The Importance of Local Weight Adjusted Biomarkers of First-Trimester Pregnancy Screening Test for Trisomy 13 and 18 on Detection Rate

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Abstract

The combined test plays an important role in reducing the number of new born babies with aneuploidies like trisomy's 21(T21), 13(T13) and 18(T18). The T13, T18 risk assessment, using the weightadjusted values of local Multiple of Medians(MoM_{corr}) of free-β-HCG and PAPP-Amaternal serum concentrations, compared to the risk calculated based on default values, will help to improve the accuracy levels. The correction is based on log-linear method.297 pregnant Albanian women were selected for serum screening with intention to calculate the risk for T13 and T18. Concentration of PAPP-A and free-β-HCG were measured with COBAS6000. Calculations for fetus T13, T18 risk were computed with ssdlab5. The MoM_{corr} were calculated based on gestational age groups. The formula for free β-HCGMoM_{corr} is: free-*β*-HCG MoM = free-*β*-HCG MoM/$10^{(0.276-0.0040 \text{ x weight})}$ and for PAPP-AMoM_{corr} = PAPP-AMoM/$10^{(0.4416-0.0066 \text{ x weight})}$. The standard deviations ofMoM_{corr} values vary from *0.656to1.57*; relative error between risk assessments from 0% to 0,15%. By means of Albanian MoM_{corr} only for two cases the results of risk assessment didn't match with the one calculated with default MoM_{corr}, and the patients moved from high risk zone to low risk zone. The localMoM_{corr} doesn't have any impact on detection rate.

KEYWORDS: Detection rate, MoM, PAPP-A, free β-HCG, screening test.

INTRODUCTION

The combined test is a screening test for the first trimester of pregnancy, to calculate the fetus risk for trisomy 21 (T21), trisomy 13 (T13) and trisomy 18 (T18). The combined test plays as well an important role in avoiding the invasive tests like Chorionic Villi sample and amniocentesis, both methods do represent a threat for mother's health and for premature pregnancy termination. Nowadays, due to its low costs, a higher number of Albanian pregnant women of broad age range perform this test. The performance of this test depends on the selected method and the accuracy of the maternal serum concentration values of the two biochemical markers; free beta sub units of human Chorionic Gonadotropin (free β-hCG), Pregnancy Associated Plasma Protein-A (PAPP-A) and that of sonographic marker the Nuchal Translucency (NT). The risk estimations for T13 and T18 are carried out by means of the risk calculator which enables the use of Albanian (local) Multiples of Median (MoM) weight adjusted values calculated with log-linear correction method for biochemical markers of maternal serum concentrations. First

important purpose of the study is the calculation of the MoM weight adjusted value of both serum markers for the sample of the Albanian pregnant women with singleton pregnancies. Second important purpose of the study is the risk recalculation for trisomy by substituting in the risk assessment calculator (statistical ssdlab5 program) the default MoM weight adjusted values with the respective values of MoM weight adjusted calculated based on Albanian women serum sample. In both cases the concentration of the serum markers were measured with COBAS6000 and the default MoM of NT values were use in the risk calculator for the risk assessment of the patient. The outcome of this study will also contribute to improve the accuracy levels of the risk assessment by lowering the false positive and negative rates. The test performance of the test will also improve by using the default MoM_{corr}values in Albanian population if the screening will increase the number of true positive cases.

MATERIALS AND METHODS

A sample of 297 Pregnant Albanian women (singleton pregnancy) has been selected for serum screening with intention to calculate the risk for T21,T13 and T18.The results of risk assessment for T21has already been reported (*Xhuvani E. et al. OIIRJ, 2014*). In this study we are partially reporting the results for screening T13 and T18. The selecting criteria for the women around the country to undergo the serum screening were nonsmoker, no twins' pregnancies, no anamnesis and family history for the T21, T18 and T13, mother's WGA not less than 9 weeks but less than 14 weeks, non-diabetic and insulin dependent, confirmed pregnancy outcome (through karyotyping etc.). In the laboratory are performed karyotyping analysis (RHG, ~350 bands) from at least 16 metaphases derived from two independent cultures in order to ensure maximum reliability of test results. The test reporting period was 24-48 hours for aneuploidy PCR and detection of F508del and up to 2 weeks for karyotype results. Fresh blood samples were drawn in the Intermedica Tirana clinic on the same day of ultrasound scanning (measurement of CRL, Crown-to-rump length and NT, Nuchal translucency). Concentration values of PAPP-A and free-β-HCG were measured with COBAS6000 and standardized echography techniques were used from specialized sonographer for measuring NT, CRL values. All women who were missing one of the parameters (serum markers concentrations, patient weight and age) necessary for the risk calculation were excluded from the study. Calculations for fetus T13, T18 risk were computed with risk calculator ssdlab5 and the relative error (η), standard deviation (STDEV), regression analysis with Excel Data analysis and Medcalc. All necessary lab materials were provided from Roche for cobas e analyzer.

