The research for the relationship between nutritional status of vitamin A in infant's body and immunization effect of hepatitis B and measles vaccine

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Abstract: This paper is to discuss the relationship among the nutritional status of vitamin A in infant's body, antibody level of hepatitis B's surface and antibody concentration of measles IgG. 2ml of infant's venous blood is collected according to the inclusion criteria in planned immunization agencies at the local hospital; the method of combining dietary record review for 24 hours and diet records of 2 days is performed on infant caregivers, so as to know the eating food and its quantity for infants in successive 72 hours. Thus we conclude that the vitamin A in serum maybe has certain relationship with maintaining effective protection level of anti - HBs antibody and measles IgG antibody.

Keywords: Baby, vitamin A, the hepatitis B vaccine, the measles vaccine, the immune response.

INTRODUCTOIN

In recent years, the regulating effect of vitamin A on the innate immunity and adaptive immune system of human body has become a hot spot of research. The clinical manifestation of vitamin A deficiency (VAD) is the change of skin mucous membrane and night blindness (Aiqing *et al.,* 2011). Before this, the appeared immune function damage has resulted into the rise of susceptibility, which is called sub clinical vitamin A deficiency (SVAD). SVAD could inhibit the normal regeneration of impaired mucosal barrier and suppress the function of neutrophils, macrophages and natural killer cell, thus damage inherent immune function. In the adaptive immune system, vitamin A is necessary to the development of T helper cells and B cells. According to the survey, the high protection level of infant's vaccinal measles vaccine in China is not ideal (22%~80%) and the immune failure rate resulted from non-response or weak response of infants and young children to the hepatitis B vaccine is 1.89%~63.06%. This paper is to conduct research about whether vitamin A malnutrition is the influencing factor or not, by which to provide reference opinions and suggestions for enhancing the protection rate of inoculating measles vaccine on infants in China, thus improve their immune competence after inoculating measles.

Research object

In August 2008 to October 2010, in MeiBu town of Linyi city in Shandong province (hereinafter referred to as place A) and the people's hospital of MengYin County (hereinafter referred to as the place B), immunization institutions are planned by local bureau, the local infants of 9~10 months are extracted to participate in the survey. The condition enrolled in the study are: 1. Parents volunteer to participate and sign informed consent; 2. the antigen on infant's hepatitis b surface is negative and their parents have no history of hepatitis b; 3. they are full-term birth and normal birth weight; 4. According to the national immunization program, the infants after the birth of 0, 1, 6 months have to complete hepatitis b vaccination of 3 needle restructuring (yeast), all three doses are 5 ug; the measles vaccination will be completed on August, and two weeks after inoculation there appears no rash; 5. There is no immune system disease and no liver or kidney disease; 6. There is no fever and infectious diseases in nearly one month.

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THE RESEARCH METHOD

The investigation on babies' birth, feeding after birth, and economic conditions data of family is performed through using the questionnaire method, which is provided by the infant caregivers (Newton *et al*., 2010). The food intake situation of infants in successive 72h is known through using the method of combing dietary record review for 24h and diet records of 2 days. NCCW application software is used to calculate the estimated average requirements of vitamin A, and the percentage it accounts for in the recommended intake (RNI).

Sample collection of blood and laboratory tests

(1) Blood samples: for the infants of 9~10 months age, 2mL limosis venous blood is extracted through scalp venipuncture, then it is placed in the test tube of heparin anticoagulation coated with aluminium, the separation of serum is conducted on the scene (it is placed at room temperature away from light for 60 min, 3000 r/min, the centrifuge is conducted for 15 min), each sample is separated into three different parts. They are placed in

20□ refrigerator for TS (temporary storage) on the scene, then they will be transferred to the -70^{o} refrigerator under test. Serum transferring and other processing operations are all conducted under the dark environment and red light.

(2) The concentration determination of vitamin A in serum: the vitamin A concentration is determined though using HPLC method. Instruments: Waiers HPLC2695 system. With reference to the assessment standard of "national academic conference on improving children's quality of life": vitamin A concentration in serum <0.70 umol/L indicates subclinical vitamin A deficiency: vitamin A concentration in serum <0.35 umol/L (100 ug/L) indicates high deficiency of vitamin A; 0.35~0.69 umol/L (100~190ug/L) indicates the absolute deficiency of vitamin A; ② That the vitamin A concentration in serum between 0.70~1.05 umol/L (200~300ug/L) could be determined as suspicious subclinical vitamin A deficiency. ③ >1.05umol/L (>300ug/L) indicates it has no vitamin A deficiency.

