

Six-Year Trends on Prevalence of Myopia among School Children in Liuyang, Central South China: Screening Data from 2013 to 2018

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Abstract

Background

Myopia is the most common and leading cause of eye problems affecting people of all ages and yet a preventable cause of blindness. The prevalence of myopia in China varied a lot, and related study is lacking from Central South China. So the purpose of this study was to investigate the trends in the prevalence of myopia among under-college school children from 2013 to 2018 in Liuyang district, Central South of China.

Methods

This was a population-based retrospective study. Uncorrected visual acuity test with the Standard Logarithmic Visual Acuity E chart was used to detect myopia. The yearly age-specific prevalence was calculated and compared.

Results

A total of 854907 children from Grade 1 to 12 were included. The prevalence of myopia increased from 24.3% (2013) to 35.9% (2018), with a significant trend of increase ($p < 0.001$). Persistence higher prevalence was seen in girls than boys, urban dwellers than rural dwellers, and senior high school than that of junior high or primary school children. Significant increase trends in the prevalence of severe myopia were seen across time (from year 2013 to 2018) in each age group among students from 11 to 18 years old ($p < 0.001$).

Conclusions

There was a remarkable yearly increase in myopia prevalence among school children of all ages and across all grades in China from 2013 to 2018, especially in female, urban and high school students. It is urgently needed for an effective public health intervention for the control of myopia among all Chinese school children.

Background

Myopia is the most common and leading cause of eye problems affecting people of all ages and yet a preventable cause of blindness¹. There have been tremendous increases in myopia prevalence in most parts of the world recently, especially among school children due to the increase pressure in education. This increase is however with variation in different parts of the world, East Asia and South Asia are among the most hit^{1,2}. About 80% of the global myopia prevalence among children 0–19 years can be found in Asia with 35% of the cases in Southeast Asia³. There exists a varying prevalence among Asian countries. The lowest prevalence was reported in Laos and Nepal, with the myopia prevalence of 0.8% among 6 to 11 years old children⁴ and 1.2% among 5 to 15 years old children⁵ respectively. Contrarily, high prevalence was reported from China², with the highest prevalence of 86.5% reported among adolescents of 13–17 years old from Yangxi, rural southern China⁶.

However, the prevalence of myopia in China varied a lot, from 2.5% among 3 to 6 years old children in Guangzhou⁷ to 86.5% in Yangxi⁶. Furthermore, dramatic increases were reported in individual studies among children across China during the past several years, with 14.9% among 5 to 15-year-olds in Beijing^{8,9} and 82.7% among 13 to 18-year-olds adolescents in Anyang¹⁰. Although the variation of myopia prevalence observed among different studies could be contributed to the heterogeneity of age, population, area and different criteria or test for myopia. Time trend is another potential contributor for the variation in myopia prevalence¹¹.

As large variation in myopia prevalence was seen among different regions in China², and related study is lacking from Central South China, it is worthwhile to investigate the myopia prevalence in this region. In this current study, we analyzed the longitudinal screening data obtained from primary to senior high school students in Liuyang district, Hunan province, China from 2013 to 2018, to determine the time series trends in myopia prevalence among school children, and to explore the potential impact factors along the education continuum to provide additional evidence from Central South China for early management, prevention and control of myopia in China.

Materials And Methods

Study population

A retrospective study was conducted from 2013 to 2018 in Liuyang, which is a county-level city located in the Northeast of Hunan province, China. Liuyang has jurisdiction of 4 city districts and 33 towns, including 1.49 million populations. Liuyang was ranked as 19th among the 'top 100 national strong counties' in China in 2016¹². From 2006, Liuyang Health Bureau has appointed the Liuyang Center for Disease Control and Prevention (CDC) to conduct annual physical examination among school students from grades 1 to 12. As a health policy aimed to improve the health of school children, the annual physical examination is requested to cover all the students in primary, junior and senior high schools under Liuyang jurisdiction. As part of the yearly compulsory routine physical examination that all students are requested to undergo, the eye examination records of all students were routinely collected each year by Jili hospital (also named as Liuyang Eye Hospital) in Liuyang City. The facility mandated for the physical examination for the students in the district. In this study, data related to the eye examination from 2013 to 2018 were retrieved for analysis.

