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Abstract

Introduction:

Total alkaline phosphatase (tALP) levels rise physiologically in maternal serum during pregnancy, and excessively so in certain conditions. However, current reference values are dated, nonlinear, and based on small samples. Factors related to variation in tALP remain unexplained. Thus, our goals in this study were to establish a physiological development curve for tALP within low-risk pregnancies and to evaluate the factors influencing tALP values.

Methods:

This was a single-center, retrospective, observational study. All patients who delivered a live singleton infant at our center from January 1, 2011 to May 31, 2019, and had a tALP assay during pregnancy, were included regardless of the gestational age at which the assay was conducted.

Results

A total of 2415 pregnancies were included. Median tALP decreased during the first trimester, it increased slightly during the second trimester, and then increased sharply during the third trimester. Factors associated with a significant increase in tALP were chronic histiocytic intervillositis, cholestasis, multiple pregnancies, liver disease, preeclampsia, smoking, and low weight for gestational age. Conversely, gestational diabetes was associated with a discrete decrease in tALP.

Discussion

Our large sample allowed establishment of tALP reference curves based on gestational age. To interpret these results more thoroughly, factors that influence tALP rates should be further scrutinized.

Keywords: alkaline phosphatase, pregnancy, smoothed curves

1. Introduction

Alkaline phosphatases (ALP) are ubiquitous enzymes that may originate in the liver, bone, intestine, kidney, germ, or placenta [1]. During pregnancy, high total ALP (tALP) levels are often associated with unfavorable obstetric prognosis [2–4], although this association is debated regarding both preeclampsia [5–12] and small for gestational age (SGA) neonates [2,13,14]. Recently, an association was shown between elevated tALP and chronic histiocytic intervillositis, a rare and severe disease of the placenta that is responsible for miscarriage, severe intrauterine growth restriction, and in utero death [15].

Although the search for abnormal variations in tALP during pregnancy has been the subject of much research, it is accompanied by several challenges. First, tALP rises physiologically during pregnancy, primarily due to increases in placental and bone isoenzymes [16], which means that gestational age must be accounted for as accurately as possible when analyzing changes in tALP levels. Second, no high-quality tALP curves are available throughout pregnancy; most current reference values are dated, derived from small samples, and often reflect only one or two values per trimester [16–24]. Moreover, large differences exist between published values—as high as three times the median or mean—making interpretation difficult [16,19,22,24].

Accordingly, our goals in this study were to establish a reference curve reflecting the natural course of tALP throughout pregnancy from a large sample of low-risk pregnancies, and to study the factors that influence these values.

2. Materials and methods

This was a single-center, observational, retrospective cohort study conducted at our Level III university maternity hospital over eight consecutive years (January 1, 2011–May 31, 2019). Pregnancies were eligible if their ICD-10 (10^{th} International Classification Diseases) codes indicated a single, live birth after at least 22 weeks of gestation (WG), and if they had at least one tALP assay performed during pregnancy, regardless of the gestational age at which it was performed. To eliminate situations that may cause variations in tALP [2,5–10,13,25,26], pregnancies were excluded if their ICD-10 codes indicated one or more of the following: preeclampsia, intrauterine growth restriction, cholestasis, another liver disease, gestational or prepregnancy diabetes, chronic histiocytic intervillositis, in utero death or medical termination of pregnancy, alcoholism, or bone disease.

The study sample was selected from our center's medical and care information system database (Sillage®, SIB, Rennes, France) and biological data server (Molis®, CGM, Brussels, Belgium). These data were cross-referenced to PMSI-ICD10 database codings. The medical information collected included maternal characteristics, gravida, prepregnancy medical history (e.g., diabetes, hypertension), pregnancy-related history (e.g., hospitalization), gestational age at current delivery, delivery mode, and neonatal birth weight. Our center routinely collects patient consent for the anonymous use of the data in their medical records for clinical research, which is administered through a routine document signed at admission to the maternity ward. Our study was approved by our national ethics committee

(Comité d'Ethique de la Recherche en Obstétrique et Gynécologie, CEROG n°2019-OBST-0703).