(3) Anti-HBs concentration of serum (Agou and Junliang, 2009): electrochemiluminescent immunoassay (ECLIA) method is adopted in measurement. Instrument: i2000sr Architect Automatic electrochemical luminescence instrument (Abbott Laboratories). Kit: anti-HBs measure kit (i20005sr Architect instrument mating reagents of Abbott Laboratories). Judgement standard: anti-HBS concentration < 10 U/L is negative, < 2.1 U/L is no immune response, anti-HBS concentration between 2.1 U/L~10 U/L is weak immune response); ≥ 10 U/L is positive (normal immune response) (Zhenyu *et al*., 2008).

(4) The surface antigen (HBsAg) of hepatitis B virus applies colloidal gold diagnostic kit of surface antigen in hepatitis B virus produced by YiKang Biological Technology (Hangzhou) Co., Ltd.

(5) Hypersensitive c-reactive protein (hs CRP) is measured through using endpoint nephelometry. Instruments: Germany's Siemens BN - II specific protein analyzer. Judging criteria: Normal value 0.8~8.0 mg/L. Suppose CRP > 5.0mg/L is positive, according to the reference value provided by literature and testing instruments.

(6) Measles specificity IgG antibody is measured with ELISA method (Ning *et al*., 2009), <1 : 200 indicates it is negative, ≥ 1 : 200 indicates it is positive, ≥1 : 800 indicates it has reached protection level.

Quality control

Dietary survey consists of nutrition professionals, estimation training for food weight is performed before survey. All the measurement of babies' body length and body weight is conducted by the same researchers. Experiment data is entered through taking the method of EpiData software and double repeating entry, consistency check is performed after completing entry, then it will be inspected. Blood sampling is collected by specialists, the whole process is taken under the weak red light. Serum is separated by specialist, testing work of laboratory is done by specialist operations. Part of the sample is randomly selected for parallel detection, in order to ensure reliable testing data.

Statistical approach

Database is constructed with Excel and analyzed with SPSS115 statistical software. Before statistic analysis, normality test will be carried on all the measurement data. Nutrient intake is described through taking median and inter-quartile range; vitamin A concentration in serum is indicated with □±s (Aiping, 2008); anti-HBs concentration in serum is indicated with Geometric Mean Concentration (GMC); antibody concentration of measles IgG in serum is indicated with geometric mean reciprocal titer (GMRT); the comparison between the nutrients shortage rate and antibody positive rate of two sexes in two places is inspected with chi-square (Aiping, 2008); the comparison of vitamin A in serum of two places for female and male is analyzed with t-test and variance; the correlation analysis of vitamin A level in serum and antibody concentration of measles IgG is conducted with simple correlation method. $P \leq 0.05$ is the difference with statistical significance.

RESULT

General information would investigate 217 cases of infants about 9~10 months age in total, where 204 cases of adequate blood sample are collected, 5 cases of increased c-reactive protein and 1 case of HBsAg positive people are excluded, 198 cases entered into final analysis in total. After test, there are no statistical differences in the distribution of month age and sex for the infants in two places, as shown in table 1.

The nutritional status of vitamin A: The infants' vitamin A concentration of serum in place A is higher than it in place B ($P<0.05$). The detectable rate of vitamin A malnourishment in place B is higher than place A ($P<0.05$). As shown in table 2.

Detecting result of anti-HBs: the geometric mean concentration (GMC) of anti-HBs for the infants in two places is 402.8 U/L, t-test is carried on its Log Koc (LgGMC), there appears no significant difference between two places and sexes.Anti-HBS positive rate in two places is 98.0% on average, there appears no significant difference between two places and sexes. As shown in table 3.

Table 1: The comparison of the condition about infants' sex and month age in two places

Grouping	Case number	Male	Female	Month age
		Case (%)	Case (%)	(± , month)
Place A	113	65(57.5)	48(42.5)	9.9±0.5
Place B	85	52(61.2)	33(38.8)	9.8±0.4
P value	0.60		0.17	

Table 2: The comparison of the condition about vitamin A concentration in serum and detectable rate of malnutrition for infants in two places

Project	Sex	Reciprocal	VA concentration in serum (± umol/L)	Subclinical Vitamin A shortage case (%)	Suspicious subclinical vitamin A shortage case (%)	Total case (%)
Place A	Male	65	0.81±0.43 [a]	24(36.9)	12(8.5)	36(54.4) [i]
	Female	48	0.72±0.45 [b]	21(43.8)	11(22.9)	32(66.7) [j]
	Sub-total	113	0.77±0.44 [c]	45(39.8)	23(20.4)	68(60.2) [k]
Place B	Male	52	0.70±0.45 [d]	22(42.3)	15(28.8)	37(71.2) [l]
	Female	33	0.55±0.40 [e]	19(57.6)	9(27.3)	28(84.8) [m]
	Sub-total	85	0.64±0.43 [f]	41(49.4)	24(28.9)	65(78.3) [n]
Total	Male	117	0.76±0.44 [g]	46(53.5)	27(57.4)	73(54.9) [o]
	Female	81	0.65±0.44 [h]	40(46.5)	20(42.6)	60(45.1) [p]
	Sub-total	198	0.72±0.44	86(43.4)	47(23.7)	133(67.2)