All students' records were electronically registered by file numbers, name, gender, age, ethnicity, school, and address. All the data were submitted to the Liuyang CDC, the institution that was in charge of the physical examination. The Liuyang CDC is the custodian of the database and data retrieval was conducted with de-identification of all students in May 2019, with the official permission from the Liuyang CDC.

Ethical approval

Since this study was based on routing data with all the subjects being de-identified. Ethical approval was waved from the Xiangya School of public health, Central South University and Liuyang CDC. The study followed the guidelines of the Declaration of Helsinki¹³.

Data collection

The following data were retrieved from the dataset: age, sex, residency type (rural/urban), education level, height, weight and results of eye examination. Details about height and weight measurements were presented in Appendix 1. Any record with missing data regarding the above listed variables, logic errors or had an age lower than 6 or larger than 18 was excluded from the final data analysis.

Eye examination

The Eye examination was conducted by an experienced group of ophthalmologists optometrists and nurses according to the Chinese National standard for students' physical examination (GB11533). All were staffs from the department of ophthalmology in Jili Hospital. Each student's uncorrected visual acuity (UCVA) was measured monocularly at a distance of 5 meters using the Standard Logarithmic Visual Acuity (LogMAR) Chart with tumbling-E optotypes under room lighting. The acuity of the LogMAR Chart that can be measured ranges from 4.0 to 5.3. If a score lower than 5.0 UCVA and a slit lamp examination as well excluded opacity of optical media and other eye conditions, then it was recorded abnormal vision (myopia). Students who have UCVA lower than 5.0, were further classified as slight myopia ($4.9 \leq UCVA < 5.0$), moderate myopia ($4.6 \leq UCVA \leq 4.8$) and sever myopia ($UCVA \leq 4.5$).

Body mass index (BMI) calculation

BMI for age was calculated by the formula weight (kg)/height (m^2). BMI was further categorized using the World Health Organization's Z score chart for children 5-19 years (appendix 2)¹⁴.

Statistical analysis

Mean with standard deviations (SD), and frequencies with percentages were reported in the descriptive statistical analyses for the continuous variables and the categorical variables, respectively. The data from the left eye was used for the estimation of myopia prevalence as a highly significant correlation of 0.89 ($p > 0.001$) (Appendix 3) between the left and right eyes was reported¹⁵.

Myopia prevalence was calculated one-time for each year and by age group from 2013 to 2018. Line graphs were used to describe and compare the annual prevalence trends of myopia, within each sex and one-year time age interval (6 to 18years) from 2013 to 2018. Linear-by-Linear association test was used to test the trends of myopia prevalence at each age group with the increase of age (from 6 years to 18 years) and year going on (from 2013 to 2018). Differences in myopia prevalence among different groups were compared with Chi-squared test. Multivariable logistic regression analysis was used to identify the association between demographic variables and myopia within each year from 2013 to 2018, and the total population. Among each logistic regression analysis, myopia status was used as the dependent variables; sex, BMI status, grade and residency type were used as the independent variables. Sensitivity analyses on multivariable logistic regressions were conducted by excluding students from senior high schools as enrollment rates decreased from over 99% in primary school and junior high school to around 88% in senior high school¹⁶, and a relative low eye examination rate was observed in senior high school. Age was not included in the logistic regression for its collinearity with grade group. The model fitting was checked with Hosmer and Lemeshow (HL) test, with P -value greater than 0.1 being considered as adequate model fit. Adjusted Odds Ratio (OR) with 95% confidence interval (CI) was calculated. A P -value of less than 0.05 (two-tailed) was accepted as statistically significant (except for HL test). All data analyses were performed using SPSS statistics version 21.0 (SPSS Inc., Chicago, Illinois, USA) and Graphical presentations by Microsoft Excel version 10.

Result

Characteristics of the study population

A total of 887,496 records from the dataset, after excluded records based on the inclusion criteria, 854,907 records were included in the final data analysis (Figure 1). By comparing with the statistical data from the Liuyang Education Bureau, from 2015 to 2018, 95.00%-96.71% students from primary school, 92.81%-94.22% students from junior high school, and 45.98%-98.22% students from junior high school received the physical examination and were included in this study (Appendix 4). Statistical data on total number of students in 2013 and 2014 is not available.