If a patient had more than one pregnancy during the study period, each pregnancy was included and analyzed individually according to the inclusion and exclusion criteria above. When several tALP assays were available for the same pregnancy, only the first was retained and analyzed. All tALP determinations were performed using the International Federation of Clinical Chemistry (IFCC) reference technique [27], using the colorimetric method with spectrophotometric measurement of *p*-nitrophenyl phosphate hydrolysis at 450/480 nm ; the same machine was used during the whole study period (COBAS 8000 system, Roche Diagnostics, Meylan, France).

Categorical variables are described as values and percentages, and quantitative variables as medians and interquartile values [IQ25; IQ75]. At each gestational age, tALP reference intervals were constructed using the parametric smoothing method of Royston and Wright [28] to obtain the smoothed 2.5th, 10th, 50th, 90th, and 97.5th percentiles. Each tALP value is also expressed as a multiple of the median (MoM) by relating it to its median for gestational age. Median tALP values among excluded patients were compared with reference values from the included sample. Then, the influence of smoking and history of chronic hypertension were investigated in the included sample.

Data were analyzed using Epi-info (Epi-info version 7.1.5, CDC, Atlanta, USA), SAS version 9.4 (SAS Institute, Cary, USA), and R Core Team version 2019 (R Core Team, Vienna, Austria). All median comparisons used the nonparametric Kruskal–Wallis test. Significance levels for all tests were set at 5%.

3. Results

Among the 66,136 pregnancies lasting at least 22 weeks in our center during the study period, 6825 had at least one tALP assay (10.3%) (Fig. 1). According to our criteria, we excluded those with gestational and prepregnancy diabetes (n=3209), preeclampsia (n=813), intrauterine growth restriction (n=707), multiple pregnancy (n=358), cholestasis (n=328), in utero death or medical termination (n=120), other liver pathologies (n=38), chronic histiocytic intervillositis (n=28), alcoholism (n=3) and bone pathologies (n=1). As some women had more than one exclusion criteria, a final sample of 2415 pregnancies was included, with a single tALP measurement for each. The number of women with an available assay ranged from 30 to 118 for each gestational age between 2 and 41 SA, with a median of 48 assays per week of gestation IQ25-75[41; 84].

The sample characteristics are presented in Table 1. The median maternal age was 30 years and 12.8% of pregnant women smoked; 42% were primigravida and 2.1% had prepregnancy hypertension. The cesarean delivery rate was 21.5%; 8.6% were premature deliveries and 7.6% of neonates were macrosomic (birth weight >4000 g).

The distribution of 2415 tALP values as a function of gestational age is shown in Fig. 2, with curves for the 2.5th, 10th, 50th, 90th, and 97.5th percentiles after Royston and Wright parametric smoothing. After a slight decrease between gestational weeks 2–13 (first trimester), these values increased slightly in weeks 14–28 (second trimester), and then increased sharply in weeks 29–41 (third trimester). Smoothed tALP distribution values at each gestational age are shown in Table 2.

Median tALP values were 0.96 [0.79; 1.24] for the 2415 included pregnancies (Table 3). Factors associated with significantly higher median tALP values, in descending order of MoM values [with 1st and 3rd interquartile ranges], were chronic histiocytic intervillositis (1.61 [1.22; 2.86]), cholestasis (1.43 [1.04; 1.90]), multiple pregnancy (1.32 [0.96; 1.79]), another liver disease (1.31 [1.00; 2.06]), preeclampsia (1.12 [0.88; 1.51]), smoking during pregnancy (1.10 [0.86; 1.45]), and SGA (1.08 [0.88; 1.51]). Conversely, the occurrence of gestational diabetes was associated with slightly but significantly lower tALP values (0.95 [0.78; 1.18]). Compared with the included sample, tALP values did not differ significantly among excluded pregnancies with prepregnancy diabetes, preexisting hypertension, or in utero death or medical termination of pregnancy.

4. Discussion

We plotted reference tALP values for a large sample of low-risk singleton pregnancies. Several factors were significantly associated with an increased tALP value (chronic histiocytic intervillositis, multiple pregnancy, cholestasis and other liver diseases, preeclampsia, smoking, and SGA), whereas the presence of gestational diabetes was associated with a slightly decreased tALP value.