Note: the comparison between place A and place B, ^{ad}t=1.42, P = 0.160; ^{be}t= 1.74, P = 0.086; ^{ef}t = 2.12, P = 0.036; $^{il}X^2$ =3.06, P=0.080; $^{jm}X^2$= 3.37, P=0.067; $^{kn}X^2$= 5.84, P = 0.016; The comparison between sex, ^{ab}t= 1.15, P = 0.252; ^{de}t= 1.58, P = 0.118; ^{gh}t = 1.80, P = 0.073; $^{il}X^2$=1.47, P=0.23, $^{lm}X^2$= 2.10, P = 0.147; $^{op}X^2$ = 2.96, P = 0.085;

Table 3: The comparison of anti-HBs' geometric mean concentration and anti-HBs' positive rate in two places

Projects		Case number	GMC (U/L)	Lg GML	Positive cases	Positive rate (%)
Place A	Male	65	447.8	2.584±0.443[a]	65[i]	100.0
	Female	48	383.9	2.651±0.424[b]	47[j]	97.9
	Sub-total	113	419.4	2.623±0.432[c]	109[k]	96.5
Place B	Male	52	420.0	2.516±0.544[d]	51[l]	98.1
	Female	33	328.3	2.623±0.535[e]	31[m]	93.9
	Sub-total	85	381.7	2.582±0.538[f]	82[n]	96.5
Total	Male	117	435.2	2.639±0.474[g]	116[o]	99.2
	Female	81	360.2	2.557±0.485[h]	78[p]	96.3
	Sub-total	198	402.8	2.557±0.485	194	98.0

Note: GMC: geometric mean concentration The comparison between place A and place B, ^{ad}t=0.31, P=0.754; ^{be}t=0.62, P=0.537; ^{ef}t=0.60, P=0.552; $^{il}X^2$=1.62, P=0.444; $^{jm}X^2$= 0.85, P=0.564; $^{kn}X^2$=1.00, P=1.0000; The comparison between sexes, ^{ab}t=0.81, P=0.419; ^{de}t=0.89, P=0.374; ^{gh}t=1.19, P=0.237; $^{il}X^2$=1.72, P=0.425; $^{lm}X^2$=0.98, P=0.557; $^{op}X^2$=1.96, P=0.307;

The relationship between vitamin A concentration in serum and anti-HBs concentration: There exists significant difference among the anti-HBs concentration Log Koc of sub clinical vitamin A shortage group, suspicious sub clinical vitamin A shortage group and normal group (F=15.8, P=0.000), there appears no significant difference in two places (t=0.60, P=0.552), the correlation coefficient of vitamin A concentration in serum and anti-HBs concentration Log Koc is 0.441 (P=0.01). As shown in table 4.

The relationship between the average daily intake of vitamin A and anti-HBs concentration: the average daily intake of infants' vitamin A in two places accounts for 52.0% in RNI median, only 19.7% of the infants reach 80% of RNI in total. Through X^2test, there appears no significant difference in two places, P=0.436. There appears no significant correlation between the percentage of average daily intake of vitamin A in RNI and anti-HBs concentration Log Koc: r=0.052 (P=0.387), as shown in table 5.

The test result of vitamin A nutriture and measles IgG antibody in serum of two different places. As shown in table 6.

The relationship between vitamin A concentration and antibody concentration of measles IgG in serum. There exists statistical differences in the GMRT Log Koc of measles IgG antibody among SVAD group, suspicious SVAD group and normal group (F=33.77, P=0.000), there is no statistical differences in the comparison of two

Table 4: The influence of vitamin A level distribution in serum on anti-HBs concentration