There were more students from primary school (ranged from 59.6% in 2018 to 69.3% in 2014) than that of junior high school (ranged from 22.2% in 2013 to 27.1% in 2015) and senior high school (ranged from 5.2% in 2014 to 14.2% in 2018). There were more students from rural area each year, with the proportion ranged from 71.9% (year 2013) to 74.9% (year 2014). Male students account for an average of around 50.9%, with a stable gender proportion across the

studied six years (Table 1, Appendix 5). There is a slight trend in the prevalence of overweight and obesity from 2013 (11.9%) to 2018 (14.1%). Details regarding the demographic characteristics and distribution on schools of the students for each year from 2013 to 2018 are presented in Table 1.

Results of UCVA score from 2013 to 2018

Generally slight trends of decreasing in mean UCVA score were observed from 2013 to 2018 among each age group. Furthermore, the decreasing trends in mean UCVA score were more obvious as the students grow, with the mean UCVA score of 4.99 (SD=0.12) on 6 years old and of 4.53 (SD=0.41) on 18 years old (Appendix 6).

Prevalence of myopia from 2013 to 2018

The total prevalence of myopia increased each year from 2013 (24.3%) to 2018 (35.9%), with a significant trend of increase in myopia prevalence across the past six years ($p < 0.001$). Similar trends were observed in each educational level subgroup from primary school to senior high school ($p < 0.001$). Within each educational level subgroup, girls showed statistically significant higher prevalence of myopia each year ($p < 0.001$). Details are reported in Table 2.

From year 2013 to 2018, lowest prevalence of myopia was observed in age eight (ranging from 10.5% in 2015 to 16.6% in 2018) and highest prevalence in age 18 (ranging from 69.8% in 2013 to 79.9% in 2018) (Appendix 7A). By using age eight as the cutoff point, a negative association between age and prevalence of myopia was observed for students from six years old to eight years old each year, while a positive association between age and prevalence of myopia was observed for students from eight years old to 18 years old each year (Figure 2A). That positive association also applied to different subgroups including male or female students (Figure 2B, Appendix 7A), and students from rural area or urban area (Figure 2C, Appendix 7B), although respectively female students, students from urban area showed higher prevalence of myopia than that of male students (Figure 2B, Appendix 7A), students from rural area (Figure 2C, Appendix 7B) at each age group within each year.

Figure 2 Myopia Prevalence from 2013 -2018

A: prevalence of myopia within each age group for each year from 2013 to 2018. B: prevalence of myopia for male or female students within each age group for each year from 2013 to 2018. C: prevalence of myopia for students from rural area or urban area within each age group for each year from 2013 to 2018.

Changes in severity of myopia from 2013 to 2018

There are different trends in severity of myopia across age groups from 2013 to 2018. A decrease in prevalence of mild myopia across age groups (from six years to 18 years) was observed each year, with the highest prevalence of mild myopia being observed in age six (ranging from 10.9% in 2014 to 19.7% in 2018) and lowest prevalence of mild myopia in age 18 (ranging from 5.3% in 2016 to 7.0% in 2013) (Figure 3A, Appendix 8). On the other hand, increase trends in prevalence across age groups were (from six years to 18 years) observed for both moderate (Figure 3B, Appendix 8) and severe myopia (Figure 3C, Appendix 8), especially for severe myopia. The prevalence of severe myopia ranged from 0.6% (year 2013) to 1.3% (year 2018) in six years old students, while increased to 46.4% (year 2013) to 60.8% (year 2018) in 18 years old students (Figure 3C, Appendix 8). Similar trends were observed in both male and female students. Trends of increase in prevalence of severe myopia from year 2013 to 2018 were observed in each age group among students aged from 11 to 18 years old (Figure 3C, Appendix 8).

A: prevalence of mild myopia for all the students, female students or male students within each age group for each year from 2013 to 2018. B: prevalence of moderate myopia for all the students, female students or male students within each age group for each year from 2013 to 2018. C: prevalence of severe myopia for all the students, female students or male students within each age group for each year from 2013 to 2018.

