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Establishment of Reference Range for Serum Concentration of Vitamin A and Vitamin E in Southern Sichuan Area of China

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ABSTRACT

Objective: To establish the reference range of serum concentration of vitamin A (VA) and vitamin E (VE) in Southern Sichuan area of China.

Methods: From August 1, 2021, to May 31, 2023, 9482 blood tablets were received for the screening of VA and VE. The information was divided into four different age groups: ≤1 year old, 1< to ≤6 years, 6< to ≤17 years, and 17< to ≤59 years. In each age group, the four seasons were further subdivided into spring, summer, autumn, and winter, as well as male and female genders. The serum concentration of VA and VE was detected by liquid chromatography—tandem mass spectrometry (HPLC-MS), and the reference range was established for verification.

Results: The concentration of VA and VE in 9482 cases showed skewed distribution. When comparing between different age groups, the serum concentration of VA and VE was statistically significant ($p < 0.05$). While comparing different seasons, the serum VA levels in different seasons were significantly different ($p < 0.05$) except in summer and autumn. There was statistical significance in VE level in different seasons ($p < 0.05$). And while comparing different genders, there was no statistical significance in VA concentration levels ($p > 0.05$). The VE concentration levels were statistically significant ($p < 0.05$). The established reference range was established and verified, and the results were in accordance with the standard.

Conclusion: The reference range of VA and VE should be set according to different ages, different seasons, and different genders.

1 | Introduction

Vitamins are essential trace organic substances for humans and animals, which are crucial in the process of body growth, metabolism, and development, and are also necessary conditions for maintaining normal physiological functions [1, 2]. Vitamin A (VA) and vitamin E (VE) are common vitamins that play an important role in all stages of human life, and their deficit and excess can affect human health [3, 4]. Vitamin A has a wide range of biological effects, mainly closely related to embryonic development, hematopoietic, immune function, vision, etc. [5, 6]; its deficiency can lead to eye-related diseases, such as decreased dark adaptability and night blindness, and even blindness [7], whereas its excess can cause acute, chronic, and teratogenic toxicity [8]. Vitamin E is essential for nervous system development, growth, and immunity [9, 10]. A relevant study has shown that VE deficiency mainly leads to nervous system abnormalities, anemia, cardiovascular diseases, pneumonia, asthma, and

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TABLE 1 | Normality test of vitamin A levels in different age groups.

	Kolmogorov–Smirnov			Shapiro–Wilk		
Age group (years)	Count	Degree of freedom	Significance	Count	Degree of freedom	*p* value
Age ≤1	0.09	3541	0	0.908	3541	0
1< to ≤6	0.115	1456	0	0.883	1456	0
6< to ≤17	0.15	404	0	0.855	404	0
17< to ≤59	0.108	4081	0	0.891	4081	0

TABLE 2 | Normality test of vitamin E levels in different age groups.

	Kolmogorov–Smirnov			Shapiro–Wilk		
Age group (years)	Count	Degree of freedom	Significance	Count	Degree of freedom	*p* value
Age ≤1	0.1	3541	0	0.925	3541	0
1< to ≤6	0.115	1456	0	0.922	1456	0
6< to ≤17	0.107	404	0	0.91	404	0
17< to ≤59	0.101	4081	0	0.862	4081	0

TABLE 3 | Comparison of vitamin A levels between different age groups.

Age group (years)	Testing statistic	Standard error	Standard test statistics	*p* value
Age≤1:6<to≤17	−836.102	143.7	−5.818	0
Age≤1:1<to≤6	−1232.791	85.192	−14.471	0
Age≤1:17<to≤59	−2549.317	62.846	−40.565	0
6<to≤17:1<to≤6	396.689	153.876	2.578	0.01
6<to≤17:17<to≤59	−1713.214	142.723	−12.004	0
1<to≤6:17<to≤59	−1316.525	83.533	−15.76	0

TABLE 4 | Comparison of vitamin E levels between different age groups.

Age group (years)	Testing statistic	Standard error	Standard test statistics	*p* value
Age≤1:6<to≤17	−836.102	143.7	−5.818	0
Age≤1:1<to≤6	−1232.791	85.192	−14.471	0
Age≤1:17<to≤59	−2549.317	62.846	−40.565	0
6<to≤17:1<to≤6	396.689	153.876	2.578	0.01
6<to≤17:17<to≤59	−1713.214	142.723	−12.004	0
1<to≤6:17<to≤59	−1316.525	83.533	−15.76	0

other diseases, whereas its excess leads to nausea and vomiting, blurred vision, elevated blood pressure, gastrointestinal dysfunction, muscle weakness, and other symptoms and hazards [11]. The literature on VA and VE reference intervals is few, and the reference intervals are only explored in children, almost not in adults. Therefore, in order to provide scientific and reasonable suggestions on VA and VE supplementation and to avoid any harm to human body due to either to their deficiency or excess, this study improved the reference range of VA and VE from children to adults through the concentration results from 9482 cases of VA and VE in addition to providing a scientific basis for the clinical diagnosis of VA and VE.