Our tALP curve is derived from one of the largest cohorts to date. Only one cohort was larger than ours, consisting of 13 656 Chinese women with measurements whose medians and distribution are given solely for sequential periods of 6 or 7 consecutive weeks of pregnancy. For the others, previous reference values were based on much smaller numbers, ranging from 30 to 103 women, with 3–7 tALP measurements per patient [16,18–20,22,23,29], and two previous studies were meta-analyses [17,21]. While tALP values rise in late pregnancy, none of the previous curves provided smoothed reference values corresponding to each gestational week. At best, they provided one or two reference values per trimester, and some curves had no values available for an entire trimester [16,23]. In contrast, we have established a curve informed by more than 2000 pregnancies, with a reference median for each gestational age, allowing us to avoid variation in tALP values according to pregnancy progression. Further, tALP determinations were performed using the same process throughout the study period (i.e., with the IFCC reference technique) [27].

It is difficult to compare our curves with those previously published. Some publications report the median [23,24], others the mean [16,19,20,22], and still others

the 2.5th and 97.5th percentiles for gestational periods that differ slightly between curves [17–19,21]. One curve—representing 103 women and published in 1996—is distinguished by particularly low tALP values [22], while another—carried out in 30 women and published in 2006—is distinguished by average values that well exceed the others for all trimesters [19]. Thus, certain medians or averages previously reported may vary by two to three times [19,22]. Nevertheless, the values presented herein are similar to most of those previously published, with a clearly demarked rise beginning in the third trimester [16–18,20,21,23,24].

In addition to providing reference values based on a large volume of assays, our curve allows us to clarify several points concerning changes in tALP across pregnancy, and to explore these sources of variation. An important implication of these findings concerns the decrease in tALP during the first trimester, that is, before the well-known phases of slight and then strong increases in tALP during the second and third trimesters, respectively [16,24]. We suggest that the tALP decrease in early pregnancy is related to two well known factors, namely hemodilution and increased glomerular filtration, which occur very early in pregnancy [30,31], and the « late » production of specific placental alkaline phosphatases, which does not really begin until the second trimester of pregnancy, when a rise in tALP is perceptible [16,24]. This production of placental isoenzyme is not detectable until the eighth week of pregnancy [32].

We identified several sources of variation in tALP. The observed elevated tALP in women with cholestasis, other liver diseases, or who have a multiple pregnancy was as expected. Regarding chronic histiocytic intervillositis, we previously reported an increase in tALP >600 IU/L in 10 out of a sample of 18 women with this condition [15]. The tALP elevation we observed again in the present study merits further

evaluation. Regarding preeclampsia, we found herein a significant tALP elevation, at 1.12 MoM. This is valuable, in part, because these are the first solid data on this topic to date. After initial descriptive and nonquantitative investigations [5,6], various studies have indicated a decrease in tALP in cases of hypertension or preeclampsia [7,9], sometimes without objective evidence [8,10]. More recent studies have not shown ALP changes in preeclampsia, from either total serum ALP or placental ALP measured in saliva [11,12].

We also showed a highly significant discrete tALP elevation among women whose infants were SGA (1.08 MoM). To our knowledge, this is another novel finding. Apart from a reported case of severe SGA with very high total antenatal ALP [14], a statistical link between ALP levels and low birth weight for gestational age has not been shown before. Although an increased risk of delivering an infant weighing <2500 g was demonstrated in the case of tALP ≥2 MoM in the second trimester, it is impossible to determine from these studies [2,13] whether this birth weight was related to delivery before 37 weeks gestational age or to actual growth restriction [3,4,33].

Similarly, we found a small but significant elevation of tALP with smoking during pregnancy (1.10 MoM). However, only a few studies have been reported, and they included small samples (i.e., N<50). Two of these failed to find an association between maternal smoking and tALP [34,35], and one study showed a significant increase in placental isoenzyme during the third trimester among patients who smoked [36]. While the mechanism of this increase is unknown, we speculate that placental hypoxia—linked to CO levels among smokers—may be involved with increased placental capillarization [37].

Apart from contradictory and isolated unexplained increases >1000 IU/L [38–41], there are few data in the literature with which to compare our finding of a very small decrease in tALP with gestational diabetes. In one modest-sized case–control study, ALP levels were unaltered during the first trimester in gestational diabetes cases [42]. In contrast, a large prospective Chinese study (N=2073 patients) showed a significant increase in gestational diabetes risk with higher serum ALP before 20 days gestation [43].