Age groups of populations were selected based on gestational age (GA) from 73 to 98 gestational days. Then all the MoM data were grouped into week gestational age (WGA) from 11 weeks to 14 weeks. The figures and tables presented in this paper are the results of 82 cases of 13 WGA group.

The log-linear method (*Reynolds et al., 1991: Wald et al., 1981*) was selected for the calculation of the Albanian maternal weight adjusted MoM values to calculate the risk for trisomy 13 and trisomy18. The formulas used for weight corrected biomarkers (*Spencer et al., 2000b*) are as follows:

$$\textbf{free}\beta\textbf{-HCGMoM}_{\textbf{corr}} = \textbf{free }\beta\textbf{-HCG MoM}/10^{(0.276-0.0040 \times \text{weight})};$$

and for corrected PAPP-AMoM is;

$$\mathbf{PAPP\text{-}AMoM_{corr} = PAPP\text{-}AMoM/10^{(0.4416-0.0066 \times weight)}};$$

where weight values are expressed in kg.

Calculation of MoM weight adjusted values (MoMcorr) were carried out without changing the coefficients in the log-linear formula while the risk assessment has performed by substituting the own data base MoM weight adjusted values(default) of both biochemical markers, (maintaining the default value for NTMoM) with that of Albanian ones. In three cases the MoM's values above 5 were truncated at 5. The MoM_{corr} are calculated for each WGA groups. The MoM_{corr} calculations are performed based only on the data of unaffected women.

We did the comparison between both results against the standard deviation values, relative error, variation coefficient and also we compared R squared values through the regression analysis and scatterplot line.

The steps of the procedure applied by *Neveux et al. (1996)* has been followed to confirm that the weight adjusted MoM values are correct: ***a***. calculation of the median of the MoM values for the serum analytes with and without weight correction; ***b***. comparing of the weight corrected medians with uncorrected medians of the MoM values and; ***c***. the overall pre-correction and post-correction values should be more or less the same and more or less equal to 1.00 MoM.

RESULTS

The serum of 297 Albanian Pregnant women, with singleton pregnancy, has been analyzed for the biochemical markers concentrations in their first trimester of pregnancy. Figure 1 shows the distribution (relative frequency in %) of pregnant women weight of the Albanian sample and according to Kolmogorov-Smirnov test the normal distribution is accepted *(p-value = 0.2529).* The dot line represent the normalized weight distribution.

The weight median value of the Albanian women is 62kg with confidence interval (CI) from 60 to 64, the lowest value is 45kg and the highest is 88kg. The MoM's weight adjusted were calculated from 73 to 98 days GA equivalent of 11 to 14 weeks gestation age. The absolute frequency of cases for each age group (based on GA), is as follows: $f_{84}=16$; $f_{85}=10$; $f_{86}=16$; $f_{87}=11$; $f_{88}=13$; $f_{89}=21$; $f_{90}=15$; $f_{91}=21$; $f_{92}=20$; $f_{93}=11$; $f_{94}=26$; $f_{95}=16$ *and* $f_{98}=10$ *and* $d_{iffer}=91$ (total of 297cases). Only age groups with frequency above 10 cases each were taken into consideration in the statistical analysis (196 cases).

In the table 1 are presented the weight adjusted MoM values, for both default (de) and Albanian (alb) as well as the respective values of $\log_{10}$Mom values only for 13 weeks gestational age-group. The dispersion of the data set of MoMs' values (both for default and Albanian) of the same WGA assessed with standard deviations are within the same interval of values from *0.656 to 1.57*. This confirms that the entire data set of MoMs values are close to each average values.