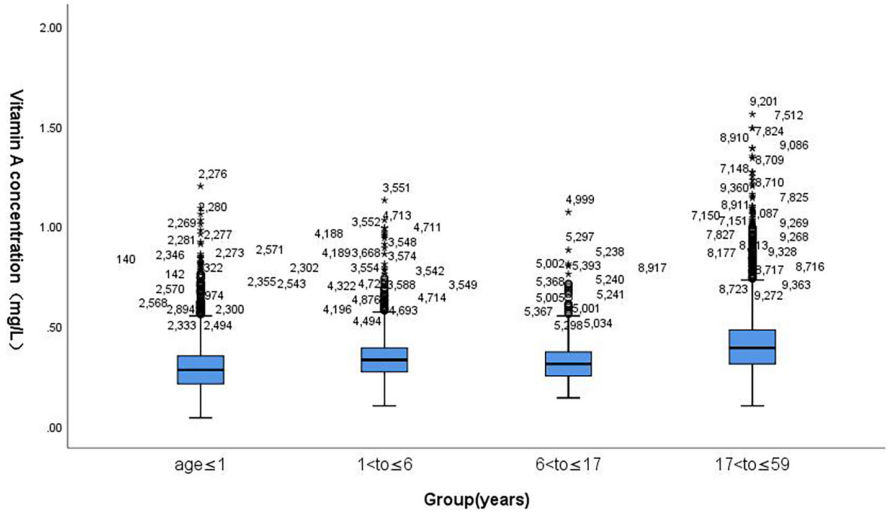

FIGURE 1 | Concentration of vitamin A was positively correlated with age.

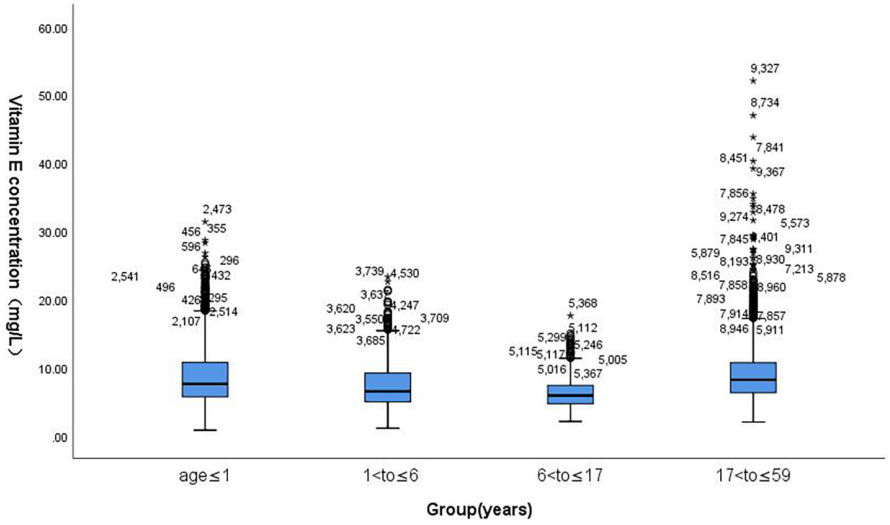

FIGURE 2 | Concentration of vitamin E was negatively correlated with age.

TABLE 5 | Comparison of vitamin A levels between different seasonal groups.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Summer–autumn	−29.68	90.229	−0.329	0.742
Summer–winter	−1546.885	80.523	−19.211	0
Summer–spring	1859.691	78.098	23.812	0
Autumn–winter	−1517.205	85.219	−17.804	0
Autumn–spring	1830.011	82.932	22.066	0
Winter–spring	312.806	72.252	4.329	0

2 | Materials and Methods

2.1 | Study Target and Design

From August 1, 2021, to May 31, 2023, 9482 blood tablets were received for screening of VA and VE. The clinical data of the enrolled subjects were collected, including name, gender, age, and collection date. Serum concentration levels in VA and VE were detected using Shimadzu 20A high-performance liquid phase-API3200MD Mass Spectrometer (HPLC-MS). Chromatographic conditions are as follows: Column: Athena C18-WP (100A, 2.1 × 50 mm, 3 μm); mobile phase: A is 0.1% formic acid aqueous solution, B is 0.1% formic acid ethanol solution; flow rate: 0.6 mL/min; column temperature: 40°C;

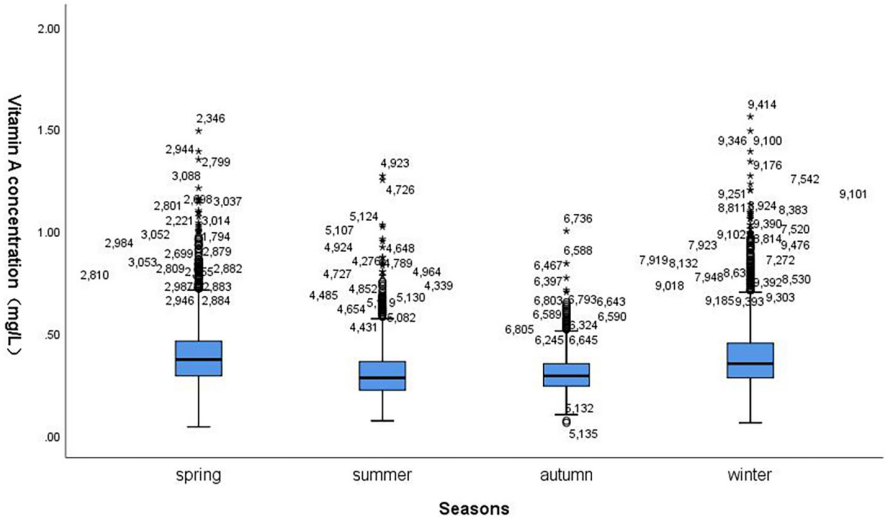

FIGURE 3 | Concentration of vitamin A was higher in spring and winter than in summer and autumn.