In sum, we found that tALP increased with chronic histiocytic intervillositis, preeclampsia, SGA, and maternal smoking, and slightly decreased in gestational diabetes, reflecting sometimes very small yet statistically significant variations. Although most of these associations are not necessarily clinically meaningful, they provide valuable knowledge. Establishing reference standards at each gestational age may enable a better understanding of potential diagnostic or prognostic roles of tALP values (e.g., in chronic histiocytic intervillositis). These findings also shed light on the origins of increased tALP during pregnancy. Whereas tALP is elevated in all placental hypoxia circumstances (i.e., preeclampsia, SGA, and maternal smoking), its rise may reflect an increase in activity and/or surface of the syncytiotrophoblast in these conditions, as the placental isoenzyme is mainly produced at this level [44]. tALP values may also reflect an increased release of placental microvesicles and exosomes containing ALP from the placental surface [44–47]. Conversely, the significance of decreased ALP in gestational diabetes is unknown. We hypothesize that it may be related to the delayed placental maturation observed in this condition [48].

A primary limitation of this study is its retrospective nature. The pregnant women in this sample had a tALP blood test without our knowing its precise reason (i.e.,

whether it was systematic-based). We tried to limit this selection bias by excluding the main known pathologies likely to modify tALP values. Further, our sample was generally characterized as low risk in terms of prematurity (8.6%), cesarean delivery (21.5%), and macrosomia (7.6%), all of which are similar to the rates observed in our country during the most recent (2016) nationally representative birth survey (prematurity, 7.5%; cesarean section, 20.4%; birth weight >4000 g, 6.8% [49]). Another limitation of our study is that tALP measured during pregnancy is the sum of hepatic, bone, placental, renal, intestinal and germinal ALP [1]. It is possible that some placental diseases are accompanied by more noticeable variations in placental ALP (PLAP) than tALP. As the determination of PLAP is more complex and expensive than that of tALP, their normal evolution during pregnancy should be the subject of studies specifically designed for this purpose.

In conclusion, our large population-based sample allowed us to establish tALP reference curves based on gestational age. These findings warrant further investigation to establish whether tALP level may be a direct or indirect reflection of the activity and/or surface of the syncytiotrophoblast, the main site of synthesis of placental isoenzyme [44].

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227 Table 1. Characteristics of the 2415 pregnancies included in the study
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	Median [IQ25; IQ75] n (%)
Maternal age	30.0 [26.0 ; 34.0]
Smoking during pregnancy (n=1839)	235 (12.8)
No previous pregnancy	1025 (42.4)
Pre-existing hypertension during pregnancy	51 (2.1)
Hospitalization during pregnancy	1393 (57.7)
Prematurity < 37 WG	208 (8.6)
Gestational age at delivery	39.7 [38.7 ; 40.7]
Cesarean section	519 (21.5)
Birthweight	3340 [3000 ; 3650]
Macrosomia > 4000 g	184 (7.6)

Table 2. Distribution of total alkaline phosphatase among the 2415 included pregnancies (IU/L)

These values were obtained after smoothing (see materials and methods)

SA	2 - 22 SA (percentile)					**SA**	23 - 41 (percentile)				
	2.5	10	50	90	97.5		2.5	10	50	90	97.5
2	34	43	70	125	177	**22**	36	44	68	110	147
3	32	41	66	115	162	**23**	37	45	70	114	152
4	31	39	63	108	151	**24**	38	47	72	118	158
5	30	38	60	103	143	**25**	40	49	75	123	164
6	30	37	59	100	137	**26**	41	51	78	128	171
7	29	37	57	97	132	**27**	43	53	81	133	179
8	29	36	57	95	129	**28**	45	55	85	140	188
9	29	36	56	93	127	**29**	46	57	89	147	198
10	29	36	56	92	125	**30**	49	60	93	155	209
11	29	36	56	92	124	**31**	51	63	98	163	221
12	29	36	56	92	124	**32**	53	66	103	173	234
13	30	36	56	92	124	**33**	56	70	109	183	249
14	30	37	57	93	125	**34**	59	74	116	195	266
15	30	37	57	94	126	**35**	63	78	123	208	285
16	31	38	58	95	127	**36**	66	83	131	223	307
17	32	39	59	97	129	**37**	70	88	140	240	331
18	32	40	61	99	132	**38**	75	94	150	258	358
19	33	41	62	101	135	**39**	80	100	161	279	389
20	34	42	64	104	138	**40**	85	107	173	303	424
21	35	43	66	107	142	**41**	91	115	187	330	465