The relative error (η) calculated between risk assessments computed with both MoMs'(de and alb) for fetus aneuploidies (T18 and T13) for the same WGA is from 0% to a maximum error of to 0,15%. The absolute error calculated between default and Albanian probability risk values by means of ssdlab5 risk calculator for the same WGA

(using the MoM_{corr} and default NTMoM values), is highly significant (p<0,0001, *Wilcoxon test* for paired samples) but practically this variation does not represent any important significance, because the computed risk probability in all cases was much less that 1:250 *(p<0.004)* that was considered as cut off value, which means that none of the positive cases (*p≥0.004*) changed their risk level.

The differences between the variation coefficients carried out of Albanian women sample and default MoM values based on WGA shows insignificant differences (table 1).

The goodness of fit, R squared coefficient value, estimated from regression analysis of the 13 week GA, in both samples is very low between 0,003 and 0,19 and is higher in the Albanian women sample (charts 1, 2, 3, 4).The regression equation for both PAPP-A and free beta HCG equation varies as slope and intercept but the trendline remains almost invariable. The linear regression line of default PAPP-AMoMcorr is *y = -0.005x + 1.725* and the *R² = 0.002*; the linear regression line for localPAPP-A MoMcorris*y = -0.039x + 3.749* and the respective value of is*R² = 0.153*(figure2).The $Log_{10}MoMPAPP\text{-}A_{de}$has y=-0.0027x+0.2571 and R² = 0.0087; the $log_{10}MoMPAPP–A_{alb}$ has y = -0.013x + 0.871 and R² = 0.190 (figure 3). Evidently the local slope and R^2 values are higher than those of the default ones. The same tendency can be observed for free beta HCG analysis (figure 4 and 5).

The *Neveux et al. (1996)* procedure has confirm that the weight adjusted MoM_{alb} values are acceptable to be used in the risk calculator..

DISCUSSIONS

Based on the above results, the substitution of the default weight corrected MoMs' with that of Albanian weight corrected MoMs', didn't lead to important changes in the values of the risk calculation for T13 and T18. None of the confirmed positive or negative cases changed their status by moving from high risk zone to low risk zone or vice-versa. The detection rate, calculated as the ratio between the total screen positive cases with the total number of the true positive cases, didn't change because the number of screen positive cases remained the same. Although the local MoM_{corr} seems to have no impact on detection rate, the results on the patient risk assessment shows an impact on false positive rate (two true negative cases, that were screened positive with default MoM_{corr}) moved from high risk zone to low risk zone when screened with local MoM_{corr}. This fact might play an important role in the continuity of the pregnancy as being very important for the psychological state of the pregnant women.

The higher value of R squared coefficient for the Albanian sample shows that all data have a better goodness-of-fit to the regression line than the default values for both linear and log-linear.The fact that there is a tendency for the local slope and R^2 values to be higher than those of the default ones may be considered as a characteristic of the Albanian population.

In order to explain these two slight differences, it would be necessary, for further and better explanations, a larger number of Albanian women to be reached. So far, we can confirm that the default weight corrected MoM values are suitable to use in the combined screening test for the Albanian population, but the use of local MoM_{corr} might increase the screening accuracy as well as improve reliability.

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Table 1- Some of MoMs' adjusted values of serum biochemical markers at 13 WGA