TABLE 6 | Comparison of vitamin E levels between different seasonal groups.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn–winter	−599.208	85.228	−7.031	0
Autumn–summer	809.269	90.221	8.97	0
Autumn–spring	1884.667	82.954	22.719	0
Winter–summer	210.061	80.524	2.609	0.009
Winter–spring	1285.459	72.289	17.782	0
Summer–spring	1075.398	78.113	13.767	0

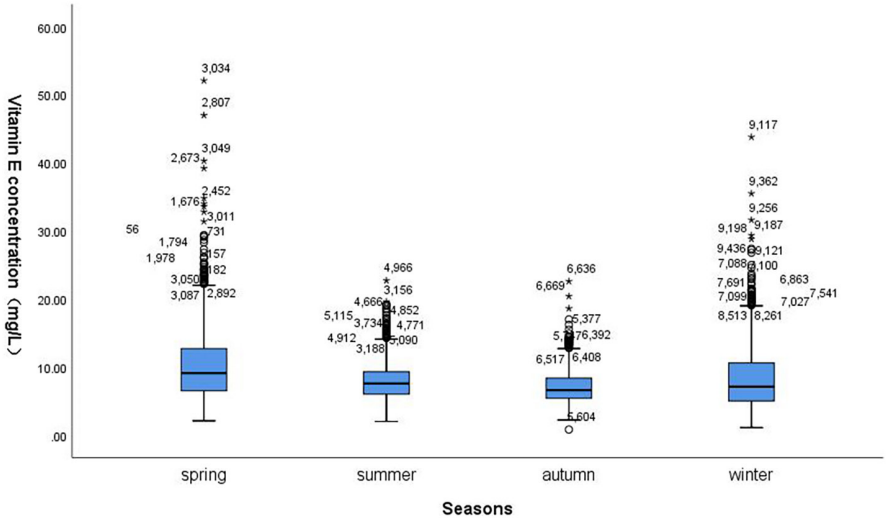

FIGURE 4 | Concentration level of vitamin E was higher in spring and winter than in summer and autumn.

and injection volume: 10 μL. Mass spectrum conditions are as follows: Ionization mode: electrospray ion source, positive and negative ions simultaneously collected; detection method: schedule, multi-reaction monitoring (MRM); and TEM: 600°C; Gas1: 60 psi. The required detection kit and the corresponding auxiliary reagents and consumables were purchased from Beijing MACE Mass Spectrometry Biotechnology Co., Ltd.

2.2 | Sample Collection and Vitamin Testing

About 2 mL of venous blood was drawn from the subjects in the early morning on an empty stomach, incubated at 37°C until the blood was completely agglutinated, and centrifuged at a relative centrifugal force of 16,000 × g for 5 min, and serum was collected and tested according to the testing procedure of supporting reagents.

TABLE 7 | Comparison of vitamin A levels between different seasons at age ≤1 year.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Summer–autumn	−58.016	56.067	−1.035	0.301
Summer–winter	−670.338	49.411	−13.567	0
Summer–spring	674.608	49.368	13.665	0
Autumn–winter	−612.322	50.963	−12.015	0
Autumn–spring	616.592	50.921	12.109	0
Winter–spring	4.269	43.484	0.098	0.922

TABLE 8 | Comparison of vitamin A levels between summer–autumn and winter–spring at age ≤1 year.

Rank test				Testing statistic	
Seasons	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	935,309.5
Winter–spring merger	2209	2013.59	4448023.5	Wilcoxon	1,823,087.5
Summer–autumn merger	1332	1368.68	1823087.5	*Z*	−18.192
Sum	3541			*p* value	0

TABLE 9 | Comparison of vitamin E levels between different seasons at age ≤1 year.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn–winter	−212.646	50.959	−4.173	0
Autumn–summer	300.484	56.029	5.363	0
Autumn–spring	727.465	50.942	14.28	0
Winter–summer	87.838	49.389	1.779	0.075
Winter–spring	514.819	43.532	11.826	0
Summer–spring	426.98	49.371	8.648	0

TABLE 10 | Comparison of vitamin E levels combined with winter and summer at age ≤1 year.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn–winter and summer merger	−246.797	47.202	−5.228	0
Autumn–spring	727.465	50.942	14.28	0
Winter and summer merger–spring	480.667	39.068	12.303	0

2.3 | Reference Range Validation Experiment

According to the age, season, and gender groups of the established reference range, 150 healthy people were randomly selected to determine the levels of VA and VE, and the established reference interval was verified.