Table 3. Factors influencing alkaline phosphatase levels in pregnant women
(IUD In utero death ; TOP Termination of pregnancy)

	MoM [IQ25-IQ75]	p
Included pregnancies (n=2415)	0.96 [0.79 ; 1.24]	
Chronic Histiocytic Intervillositis (n=28) [(a)]	1.61 [1.22 ; 2.86]	< 0.001
Cholestasis (n=328) [(a)]	1.43 [1.04 ; 1.90]	< 0.001
Multiple pregnancies (n=358) [(a)]	1.32 [0.96 ; 1.79]	< 0.001
Other liver diseases (n=38) [(a)]	1.31 [1.00 ; 2.06]	< 0.001
Preeclampsia (n=812) [(a)]	1.12 [0.88 ; 1.51]	< 0.001
Smoking (n=235) [(b,c)]	1.10 [0.86 ; 1.45]	< 0.001
Small for Gestational Age (n=707) [(a)]	1.08 [0.88 ; 1.51]	< 0.001
IUD and TOP (n=120) [(a)]	1.01 [0.82 ; 1.34]	0.148
Prepregnancy diabetes (n=417) [(a)]	1.00 [0.80 ; 1.32]	0.067
Gestational diabetes (n=2873) [(a)]	0.95 [0.78 ; 1.18]	0.036
Previous hypertension (n=51)[(b)]	0.91 [0.77 ; 1.22]	0.66