Patient Weight [kg]	MoMPAPP-A_{de}	MoMPAPP-A_{alb}	log_{10}MoM PAPP-A	log_{10}MoMPAPP-A_{alb}	Free β-HCG MoM	free β-HCGMoM$_{alb}$	Log_{10} HCG	Log_{10} HCG$_{alb}$
50	2.58	2.56	0.412	0.408	0.77	0.84	-0.11	-0.08
50	3.94	5	0.595	0.699	2.22	2.57	0.35	0.41
50	2.63	3.58	0.420	0.554	0.87	1.18	-0.06	0.07
53	1.14	1.01	0.057	0.004	0.85	0.87	-0.07	-0.06
53	0.91	1.24	-0.041	0.093	2.20	2.4	0.34	0.38
53	2.20	3.23	0.342	0.509	1.23	1.12	0.09	0.05
53	0.89	0.93	-0.051	-0.032	0.51	0.55	-0.29	-0.26
56	1.10	1.23	0.041	0.090	2.75	0.48	0.44	-0.32
56	2.73	2.96	0.436	0.471	0.50	0.59	-0.30	-0.23
57	1.55	1.93	0.190	0.286	2.79	2.63	0.45	0.42
57	1.64	1.46	0.215	0.164	2.41	2.38	0.38	0.38
58	0.85	1.08	-0.071	0.033	1.10	0.89	0.04	-0.05
58	1.30	1.62	0.114	0.210	2.38	2.04	0.38	0.31
58	1.04	1.05	0.017	0.021	0.94	1.06	-0.03	0.03
58	1.42	0.91	0.152	-0.041	0.81	0.88	-0.09	-0.06
58	0.75	0.65	-0.125	-0.187	1.69	1.63	0.23	0.21
58	1.41	1.22	0.149	0.086	3.42	3.29	0.53	0.52
59	3.65	3.66	0.562	0.563	1.46	4.07	0.16	0.61
59	0.83	1.01	-0.081	0.004	1.34	1.11	0.13	0.05
59	0.94	1.13	-0.027	0.053	1.89	1.56	0.28	0.19
59	0.97	1	-0.013	0.000	0.62	0.54	-0.21	-0.27
59	1.47	1.22	0.167	0.086	1.24	1.16	0.09	0.06
60	1.64	1.11	0.215	0.045	0.75	0.64	-0.12	-0.19
60	1.06	1.09	0.025	0.037	1.74	2.11	0.24	0.32
60	1.15	1.11	0.061	0.045	1.11	0.87	0.05	-0.06
60	0.52	0.61	-0.284	-0.215	0.59	0.47	-0.23	-0.33
60	0.44	0.41	-0.357	-0.387	1.36	1.47	0.13	0.17
60	2.04	1.37	0.310	0.137	0.70	0.66	-0.15	-0.18
61	0.44	0.52	-0.357	-0.284	1.57	1.21	0.20	0.08
62	2.01	1.07	0.303	0.029	1.92	1.73	0.28	0.24
62	2.52	1.13	0.401	0.053	1.17	1.14	0.07	0.06
62	2.34	1.49	0.369	0.173	4.92	4.07	0.69	0.61
62	0.84	0.76	-0.076	-0.119	2.07	0.64	0.32	-0.19

62	1.19	1.08	0.076	0.033	0.59	0.84	-0.23	-0.08
62	0.72	0.82	-0.143	-0.086	1.86	1.38	0.27	0.14
62	1.44	1.08	0.158	0.033	0.86	0.75	-0.07	-0.12
62	0.53	0.4	-0.276	-0.398	0.73	0.65	-0.14	-0.19
63	0.63	0.73	-0.201	-0.137	3.36	2.11	0.53	0.32
63	1.73	1.61	0.238	0.207	0.35	0.24	-0.46	-0.62
63	1.39	0.86	0.143	-0.066	1.31	1.06	0.12	0.03
63	2.40	2.09	0.380	0.320	1.08	1.06	0.03	0.03
63	1.20	1.33	0.079	0.124	0.59	0.42	-0.23	-0.38
63	3.08	2.22	0.489	0.346	3.49	2.12	0.54	0.33
64	1.72	1.64	0.236	0.215	1.30	1.14	0.11	0.06
64	0.85	0.71	-0.071	-0.149	0.40	0.24	-0.40	-0.62
64	1.10	0.9	0.041	-0.046	1.08	1.07	0.03	0.03
64	1.13	1.08	0.053	0.033	0.76	0.83	-0.12	-0.08
65	0.99	0.5	-0.004	-0.301	0.57	0.47	-0.24	-0.33
65	0.77	0.62	-0.114	-0.208	3.82	0.55	0.58	-0.26
65	1.52	1.2	0.182	0.079	1.07	1.04	0.03	0.02
65	0.45	0.3	-0.347	-0.523	1.38	1.14	0.14	0.06
65	0.66	0.44	-0.180	-0.357	0.40	0.33	-0.40	-0.48
66	0.75	0.47	-0.125	-0.328	1.44	1.04	0.16	0.02
66	0.64	0.67	-0.194	-0.174	1.36	0.88	0.13	-0.06
66	1.60	1.04	0.204	0.017	1.33	1.08	0.12	0.03
67	1.40	1.43	0.146	0.155	0.60	0.38	-0.22	-0.42
67	0.67	0.48	-0.174	-0.319	0.70	0.6	-0.15	-0.22
68	2.49	3.38	0.396	0.529	3.18	1	0.50	0.00
68	0.89	0.89	-0.051	-0.051	2.43	1.46	0.39	0.16
68	1.16	0.83	0.064	-0.081	3.35	3.05	0.53	0.48
68	1.81	0.97	0.258	-0.013	0.88	0.69	-0.06	-0.16
68	1.19	1.55	0.076	0.190	0.89	0.64	-0.05	-0.19
68	1.85	1.12	0.267	0.049	1.30	1.01	0.11	0.00
68	1.85	1.12	0.267	0.049	1.30	1.01	0.11	0.00
69	1.14	0.57	0.057	-0.244	0.66	0.48	-0.18	-0.32
69	2.25	1.81	0.352	0.258	2.00	1.99	0.30	0.30
76	0.49	0.42	-0.310	-0.377	1.44	0.67	0.16	-0.17
76	1.60	0.87	0.204	-0.060	1.63	1.27	0.21	0.10