2.4 | Statistical Method

SPSS 26.0 software was used for statistical analysis of experimental data. All group comparison data were tested for normality using the "one-sample Kolmogorov–Smirnov test." For those with normal distribution, the independent sample *t*-test was used for comparison between two groups, and single factor analysis of variance was used for comparison between multiple groups. Nonparametric tests were used for non-normal distributions. The outliers were removed by Tukey's method, and the reference interval was represented by *P*2.5–*P*97.5. $p < 0.01$ or $p < 0.05$ was considered statistically significant. If there is no statistical significance, the two sets of data can be combined for statistical processing. If the two were statistically significant, they were processed separately.

TABLE 11 | Comparison of vitamin A levels between different seasons at 1< age ≤6 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Summer–autumn	−69.12	35.431	−1.951	0.051
Summer–winter	−376.203	30.329	−12.404	0
Summer–spring	387.287	31.142	12.436	0
Autumn–winter	−307.084	32.993	−9.308	0
Autumn–spring	318.167	33.742	9.429	0
Winter–spring	11.083	28.338	0.391	0.696

TABLE 12 | Comparison of vitamin A levels between summer–autumn and winter–spring at 1< age ≤6 years.

Rank test				Testing statistic	
Seasons	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	130,839
Summer and autumn merger	883	866.82	765,406	Wilcoxon	295,290
Winter and spring merger	573	515.34	295,290	*Z*	−15.592
Sum	1456			*p* value	0

TABLE 13 | Comparison of vitamin E levels between different seasons at 1< age ≤6 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn–winter	157.211	35.449	4.435	0
Autumn–summer	−267.887	33.009	−8.116	0
Autumn–spring	309.249	33.759	9.16	0
Summer–winter	−110.676	30.344	−3.647	0
Summer–spring	152.038	31.158	4.88	0
Winter–spring	41.362	28.352	1.459	0.145

TABLE 14 | Comparison of vitamin E levels combined with winter and spring at 1< age ≤6 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn–summer	157.211	35.449	4.435	0
Autumn–winter and spring merger	−287.28	30.214	−9.508	0
Summer–winter and spring merger	−130.069	27.277	−4.768	0

3 | Results

3.1 | Correlation Between Serum VA and VE Concentrations and Age

The results of the normality test of the data at different age stages showed that the significance of the four age groups was <0.05 whether it is VA or VE levels, so the distribution of VA and VE levels was skewed (Tables 1 and 2). The comparison between vitamin concentrations in the four age groups showed a statistical significance of $p < 0.05$ (Tables 3 and 4). As age increased, the levels of VA and VE did not simply increase or decrease in the four age groups, and VA and VE levels show periodic changes (Figures 1 and 2).

3.2 | Correlation Between Serum VA and VE Concentrations and Seasons

In general, VA levels were compared in different seasons, and the concentration levels in other seasons had statistical significance except in summer–autumn ($p > 0.05$; Table 5), and the

TABLE 15 | Comparison of vitamin A levels between different seasons at 6< age ≤17 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Summer–autumn	−0.027	17.321	−0.002	0.999
Summer–spring	90.784	14.993	6.055	0
Summer–winter	−119.246	15.731	−7.581	0
Autumn–spring	90.757	18.715	4.849	0
Autumn–winter	−119.218	19.311	−6.174	0
Spring–winter	−28.461	17.254	−1.65	0.099

TABLE 16 | Comparison of vitamin A levels between spring–winter and summer–autumn at 6< age ≤17 years.

Rank test				Testing statistic	
Seasons	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	9827
Winter and spring merger	184	259.09	47673	Wilcoxon	34,137
Summer and autumn merger	220	155.17	34137	*Z*	−8.915
Sum	404			*p* value	0

TABLE 17 | Comparison of vitamin E levels between different seasons at 6< age ≤17 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn–winter	−37.43	19.322	−1.937	0.053
Autumn–summer	44.303	17.331	2.556	0.011
Autumn–spring	66.353	18.726	3.543	0
Winter–summer	6.873	15.74	0.437	0.662
Winter–spring	28.923	17.264	1.675	0.094
Summer–spring	22.05	15.002	1.47	0.142

TABLE 18 | Comparison of vitamin E levels combined with spring, summer, and winter at 6< age ≤17 years.

Rank test				Testing statistic	
Seasons	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	8240.5
Autumn	64	161.26	10320.5	Wilcoxon	10,320.5
Spring, summer, and winter merger	340	210.26	71489.5	*Z*	−3.08
Sum	404			*p* value	0.002

concentration levels in spring and winter were higher than those in summer and autumn (Figure 3). There was statistical significance in VE levels in different seasons ($p < 0.05$) (Table 6), and the concentration levels in spring and winter were higher than those in summer and autumn (Figure 4).

