that the abortions in this group were induced (Table 2). From the above, the newborns of patients with LR-T21 showed a weight of 3010.1±358.3, which was statistically higher than that presented by patients with intermediate risk (2972.8±318.3) and high for T21 (2853±749.3). Regarding height and APGAR (1m and 5m), there were no significant differences between the patients of the different risks for T21 (Table 2).



Graphic 1. Cal≤10%, Calcifications ≤10%, IC≤10: Ischemic Changes ≤10%, ASCV: Atrophic Small Chorionic Villus, CDV: Congested Dilated Vessels, AH: Acute Hemorrhage, Hema: Hematoma, CA: Chorioangioma, EE: Extravasation of Erythrocytes, SE: Stromal Edema and CU: Umbilical Cord. p≤0.05, Chi^2.

Finally, the histopathological findings of the placentas showed that IR-T21 presented a numerical increase in the prevalence of Cal≤10% [90.9% (20/22) vs 74.3% (29/39)], ASCV [45.4% (10 /22) vs 38.4% (15/39)], CDV [31.8% (7/22) vs 25.6% (10/39)], AH [22.7% (5/22) vs 15.3% (6/39)] , Hema [45.4% (10/22) vs 38.4% (15/39)], CA [9.09% (2/22) vs 0% (0/39)], EE [31.8% (7/22) vs 15.3 % (6/39)], infarcts [54.5% (12/22) vs 15.3% (6/39) and SE [33.3% (7/22) vs 15.3% (6/39)], compared to LR-T21. In parallel, IR-T21 had a higher prevalence of Cal≤10% [90.9% (20/22) vs 33.3% (4/12), IC≤10% [45.4% (10/22) vs 0% (0/ 12)], ASCV [45.4% (10/22) vs 0% (0/12)], CDV [31.8% (7/22) vs 0% (0/12)], AH [22.7% (2/22)) vs 0% (0/12)], Hema [45.4% (10/22) vs 0% (0/12)], CA [9.09% (2/22) vs 0% (0/12)], EE [31.8% (7/22) vs 0% (0/12)] and Infarcts [54.5% (12/22) vs 33.3% (4/12)], compared to HR-T21. On the contrary, LR-T21 presented a higher IC≤10% [51.2% (20/39) vs 45.4% (10/22)], compared to IR-T21 (Graphic 1).

Discussion

One of the main objectives of the CT is to identify the risk of having a child with Down Syndrome (T21), a result that can be complemented with the performance of the cffDNA, giving the doctor the opportunity to propose the performance of an amniocentesis, either of amniotic or chorionic villus fluid. In the particular case of this study, only 25% (3/12) of HR-T21 (CT) were found to be positive for T21 in the cffDNA and karyotype tests of embryonic remains (Table 2). In addition to this, 100% of RB-T21 and RI-T21 (CT) were negative at T21 by cffDNA analysis, which ruled out the performance of invasive tests (Table 3). For its part, the prevalence of women with IR-T21 and HR-T21 may be due to the fact that 67.7% of the population is ≥35 years old, maternal age associated in the literature with an increased risk of chromosomal abnormalities in offspring [24].

As already described in the introduction to this research, the increase in peripheral blood

concentrations of β-hCG and the decrease in PAPP-A can place pregnant women at greater risk of giving birth to a child with T21 by combined test [20], as can be seen in Table 2 of this work. However, we observed that 100% (22/22) of the patients with IR-T21 and 66.6% of the patients with HR-T21 by CT, in the cffDNA test, were negative for T21. Situations in which the concentrations of β-hCG and PAPP-A could not reflect the risk for T21, but that the fetoplacental function is related to alterations such as FGR, PA, PDM, preeclampsia, hypertension, PB and PL [20].

In our case, higher concentrations of β-hCG and lower concentrations of PAPP-A in RI-T21, may be associated in 23.18% with complications that occurred during pregnancy [FGR (4.5%), PP (4.5%), PG (9.09%) and Death (9.09%)]. Coinciding with Antsasklis P. et al., 2019 [25], who in their review found that PAPP-A concentrations ≤0.2 (MoM) in the first trimester of pregnancy increase the risk of developing FGR, preeclampsia and death, López A. et al., 2016 [26], a retrospective study that included 285 patients found that PAPP-A concentrations (5th percentile) ≤ 0.4 MoM in the first trimester increased the risk of PL, FGR, hypertensive diseases, hypertension and gestational diabetes. In the case of β-hCG, Park H. et al., 2014 [27], observed significantly higher levels of β-hCG (1.66 vs 1 MoM) in patients who developed preeclampsia, compared to control, Mikat B. et al., 2012 [28], find, in a study conducted on 155 pregnant women, that first trimester concentrations of β-hCG were higher in patients who developed preeclampsia later in pregnancy and Rivas M. et al., 2014 [29], in a study that brought together patients with preeclampsia, FGR, PB and PL, to form a group with maternal perinatal complications (CPM, n=10), observed an increase in β-hCG (0.76 vs 0.60 MoM) in the first trimester, in comparison to control. In contrast, Karahasanovic A. et al., 2014 [30], detected a significant reduction in β-hCG levels (0.75 vs 0.97 MoM) in 161 women with preeclampsia, Pornwattanakrilert W. et al., 2020 [31], in work that included 500 women with PL, observed a decrease in β-hCG concentrations, compared to the control group (1.12 vs 1.23 MoM) and Litwińska E. et al., 2017 [23], in a study that included 94 pregnant women with early preeclampsia (n=22), late preeclampsia (n=29) and FGR (n=43), found no significant difference in β-hCG concentrations (1.08, 1.25 and 1.12 vs 1.14 MoM) when compared to control.