a- Compared to the 2415 included pregnancies

b- Comparison within included pregnancies

c- 576 missing values for tobacco use during pregnancy

Figure 1: Flow chart of the study

* Several possible exclusion criteria

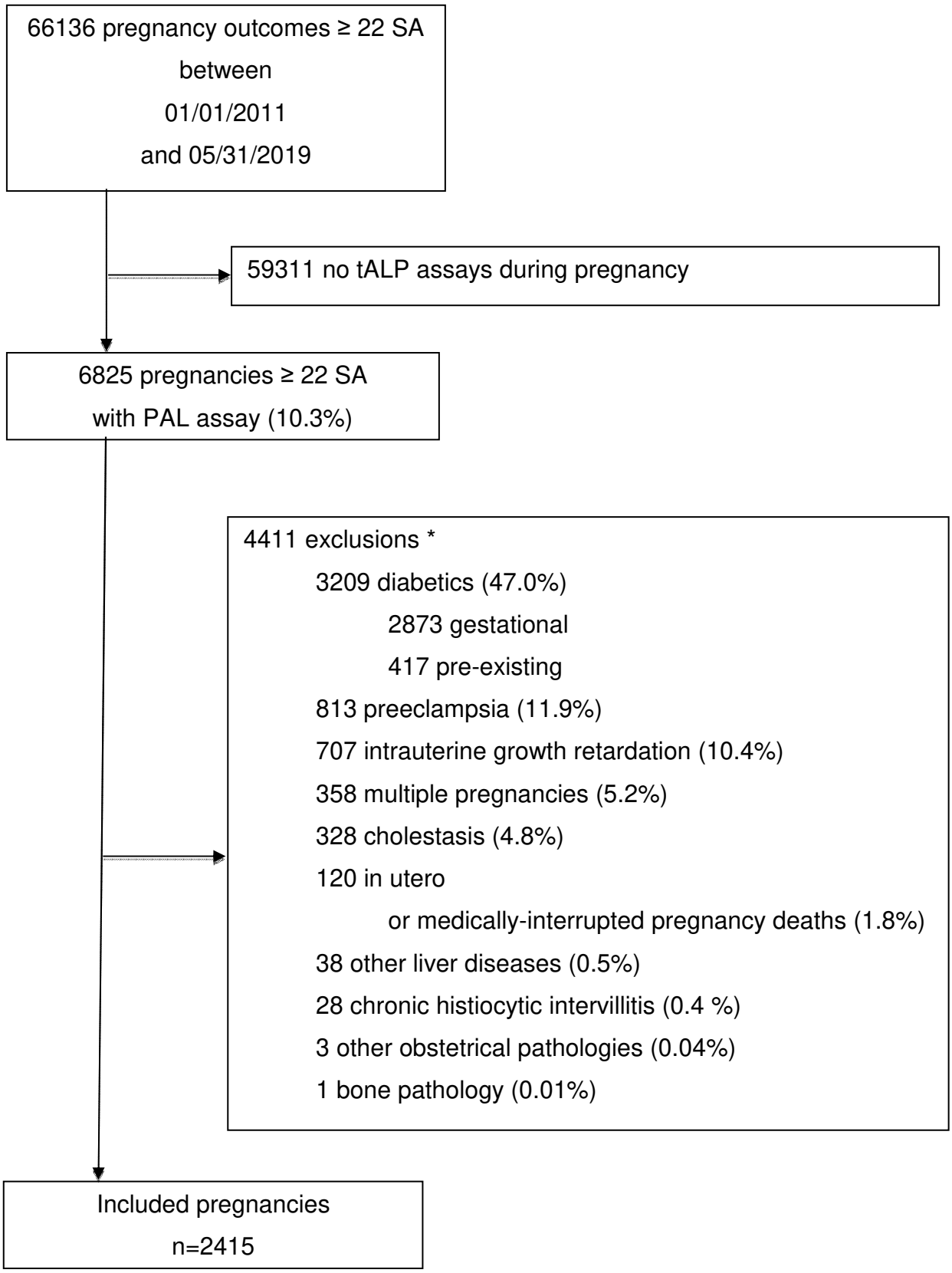
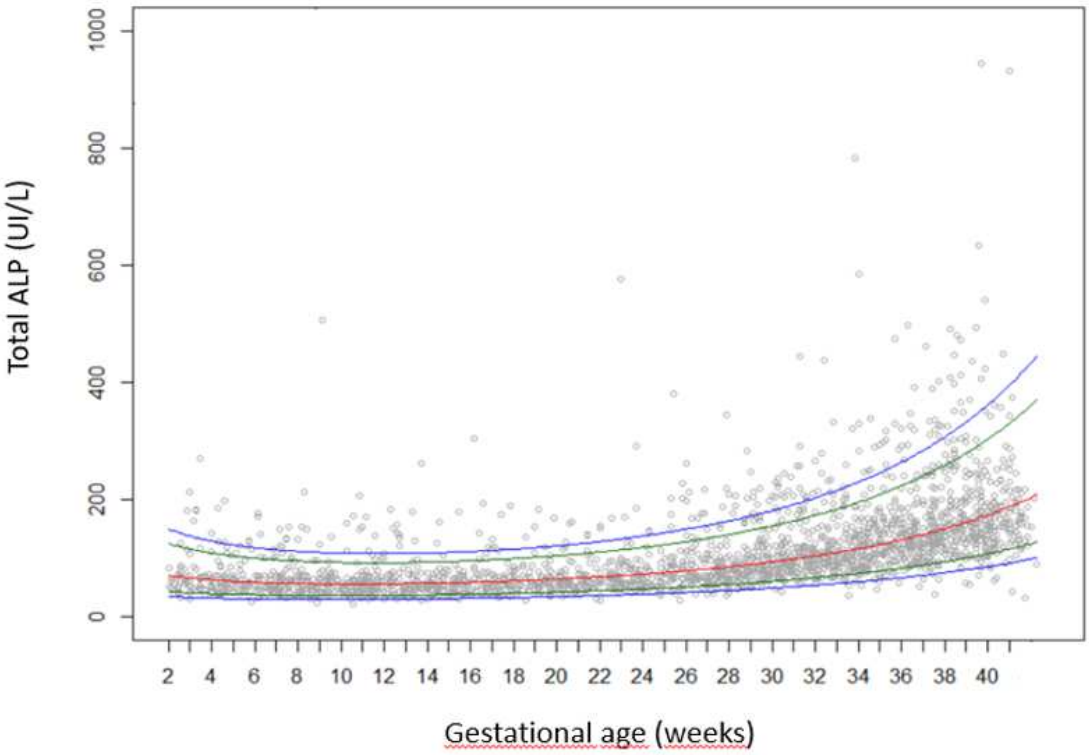


Figure 2. Distribution of total alkaline phosphatase (tALP) values by gestational age among the 2415 included pregnancies.

The solid line curves represent the 2.5th , 10th , 50th , 90th and 97.5th percentiles obtained after modeling (from bottom to top). The median is shown in red.



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