Specifically, when the age ≤1 year, the comparison of VA level between summer and autumn and between winter and spring has no statistical significance ($p > 0.05$), whereas the comparison of VA level between other seasons has statistical significance ($p < 0.05$) (Table 7). Combined with the VA concentration levels between summer and autumn and winter and spring, the comparison difference has statistical significance ($p < 0.05$) (Table 8). There was no statistically significant difference in VE level between winter and summer ($p > 0.05$), whereas there was a statistically significant difference between other seasons ($p < 0.05$) (Table 9). The VE levels from winter to summer were combined, and the difference was statistically significant ($p < 0.05$) (Table 10). When the age was 1< to ≤6 years, there was no statistically significant difference in VA level between summer–autumn and winter–spring ($p > 0.05$), whereas there was statistically significant

TABLE 19 | Comparison of vitamin A levels between different seasons at 17<age ≤59 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn–summer	76.677	59.285	1.293	0.196
Autumn–winter	−714.481	57.058	−12.522	0
Autumn–spring	935.629	53.192	17.59	0
Summer–autumn	−637.803	54.681	−11.664	0
Summer–spring	858.952	50.634	16.964	0
Winter–spring	221.148	48.008	4.606	0

TABLE 20 | Comparison of vitamin A levels combined with summer and autumn at 17< age ≤59 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Summer and autumn merger–winter	673.245	47.32	14.228	0
Summer and autumn merger–spring	894.393	42.579	21.006	0
Winter–spring	221.148	48.008	4.606	0

TABLE 21 | Comparison of vitamin E levels between different seasons at 17< age ≤59 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn–winter	−52.344	57.073	−0.917	0.359
Autumn–summer	434.308	59.3	7.324	0
Autumn–spring	775.474	53.206	14.575	0
Winter–summer	381.964	54.696	6.983	0
Winter–spring	723.13	48.021	15.059	0
Summer–spring	341.167	50.648	6.736	0

TABLE 22 | Comparison of vitamin E levels combined with autumn and winter at 17< age ≤59 years.

Seasons	Testing statistic	Standard error	Standard test statistics	*p* value
Autumn and winter merger–summer	403.918	49.179	8.213	0
Autumn and winter merger–spring	745.085	41.629	17.898	0
Summer–spring	341.167	50.648	6.736	0

difference between other seasons ($p < 0.05$) (Table 11). The VA concentration levels were combined in summer–autumn and winter–spring, and the differences were statistically significant ($p < 0.05$) (Table 12). There was no statistically significant difference in VE level between winter and spring ($p > 0.05$), whereas there was statistically significant difference between other seasons ($p < 0.05$) (Table 13). The concentration levels of VE from winter to spring were combined, and the differences were statistically significant ($p < 0.05$) (Table 14). When the age was 6< to ≤17 years, there was no statistical significance in VA level between summer and autumn and between winter and spring ($p > 0.05$), but there was statistical significance in VA level between other seasons ($p < 0.05$) (Table 15). The VA concentration levels of summer–autumn and winter–spring were combined, and the differences were statistically significant ($p < 0.05$) (Table 16). There was no significant difference in VE level between spring, summer, and winter ($p > 0.05$) (Table 17). The concentration of VE in spring, summer, and

TABLE 23 | Comparison of vitamin A levels between different genders.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	10,513,530.5
Male	3618	4715.4	17,060,301.5	Wilcoxon	17,060,301.5
Female	5864	4757.61	27,898,601.5	Z	−0.73
Sum	9482			p value	0.466

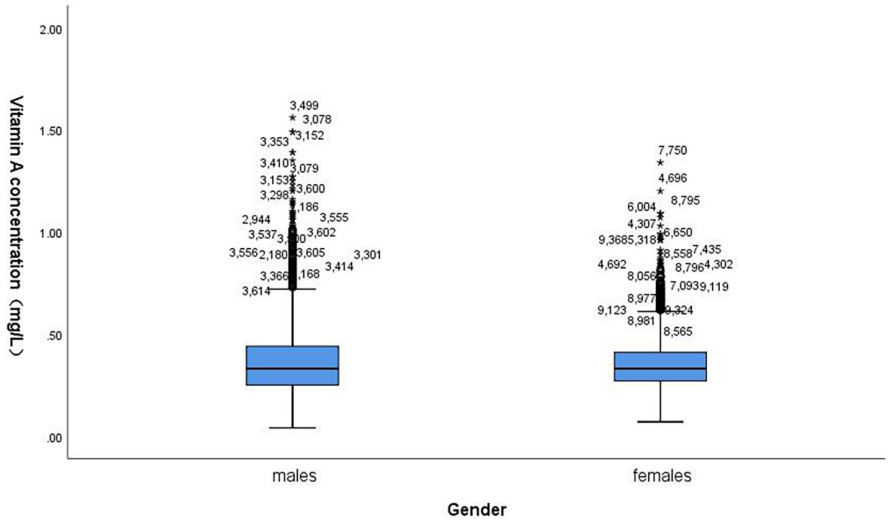

FIGURE 5 | Concentration level of vitamin E in male and female was nearly equal.

TABLE 24 | Comparison of vitamin E levels between different genders.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	9,281,477.5
Male	3619	4374.65	15,831,867.5	Wilcoxon	15,831,867.5
Female	5863	4967.94	29,127,035.5	Z	−10.253
Sum	9482			p value	0

winter was significantly higher than that in autumn ($p < 0.05$) (Table 18). When the age was 17< to ≤59 years, the VA level was not statistically significant between autumn and summer ($p > 0.05$), and the differences between other seasons were statistically significant ($p < 0.05$) (Table 19). Combined with the concentration of VA from autumn to summer, the difference was statistically significant ($p < 0.05$) (Table 20). There was no statistically significant difference in VE level between autumn and winter ($p > 0.05$), whereas there was statistically significant difference between other seasons ($p < 0.05$) (Table 21). Combined with the concentration levels of VE in autumn and winter, the difference was statistically significant ($p < 0.05$) (Table 22).