For its part, in the literature the development of alterations during pregnancy such as FGR, preeclampsia, death, hypertension and gestational diabetes are associated with macroscopic and microscopic placental changes [32, 33]. Findings in studies such as the one carried out by Vedmedovska N. et al., 2011 [34], in patients with FGR, a higher incidence of alterations in villi, such as thickening of the trophoblastic basement membrane, infarcts, thrombi and hematomas, compared to the control group. Voicu N. et al., 2020 [33], in a study carried out on 30 placentas of women with FGR, found a higher prevalence of macroscopic changes (fibrin deposits, calcifications and infarcts) and microscopic changes (massive infarcts caused by vascular ischemia, fibrin deposits intra and extravenous, calcifications and vascular thrombosis), when compared with a group of patients who did not develop FGR and who presented thrombophilias, Ogge G. et al., 2011 [35], in a retrospective case-control study that included 8307 women who gave birth after 20 weeks of gestation found that women who developed preeclampsia had a higher incidence of alterations in placental villi (infarcts, fibrin, distal hypoplasia, agglutination and syncytial knots), Devisme L. et al. 2013 [36], in a retrospective study of control cases that included 173 women who developed preeclampsia observed a greater presence of syncytial knots, infarcts, basal decidual vasculopathy, hi villous permaturation and placental erythroblastosis, compared to control.

Regarding Sarafzadeh A. et al., 2018 [37], in a study that included 118 patients who had PB, they found that only 10% presented syncytial knots, chorioangiosis and microcalcifications, Azizi M. et al., 2014 [38], in a case-control study of 100 placentas obtained from patients who had PL reported a greater presence of calcifications, inflammatory lesions and thrombi, compared to the control group. Odendal H., 2021 [39], in review, refer to the pathology of 13 placentas of late abortions (19-25 SDG), in which a prevalence of PA of 46% and acute chorioamnionitis of 30% could be observed. In turn, Lema G. et al., 2020 [40], in a case-control study, which included 96 patients with gestational loss at week 33.8±3.2, showed a higher incidence of vascular alterations in the uterus and placenta (73 vs. 3.4%). In addition, acute chorioamnionitis (8 vs 4%), when compared to the control group. For this reason, it could be thought that the higher prevalence of Cal≤10% [90.9% (20/22) vs 74.3% (29/39)], ASCV [45.4% (10/22) vs 38.4% (15/39)], CDV [31.8% (7/22) vs 25.6% (10/39)], AH [22.7% (5/22) vs 15.3% (6/39)], Hema [45.4% (10/22) vs 38.4% (15/39)], CA [9.09% (2/22) vs 0% (0/39)], EE [31.8% (7/22) vs 15.3% (6/39)], Infarcts [54.5 % (12/22) vs 15.3% (6/39) and SE [33.3% (7/22) vs 15.3% (6/39)], in HR-T21 when compared with LR-T21, could be the cause by which, the patients were classified in IR-T21, despite not developing complications during pregnancy (FGR, PA, PDM, preeclampsia, hypertension, PB and PL).

CONCLUSIONS

The concentrations of biochemical markers such as β-hCG and PAPP-A, as part of the first trimester combined test, are a good tool that can allow for the identification of T21.

The development of placental alterations such as small villi, atrophic chorionic villi, with calcifications, congested dilated vessels, acute hemorrhage, hematomas, chorioangioma, extravasation of erythrocytes, infarcts and stromal edema, in addition to the presence of alterations or complications, such as fetal growth restriction, preterm delivery, spontaneous abortion and death, could alter the peripheral blood concentrations of β-hCG and PAPP-A, which, when used to calculate the risk in the Combined Test, would place groups of patients in IH-T21 and HR -T21, really reflecting some alterations or complications during pregnancy, but not the presence of a fetus with T21.

Finally, it is recommended to perform cffDNA to reinforce the result obtained in the Combined Test that will allow the doctor to make a decision.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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