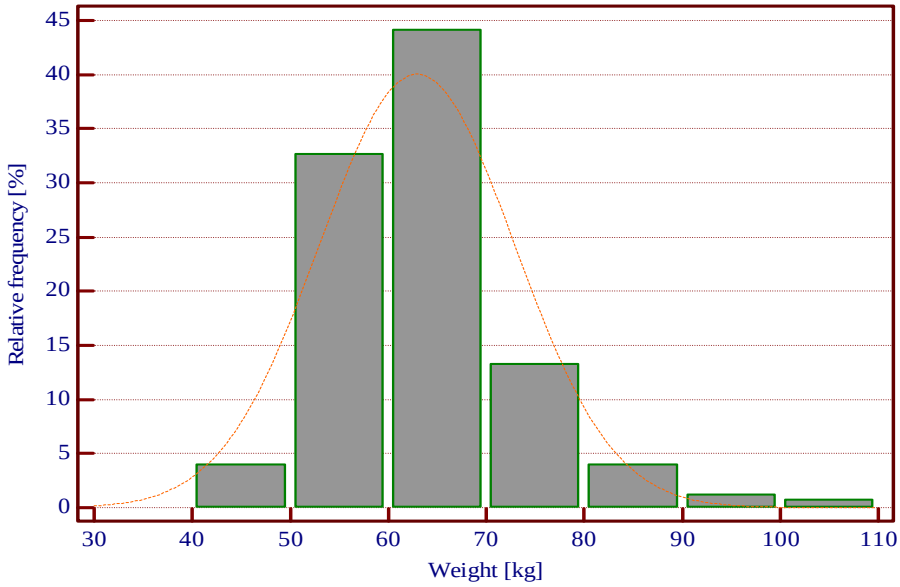

Figure 1-Histogram of weight distribution for 297 cases

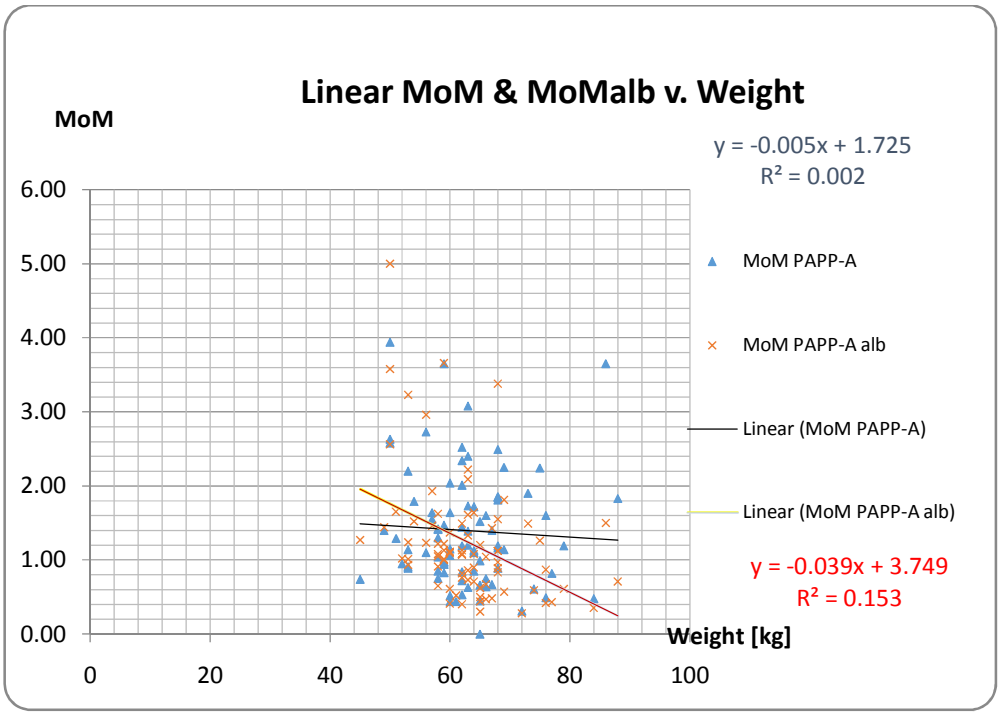

Figure 2- Linear MoM_{de}&MoM_{alb} PAPP-A