Possible factors associated with myopia

Female students (OR: 1.52, 95%CI: 1.51-1.54), students from urban area (OR: 1.82, 95%CI: 1.80-1.84) were more likely to be myopic than male students and those who are from rural area, respectively. Compared to students from primary schools, those from Junior high school (OR: 3.43, 95%CI: 3.39-3.47), senior high school (OR: 10.02, 95%CI: 9.86-10.18) were more likely to be myopic. BMI status was associated with myopia, with overweight (OR: 1.12, 95%CI: 1.10-1.15) and obese students (OR: 1.15, 95%CI: 1.13-1.18) more likely to be myopic than students with normal weight. Results from logistic regression analyses conducted independently based on each year showed consistent associations (Table 3). *P* values of all HL test were > 0.1 , indicating good model fit for all the multivariate logistic regression analyses. No substantial changes on risk factors were observed in the sensitivity analysis (Appendix 9).

Table 1
Demographic characteristics and distribution on schools of the students for each year from 2013 to 2018

	2013	2014	2015	2016	2017	2018	Total	p-value
No. of Students								
Residency Type (%)								
Rural	66782 (71.9)	96683 (74.9)	105125 (73.1)	112477 (73.1)	122599 (74.1)	122688 (72.3)	626354 (73.3)	0.001
Urban	26163 (28.1)	32425 (25.1)	38614 (26.9)	41437 (26.9)	42960 (25.9)	46954 (27.7)	228553 (26.7)	
Gender (%)								
Female	44989 (48.4)	63209 (49.0)	70437 (49.0)	75761 (49.2)	81660 (49.3)	83526 (49.2)	419582 (49.1)	0.001
Male	47956 (51.6)	65899 (51.0)	73302 (51.0)	78153 (50.8)	83899 (50.7)	86116 (50.8)	435325 (50.9)	
BMI Status (%)								
Underweight	9385 (10.1)	13340 (10.3)	14642 (10.2)	16240 (10.6)	15420 (9.3)	14103 (8.3)	83130 (9.7)	
Normal	72499 (78.0)	100860 (78.1)	112441 (78.2)	119949 (77.9)	128854 (77.8)	131604 (77.6)	666207 (77.9)	0.001
Overweight	5659 (6.1)	7484 (5.8)	8500 (5.9)	9174 (6.0)	10749 (6.5)	11842 (7.0)	53408 (6.2)	
Obesity	5402 (5.8)	7424 (5.8)	8156 (5.7)	8551 (5.6)	10536 (6.4)	12093 (7.1)	52162 (6.1)	
Grade Group								
Primary School	64179 (69.1)	89452 (69.3)	94581 (65.8)	96726 (62.8)	99780 (60.3)	101148 (59.6)	545866 (63.9)	
Junior High School	20624 (22.2)	32902 (25.5)	38890 (27.1)	40530 (26.3)	42755 (25.8)	44371 (26.2)	220072 (25.7)	0.001
Senior High School	8142(8.8)	6754(5.2)	10268(7.1)	16658 (10.8)	23024 (13.9)	24123 (14.2)	88969 (10.4)	
Age Group (%)								
6–12 years	68648(73.9)	95046 (73.6)	100150 (69.7)	102700 (66.7)	105536 (63.7)	107083 (63.1)	579163 (67.7)	
13–15 years	17104 (18.4)	27720 (21.5)	35078 (24.4)	38583 (25.1)	41512 (25.1)	42617 (25.1)	202614 (23.7)	0.001
16–18 years	7193 (7.7)	6342 (4.9)	8511 (5.9)	12631 (8.2)	18511 (11.2)	19942 (11.8)	73130 (8.6)	
Total No. of students	92945 (100.0)	129108 (100.0)	143739 (100.0)	153914 (100.0)	165559 (100.0)	169642 (100.0)	854907 (100.0)	
No. Of Schools								
Primary School	185	187	193	200	205	210	1180	
Junior High School	51	51	52	52	51	52	309	n.a
Senior High School	11	11	12	12	13	15	74	
Total No. of Schools	247	249	257	264	269	277	1563	

Table 2
Prevalence rate of myopia among school children in each educational level for boys and girls from 20113 – 2018