3.3 | Correlation Between Serum VA and VE Concentrations and Gender

Overall, there was no statistical significance in VA concentration levels between different genders ($p > 0.05$) (Table 23), and the levels were nearly equal (Figure 5). The VE concentration levels were statistically significant ($p < 0.05$) (Table 24) and higher in females than in males (Figure 6).

Specifically, when the age was ≤1 year, there was no statistical significance in the combined summer–autumn and winter–spring VA levels in males and females ($p > 0.05$) (Tables 25 and 26). In the combined winter–summer, spring and autumn VE levels, there was statistical significance for both male and female ($p < 0.05$) (Tables 27–29). When the age was 1< to ≤6 years, there was no statistical significance of VA concentration levels in the combined summer–autumn and winter–spring in males and females ($p > 0.05$) (Tables 30 and 31). In the combined VE concentration levels from winter to spring, there was statistical significance for both male and female ($p < 0.05$) (Table 32), whereas in summer and autumn, there was no statistical significance for both male and female ($p > 0.05$) (Tables 33 and 34). When the age was 6< to ≤17 years, in the combined summer–autumn and winter–spring VA concentration levels, the former had no statistical significance in

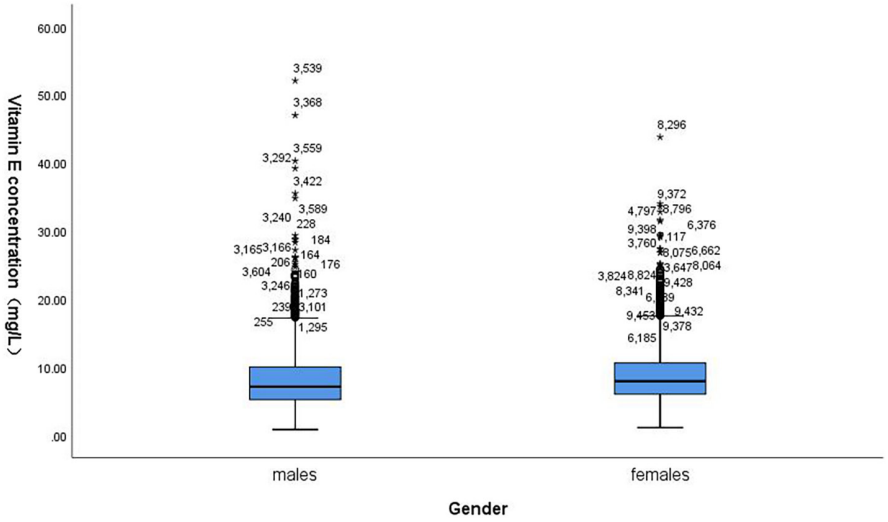

FIGURE 6 | VE concentration in female was higher than that in male.

TABLE 25 | Comparison of vitamin A levels between male and female combined with summer and autumn at age ≤1 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	221,101
Male	693	666.95	462,197	Wilcoxon	425,581
Female	639	666.01	425,581	*Z*	−0.045
Sum	1332			*p* value	0.964

TABLE 26 | Comparison of vitamin A levels between male and female combined with winter and spring at age ≤1 year.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	608,792.5
Male	1152	1104.97	1,272,920.5	Wilcoxon	1,272,920.5
Female	1057	1105.04	1,168,024.5	*Z*	−0.003
Sum	2209			*p* value	0.998

TABLE 27 | Comparison of vitamin E levels between male and female combined with winter and summer at age ≤1 year.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	361,510.5
Male	934	854.56	798,155.5	Wilcoxon	798,155.5
Female	869	952.99	828,150.5	*Z*	−4.012
Sum	1803			*p* value	0

males and females ($p > 0.05$) (Table 35), whereas the latter had it ($p < 0.05$) (Table 36). There was no statistical significance in VE concentration in male or female in the combined spring, summer, winter, and autumn ($p > 0.05$) (Tables 37 and 38). When the age was 17< to ≤59 years, there was statistical significance in VA concentration levels in both male and female ($p < 0.05$) (Tables 39–41). In the combined autumn–winter and spring VE concentration levels, there was no statistical significance in males and females ($p > 0.05$) (Tables 42 and 43), but there was statistical significance in summer ($p < 0.05$) (Table 44).