Grouping	Place A			Place B			Total		
	Case Number	GMC (U/L)	LgGMC	Case Number	GMC (U/L)	LgGMC	Case number	GMC (U/L)	LgGMC
Subclinical vitamin A shortage group	45	255.4	2.407± 0.478[a]	42	279.4	2.446± 0.613[e]	87	266.7	2.426± 0.545[i]
Suspicious subclinical vitamin A shortage group	26	444.4	2.648± 0.426[b]	25	400.3	2.602± 0.472[f]	51	422.2	2.625± 0.445[j]
Normal group	42	688.8	2.838± 0.233[c]	18	739.6	2.869± 0.271[g]	60	703.7	2.847± 0.243[k]
Total	113	419.4	2.623± 0.432[d]	85	381.7	2.582± 0.538[h]	198	402.8	2.605± 0.479[l]

Note: GMC: geometric mean concentration The comparison between place A and B, [ae]t=0.33, P=-0.741; [bf]t=0.36, P=0.719; [cg]t=-0.45, P=0.655; [dh]t=0.60, P=0.552 The comparison between different groups, place A: [d]F=13.27, P=0.000; [ae]MD=-0.431, P=0.000; [be]MD=-0.190, P=0.129; [ab]MD=-0.241, P=0.037 Place B: [h]F=4.22, P=0.018; [cg]MD=-0.423, P=0.013; [lg]MD=-0.267, P=0.225; [ef]MD=-0.156, P=0.461; Two places: [i]F=15.88, P=0.000; [ih]MD=-0.421, P=0.000; [jk]MD=-0.221, P=0.026; [ij]MD=-0.199, P=0.032

Table 5: The comparison between the ADI (acceptable daily intake) of vitamin A and anti-HBs concentration in two different places (U/L)

C	Place A			Place B			Total		
	case number	GMC (U/L)	LgGMC	case number	GMC (U/L)	LgGMC	case number	GMC (U/L)	LgGMC
<20%	6	374.7	2.574±0.453	1	369.5	2.568	7	372.1	2.571±0.201
20%~	30	401.5	2.604±0.421	16	384.8	2.585±0.527	46	393.1	2.594±0.501
40%~	31	456.3	2.659±0.383	30	412.5	2.615±0.681	61	433.8	2.637±0.489
60%~	24	414.2	2.617±0.432	21	439.2	2.643±0.335	45	426.5	2.630±0.388
80%~	12	411.5	2.614±0.581	9	384.8	2.585±0.556	21	397.9	2.600±0.562
100%~	10	510.8	2.708±0.458	8	413.2	2.616±0.454	18	459.4	2.662±0.456
Total	113	426	2.629±0.371	85	400	2.602±0.578	198	412.8	2.616±0.468

The comparison between place A and place B, X^2=4.84, P=0.436.

Table 6: The comparison between infants' vitamin A level and measles IgG anti-body in serum of twp places

Grouping		n	VA concentration in serum (umol/L)	Case number of VA malnutrition case (%)		Measles lgC antibody concentration		Case number of measles lgC antibody case (%)	
				SVAD	Suspicious SVAD	GMRT	LogGMRT	Positive	Protection
Place A	Male	65	0.81±0.43	24(36.9)	12(18.5)	832.8	2.921±0.353	62(95.4)	54(83.1)
	Female	48	0.72±0.45	21(43.8)	11(22.9)	737.0	2.867±0.373	46(95.8)	41(85.4)
	Subtotal	113	0.77±0.44	45(39.8)	23(20.4)	776.3	2.890±0.364	108(95.6)	95(84.1)
Place B	Male	52	0.70±0.45	22(42.3)	15(28.8)	703.5	2.847±0.323	49(94.2)	37(71.2)
	Female	33	0.55±0.40	19(57.6)	9(27.3)	656.0	2.817±0.333	31(93.9)	23(69.7)
	Subtotal	85	0.64±0.43	41(49.4)	24(28.9)	674.0	2.829±0.327	80(94.1)	60(70.6)
Total		198	0.72±0.44	86(43.4)	47(23.7)	730.6	2.864±0.349	188(94.9)	160(78.3)

Note: compared with place B, *P<0. 05

places (t=1.224, P=0.222); there exists positive correlation between vitamin A concentration and measles IgG antibody GMRT in serum (r=0.560, P=0.000). As shown in table 7.

The average daily intake of infants' vitamin A in two places accounts for 52.0% of RNI median, only 19.7% of the infants reach 80% of RNI in total. After $\chi 2$ test, there is no statistical differences in two places (P=0.44). There appears no correlation between the percentage of average daily intake of vitamin A accounts for in RNI and antibody GMRT of measles IgG (r=0.073, P=0.59).