against maternal weight (chart 1)

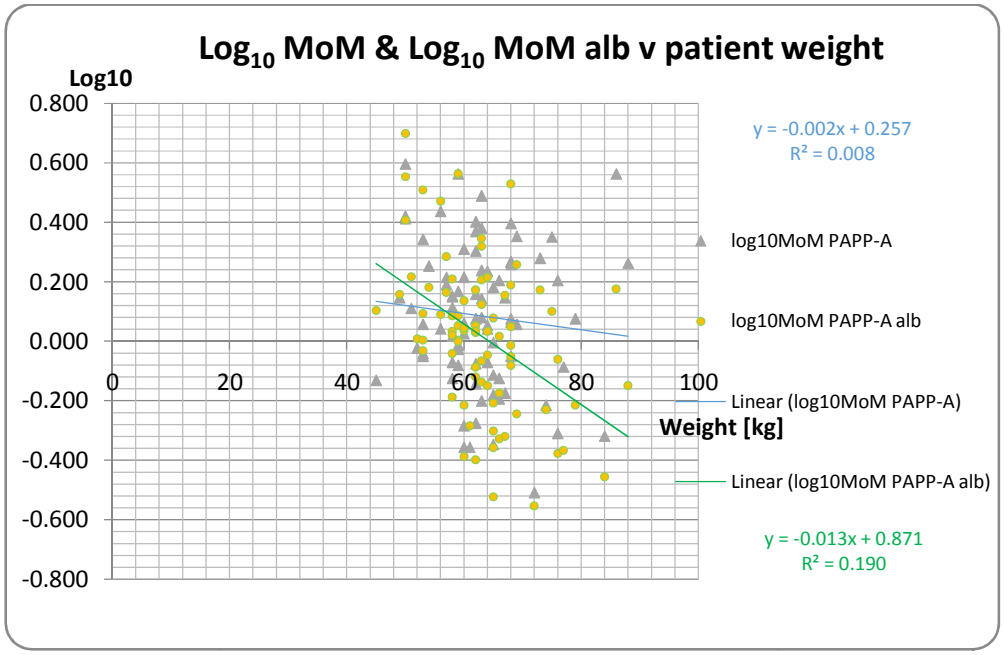

Figure 3-Log_{10} MoMPAPP-A_{de}& Log_{10} MoMPAPP –A_{alb} against maternal weight