Year	Primary School			Junior High School			Senior High School			Girls (%)
	Girls (%)	Boys(%)	Total	Girls (%)	Boys(%)	Total	Girls (%)	Boys(%)	Total	
2013	5542(17.8)	5047(15.3)	10589(16.5)	3904(40.4)	3094(28.3)	6998(33.9)	2842(68.4)	2118(53.2)	4960(60.9)	12288(2
2014	8229(18.8)	7077(15.5)	15306(17.1)	6853(43.6)	5381(31.3)	12234(37.2)	2835(74.8)	1990(67.1)	4825(71.4)	17917(2
2015	7969(17.4)	6889(14.1)	14858(15.7)	9357(49.2)	7080(35.6)	16437(42.3)	4027(72.8)	2805(59.2)	6832(66.5)	21353(3
2016	10459(22.4)	9090(18.2)	19549(20.2)	10510(53.2)	7931(38.2)	18441(45.5)	6923(74.8)	4621(62.5)	11544(69.3)	27892(3
2017	10603(22.0)	9245(17.9)	19848(19.9)	11522(54.9)	8476(38.9)	19998(46.8)	9675(77.0)	6573(62.9)	16248(70.6)	31800(3
2018	11876(24.6)	10182(19.3)	22058(21.8)	12227(56.0)	9035(40.1)	21262(47.9)	10375(78.0)	7209(66.6)	17584(72.9)	34478(4

*Linear-by-Linear association test for the prevalence of myopia from 2013 to 2018 among students in primary schools, in junior high schools, senior high schools: all the p-values are less than 0.001.

Table 3
Association between demographic characteristics and myopia from 2013 to 2018: logistic regression analyses

Factors	2013			2014			2015			2016			2017			2018
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	P	OR
Sex*																
Female	1.42	1.39–1.47	< .001	1.42	1.38–1.46	< .001	1.49	1.46–1.53	< .001	1.53	1.49–1.56	< .001	1.59	1.56–1.63	< .001	1.60
Residency[†]																
Urban	1.88	1.82–1.95	< .001	1.84	1.79–1.89	< .001	1.88	1.83–1.95	< .001	1.66	1.62–1.75	< .001	1.99	1.94–2.04	< .001	1.76
Education level[‡]																
Junior high	2.76	2.66–2.86	< .001	2.26	2.12–2.39	< .001	2.83	2.67–3.00	< .001	5.02	4.59–5.47	< .001	3.66	3.57–3.75	< .001	3.38
Senior high	8.39	7.98–9.83	< .001	4.98	4.04–6.16	< .001	4.39	3.92–4.93	< .001	1.39	1.32–1.48	< .001	9.95	9.63–10.29	< .001	9.55
BMI status[§]																
Overweight	1.16	1.08–1.24	< .001	1.16	1.10–1.23	< .001	1.13	1.07–1.20	< .001	1.11	1.06–1.67	< .001	1.10	1.05–1.15	< .001	1.07
Obesity	1.18	1.10–1.26	< .001	1.16	1.10–1.23	< .001	1.20	1.13–1.27	< .001	1.15	1.09–1.21	< .001	1.12	1.06–1.16	< .001	1.10
Underweight	0.94	0.89–0.99	< .018	0.91	0.87–0.95	< .001	0.96	0.92–1.00	< .001	0.91	0.88–0.95	< .001	0.93	0.89–0.96	< .001	1.02

Keys: CI = confidence interval; OR = odds ratio; * male was used as references; [†]Rural was used as references; [‡]Primary school was used as references; [§]norm

Discussion

The prevalence of myopia among a large-scale population-based school child ($n=854,907$) across the whole under-college education course were summarized and compared for the past six years from 2013 to 2018 in Central South China. A remarkable continuously increasing trend of myopia was seen among Chinese school children, from 24.3% in 2013 to 35.9% in 2018. A similar increases trend was also observed across age groups (from age 6 to 18) within each of the six years. This study showed remarkable increasing trends in prevalence of severe myopia from 2013 to 2018 across age 6 to 18. The same increasing trends were reported from other studies as well ^{17 18}.

It is noted that a more remarkable increase in trends were observed among female students, both in terms of longitudinal years and ages. Results from logistic regression analysis showed that being female is positively associate with being myopic (OR=1.52). That is consistent with several previous studies^{11, 13, 19-22}. It should be noted that the differences among male and female students were not found in young age (6-8 years old). Another study from Shanghai covered preschool children also did not find any significant difference between boys and girls²³. Evidence has demonstrated that near work is a risk factor for myopia ²⁴. Girls read a lot more hours than their counterparts boys ²⁵. That also helps to explain that higher education level is positively associated with myopia prevalence observed in this study.