3.4 | Reference Range of VA and VE

The statistical analysis showed that VA and VE concentrations were related to age, season, and gender. Therefore, it is necessary to establish the reference interval of serum concentration of VA

TABLE 28 | Comparison of vitamin E levels between male and female in spring at age ≤1 year.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	141,631
Male	577	534.46	308,384	Wilcoxon	308,384
Female	527	572.25	301,576	*Z*	−1.967
Sum	1104			*p* value	0.049

TABLE 29 | Comparison of vitamin E levels between male and female in autumn at age ≤1 year.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	44,778
Male	335	301.67	101,058	Wilcoxon	101,058
Female	299	335.24	100,237	*Z*	−2.304
Sum	634			*p* value	0.021

TABLE 30 | Comparison of vitamin A levels between male and female combined with summer and autumn at 1< age ≤6 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	37,432
Male	301	275.36	82,883	Wilcoxon	82,883
Female	272	299.88	81,568	*Z*	−1.773
Sum	573			*p* value	0.076

TABLE 31 | Comparison of vitamin A levels between male and female combined with winter and spring at 1< age ≤6 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	63,279
Male	379	356.96	135,289	Wilcoxon	135,289
Female	339	362.34	122,832	*Z*	−0.347
Sum	718			*p* value	0.729

TABLE 32 | Comparison of vitamin E levels between male and female combined with winter and spring at 1< age ≤6 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	88,425
Male	466	423.25	197,236	Wilcoxon	197,236
Female	417	462.95	193,050	*Z*	−2.309
Sum	883			*p* value	0.021

TABLE 33 | Comparison of vitamin E levels between males and females in summer at 1< age ≤6 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	11,534
Male	176	154.03	271,10	Wilcoxon	27,110
Female	149	173.59	258,65	*Z*	−1.87
Sum	325			*p* value	0.061

TABLE 34 | Comparison of vitamin E levels between males and females in autumn at 1< age ≤6 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	6842
Male	125	117.74	14,717	Wilcoxon	14,717
Female	123	131.37	16,159	*Z*	−1.498
Sum	248			*p* value	0.134

TABLE 35 | Comparison of vitamin A levels between male and female combined with summer and autumn at 6< age ≤17 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	5687.5
Male	109	107.18	11,682.5	Wilcoxon	11,682.5
Female	111	113.76	12,627.5	*Z*	−0.768
Sum	220			*p* value	0.442

TABLE 36 | Comparison of vitamin A levels between male and female combined with winter and spring at 6< age ≤17 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	3434
Male	92	83.83	7712	Wilcoxon	7712
Female	92	101.17	9308	*Z*	−2.211
Sum	184			*p* value	0.027

TABLE 37 | Comparison of vitamin E levels between male and female combined with spring, summer, and winter at 6< age ≤17 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	14,365
Male	174	170.94	29,744	Wilcoxon	28,226
Female	166	170.04	28,226	*Z*	−0.085
Sum	340			*p* value	0.932

TABLE 38 | Comparison of vitamin E levels between males and females in autumn at 6< age ≤17 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	497
Male	27	32.41	875	Wilcoxon	875
Female	37	32.57	1205	*Z*	−0.034
Sum	64			*p* value	0.973

TABLE 39 | Comparison of vitamin A levels between males and females combined with autumn and summer at 7< age ≤59 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	67535
Male	251	1193.94	2,99,678	Wilcoxon	9,61,988
Female	1337	719.51	9,61,988	*Z*	−15.048
Sum	1588			*p* value	0

TABLE 40 | Comparison of vitamin A levels between males and females in spring at 7< age ≤59 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	57,088.5
Male	317	1138.91	3,61,034.5	Wilcoxon	7,30,468.5
Female	1160	629.71	7,30,468.5	*Z*	−18.843
Sum	1477			*p* value	0

TABLE 41 | Comparison of vitamin A levels between males and females in winter at 7< age ≤59 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	24,133.5
Male	237	796.17	1,88,692.5	Wilcoxon	3,27,943.5
Female	779	420.98	3,27,943.5	*Z*	−17.242
Sum	1016			*p* value	0

TABLE 42 | Comparison of vitamin E levels between males and females combined with autumn and winter at 17< age ≤59 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	2,29,838
Male	334	855.64	2,85,783	Wilcoxon	2,85,783
Female	1416	880.19	12,46,342	*Z*	−0.799
Sum	1750			*p* value	0.425

TABLE 43 | Comparison of vitamin E between males and females in spring at 17< age ≤59 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	1,76,661
Male	317	716.29	2,27,064	Wilcoxon	2,27,064
Female	1160	745.21	8,64,439	*Z*	−1.07
Sum	1477			*p* value	0.285

and VE according to different age stages, different seasons, and different genders. The reference interval of serum concentration of VA and VE in the population is shown in Tables 45 and 46.

3.5 | Simple Verification of Reference Range of VA and VE

The detection results of VA and VE in 80 selected cases from the four age groups were all within the reference interval of the corresponding age group. Given that the verified results were all distributed within the corresponding reference interval, the reference interval established in this study has practical significance and can be used and promoted in clinical practice.

4 | Discussion

As an essential nutrient for maintaining normal metabolism and function of the body, fat-soluble vitamins play an important role in growth, development, immune regulation, and other aspects [12–15].