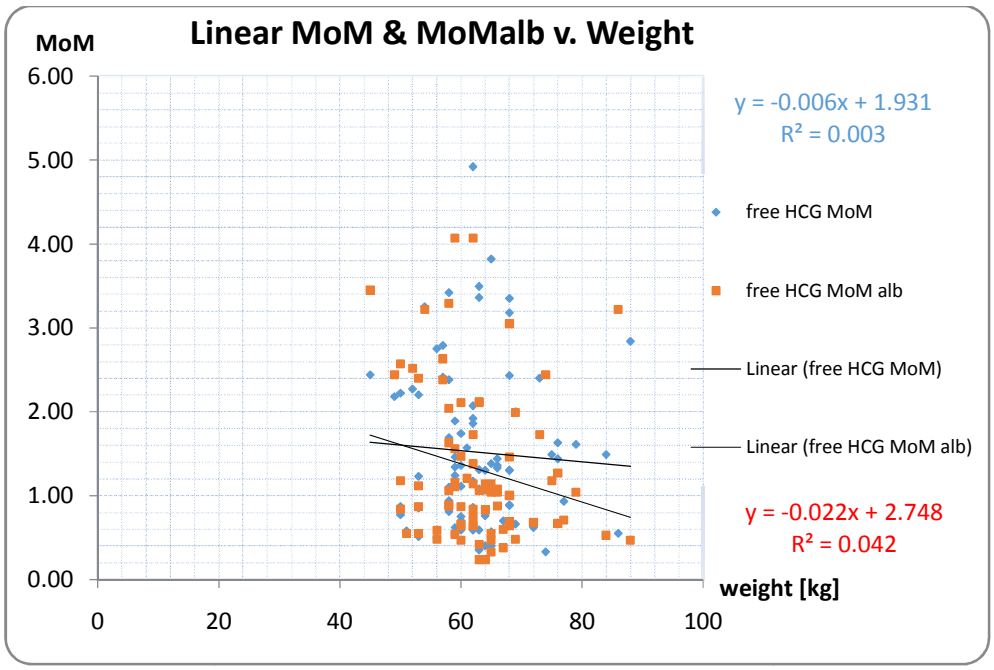

Figure 4-Linear $MoMHCG_{de}$&$MoMHCG_{alb}$ against maternal weight (chart 3)

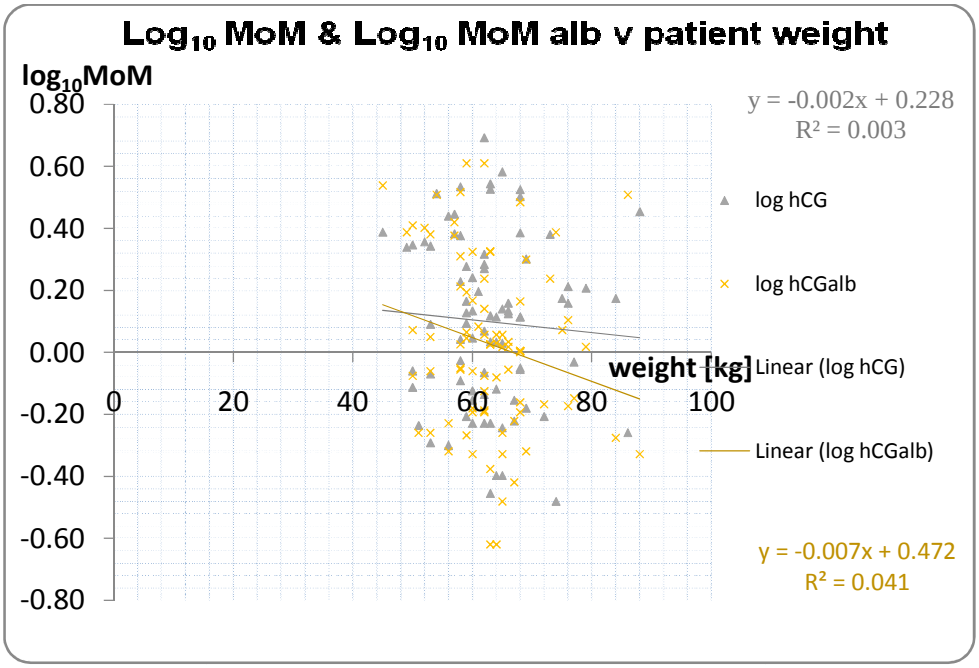

Figure 5-Freeβ-HCG $Log_{10}MoM_{de}$&Freeβ-HCG $Log_{10}MoM_{alb}$ against weight