DISCUSSION

Since our country has began to implement the vaccination of hepatitis B vaccine, it has greatly reduced the prevalence of hepatitis b in our country, but at present in

Table 7: The influence of vitamin A level distribution in serum on anti-body concentration of measles IgG

Grouping	Place A			Place B			Total		
	n	GMRT	Log GMRT	n	GMRT	Log GMRT	n	GMRT	Log GMRT
SVAD group	45	482.4	2.683±0.490	42	439.8	2.643±0.380	87	461.3	2.664±0.438
Suspicious SVAD group	26	1121.0	3.050±0.195	25	968.9	2.986±0.058	51	1043.7	3.019±0.147
Normal group	42	1029.4	3.013±0.062	18	1102.7	3.042±0.077	60	1050.8	3.022±0.068
Total	113	776.3	2.890±0.364	85	674.0	2.829±0.327	198	730.6	2.864±0.349

The relationship between average daily intake of vitamin A and antibody concentration of measles IgG in serum

our country there are still existing the phenomenon that surface antibody of hepatitis B virus (anti-HBs) is negative or it fails to get protection, thus threshold drops among 10%~25% of the population after implementing 3 doses of inoculation according to the immune program of 0, 1, 6 months (Guoxian, 2007). This research shows that for the infants who are 9-10 months age, their average geometric concentration of anti-HBs is 402.8U/L, the positive rate is 98.0%, the positive conversion rate is more ideal for the infants within 1 year old after inoculating the anti-HBs of hepatitis B vaccine, however, our team as well as other authors have found that for the children who have enjoyed successful inoculation against the hepatitis b vaccine, anti-HBs concentration also drops with the extension of inoculation time, until it is blew the effective protection concentration.

At present, some areas in the world have made remarkable progress in measles control, but there are still 30 million people becoming infected with measles worldwide each year, the estimated death cases are 610000, there else a lot of cases suffering with complications and permanent sequelae (Zhengyu *et al*., 2008). In most developing countries (Zhengyu *et al*., 2008), children are vaccinated against measles vaccine in nine months, the positive conversion rate is expected to be 80%~85% after inoculating serum antibody at that moment. This research shows that measles antibody GMRT in serum is 730.6 for the babies of 9-10 months old in rural, its positive rate is 94.9%, but positive protection rate is only 78.3%, which is close to above results and less than some developed countries (as much as 98%, these countries have postponed the time of measles vaccination until the child lost his mother antibody completely, such as 12 months old). In recent years, there are reports about the measles emission of little month age and small-scale prevalence, postponing vaccination does not conform to China's national conditions. Most domestic authors argues to turn to the following several aspects: ① improving the routine immunization coverage and the quality of vaccination; ② by improving the crowd immunization coverage, wild poliovirus circulation of measles is blocked, the inoculation rate of children in groups of big age is improved to ensure that the baby of 8 months will not be exposed to the measles infection; ③ measles vaccination of women in childbearing age will be inoculated and incorporated into the measure of controlling baby's measles.

CONCLUSION

This study shows that the average concentration of vitamin A content in serum among babies of 9~10 months old is 0.72±0.44umol/L, the average detection rate of vitamin A malnourishment is 67.2% (subclinical vitamin A deficiency is 43.4%, deficiency of suspicious subclinical vitamin A is 23.7%). Dietary survey of this study finds that the average daily intake of vitamin A accounts for 52.0% of RNI median, only 19.7% of children reaches 80% of RNI (YinZhong *et al*., 2007). The research object of this topic is the babies in infancy stage, who are in fast growth and development, their consumption of vitamin A is also relatively more, at the same time because they are in the adaptive stage of before and after adding assisted food and weaning, the intake of food rich in vitamin A is relatively less, which is the important cause of malnutrition in vitamin A. Studies have shown that the effect of VA on the incidence of measles is mainly due to the enhanced production of measles antibody and promoting the proliferation of lymphocytes. Coutsoudis etc found that vitamin A supplementation can increase the IgG antibody level after inoculating measles vaccine for children (Ross, 2007), but Semba etc hold opposing opinion. This study indicates that there exists statistical difference in GM-RT Log Koc of measles IgG among SVAD group, suspicious SVAD group and normal group; Vitamin A concentration in serum has positive correlation with antibody GMRT of measles IgG. It suggests that better nutritional status of vitamin A has significance on organism's maintaining effective protection concentration for antibody of measles IgG. In addition, this study does not indicate that there is correlation between the percentage of average daily intake of vitamin A accounts for in RNI and concentration of measles antibody (Black *et al*., 2008). Studies have also found that high dietary intake of vitamin A not only can obviously enhance the secretion of IgA and the synthesis of IL - 10, but also can reduce the concentration of IgG in serum and inhibit the synthesis of IFN -. This study does not indicate there exists correlation between the percentage of average daily intake of vitamin A accounts for in RNI and concentration logarithm of hepatitis b antibody (r=0.052, P=0.387).

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