Vitamin A, also known as retinol, is a fat-soluble vitamin [6]. It has extensive regulatory effects on body growth and development, cell proliferation and differentiation, and immune function [16–19]. Its deficiency can increase the incidence of recurrent respiratory tract infection (RRTIs) in children, lead to a decrease in the diversity of the human gut microbiome, indirectly increase the susceptibility to gastrointestinal infection or injury [20–23], and can also lead to salivary gland atrophy. It can cause hypofunction of salivary glands and reduce the oral defense ability against infection and the buffering ability of plaque acid [24–30]. The intake of VA only marginally above the recommended amount is associated with embryopathy, reduced bone mineral density in the neonate, and increased risk for hip fracture [31, 32]. Vitamin E is the general name of 4 tocopherols and 4 tocotrienols found in food [33–35], which is known for its antioxidant activity, is essential for growth and development, and is closely related to immune function [36–38].

TABLE 44 | Comparison of vitamin E levels between males and females in summer at 17< age ≤59 years.

Rank test				Testing statistic	
Genders	Number of cases	Rank mean	Sum of ranks	Mann–Whitney	33,478.5
Male	154	294.89	45,413.5	Wilcoxon	45,413.5
Female	700	456.67	319,671.5	*Z*	−7.369
Sum	854			*p* value	0

TABLE 45 | Reference range of vitamin A level between different ages, different seasons, and different genders.

Age group (years)	Seasons	Genders	Reference range (mg/L)
Age ≤1	Spring and winter merger	/	0.1300–0.6575
	Summer and autumn merger	/	0.1100–0.4600
1< to ≤6	Spring and winter merger	/	0.2100–0.7302
	Summer and autumn merger	/	0.1635–0.4600
6< to ≤17	Spring and winter merger	Male	0.1530–0.7937
		Female	0.1963–0.7100
	Summer and autumn merger	/	0.1800–0.5195
17< to ≤59	Summer and autumn merger	Male	0.2230–0.9750
		Female	0.1800–0.5600
	Spring	Male	0.3920–1.1020
		Female	0.2200–0.6900
	Winter	Male	0.3200–1.2015
		Female	0.2100–0.6600

TABLE 46 | Reference range of vitamin E level between different ages, different seasons, and different genders.

Age group (years)	Seasons	Genders	Reference range (mg/L)
Age ≤1	Summer and winter merger	Male	2.6000–17.0000
		Female	2.9125–17.2125
	Spring	Male	3.8000–20.6200
		Female	3.8000–20.8800
	Autumn	Male	3.5400–12.2600
		Female	3.7500–13.3000
1< to ≤6	Spring and winter merger	Male	2.7350–15.8975
		Female	2.7270–16.5275
	Summer	/	3.6000–11.0550
	Autumn	/	3.4000–9.0000
6< to ≤17	Spring, summer, and winter merger	/	2.7000–13.6950
	Autumn	/	2.9875–8.7125
17< to ≤59	Autumn and winter merger	/	3.5000–16.9295
	Spring	/	3.8950–21.6000
	Summer	Male	3.5750–12.7250
		Female	4.6000–15.3000

Relevant studies have shown that VE deficiency mainly leads to nervous system abnormalities, anemia, cardiovascular diseases, pneumonia, and asthma [39, 40]. An excessive intake of VE will resist the effect of vitamin K, resulting in bleeding, platelet generation, and aggregation and thus thrombosis [41]. To date, thresholds for vitamin deficiency in the general population have been established on the basis of different nutritional status and recommendations from WHO [8, 42]. The researchers believe that the threshold for vitamin deficiency should be specific within population subgroups and that it is not appropriate to have only one threshold for vitamin deficiency in all populations. Vitamin levels can vary depending on age, genetics, inflammation, and pregnancy [43]. Therefore, it is of great clinical significance to establish VA and VE reference intervals suitable for different populations in different regions to evaluate the vitamin levels of corresponding populations, to make reasonable treatment procedures for clinicians.

In this study, the serum concentration levels in VA and VE from infants to adults in Southern Sichuan, China, were measured and divided into four different age groups from age ≤1 year (3541 cases), 1< to ≤6 years (1456 cases), 6< to ≤17 years (404 cases), and 17< to ≤59 years (4081 cases). The results showed that the distribution of VA and VE in different age groups was skewed, and there were significant differences, indicating that VA and VE levels were correlated with age and that the VA concentration level was positively correlated with age. The concentration level of VE was negatively correlated with age. VA and VE are fat-soluble vitamins, and their content level can be determined on the basis of the geographical location of the population, sunshine conditions, and living habits. The results of this study showed that the levels of VA and VE were lower in summer and autumn than in spring and winter, which may be due to the accelerated loss of VA and VE in summer and autumn. Therefore, for these two seasons, vitamin supplementation should be appropriate. However, the VE concentration level is related to gender, and the level of men is lower than that of women.

5 | Conclusion

In summary, age, season, and gender are closely related because of VA and VE content. Therefore, it is necessary to establish a reference interval suitable for the local population in the northern and southern regions of Sichuan, to provide a scientific basis for the clinical prevention and diagnosis of VA and VE deficiency and formulate safe and reasonable treatment measures to avoid deficiency or excess.

Author Contributions

Qiang Zhong was involved in conception and design. Qiang Zhong, Guoping Huang, and Wen Zhang were involved in materials and samples collection, and data analysis and interpretation, and data collection and collation. All authors were involved in manuscript writing, approved the final manuscript and consented to publish this manuscript.

Acknowledgments

The authors have nothing to report.

Consent

Informed consent was taken from all individual participants.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.