This current study showed a significant association between myopia and BMI status. Possible explanation was that overweight and obesity are the results of physical inactivity, while outdoor activities have been reported to be a protecting factor for myopia ^{9, 26}. However, data on children's outdoor activity have not been collected in this study, future studies are needed to confirm whether BMI is an independent risk factor for myopia.

There are several strengths of this study. Firstly, this study was a very large-scale population-based study including 854,907 participants, covered all the under-college school children aged 6-18, provided clear information on myopia prevalence in each age and grade group. Secondly, the study was a long time series prevalence survey, which described periodic changes with time trends of myopia prevalence for the past six years. Lastly, the detailed description on the age-specific myopia prevalence provides evidence for the targeted population regarding myopia prevention strategies.

Some limitations of the study should be acknowledged as well. First and foremost, we used the LogMAR Chart to measure students' UCVA. Both cyclogenic and non-cyclogenic refractions were not utilized in this survey due to screening nature of the study with a large-scale population included ¹¹. However, evidence has suggested UCVA showed a reasonable accurate estimate of myopia prevalence and could be used for population screening purpose ²⁷⁻²⁹. A diagnostic test on LogMAR chart showed a sensitivity of 83.6% and specificity of 98.2% in detecting myopia among Chinese under-college students³⁰. Potential false positive of the LogMAR chart might lead to overestimation of the myopia prevalence in this study. However, as a uniform standard was used throughout the study years, we believed that the information on the trends of myopia and difference severity of myopia prevalence in terms of time and age progress is less likely to be influenced.

Secondly, this study focused on school children, although the Nine-year compulsory education policy is well implemented in China, and the national enrollment rate was over 99% in primary school and junior high school, and was over 88% in senior high school since 2012¹⁶. The results should not be extrapolated to the children with the same age while are not enrolled in the under-college education. Furthermore, the increase prevalence in senior high school could also be contributed by selection bias due to the decreased low enrollment rate. Further cohort studies are needed to confirm the net risk of increase education level to the development of myopia. However, as the prevalence was calculated based on majority of the high school students, especially after 2017, the results indicated immediate public health strategies are needed to improve the eye health among school students.

Lastly, the coverage of the examination among the targeted children might impact the validity of the results. Based on the official statistical data from 2015 to 2018, we believe that we sampled the majority of the students in primary and junior high school. Selection bias might be existed among students in senior high school before 2016. Based on the data from 2017 and 2018 that had optimal coverage for senior high school students, similar trend of increase in myopia prevalence was observed with the results before 2016.

Conclusions

There was a marked yearly increase of myopia prevalence among school children of all ages and across all grades in China from 2013 to 2018, prominent especially in high school. Similar increase trends were seen in severe myopia within the past six years. Girls showed higher prevalence of myopia than that of boys with each age group, especially in high school. It is urgently needed of evidence-proved effective strategies/ public health intervention programs for the control of myopia and myopia correction among all Chinese school children.

Abbreviations

Body mass index (BMI); Center for Disease Control and Prevention (CDC); Confidence interval (CI); Hosmer and Lemeshow (HL); Odds Ratio (OR); Standard deviations (SD); Uncorrected visual acuity (UCVA).

Declarations

Ethics approval and consent to participate:

Since this study was based on routing data with all the subjects being de-identified. Ethical approval was waved from the Xiangya School of public health, Central South University and Liuyang CDC. The study followed the guidelines of the Declaration of Helsinki¹³.

Consent for publication:

Not applicable.

Availability of data and materials:

Relevant supporting data aside those found in the main text will be available from the corresponding author on request.

Competing interests:

None of the following authors have any proprietary interests or conflicts of interest related to this submission.

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Authors' contributions:

MSC, XX, XYW conceived the idea for and designed this study; HJL, YTX, TL and HZ conducted the study; JA, HW and YCC performed the statistical analysis; JA wrote the first draft of the manuscript and XYW revised the article. All authors critically revised and approved the final manuscript.

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Figures

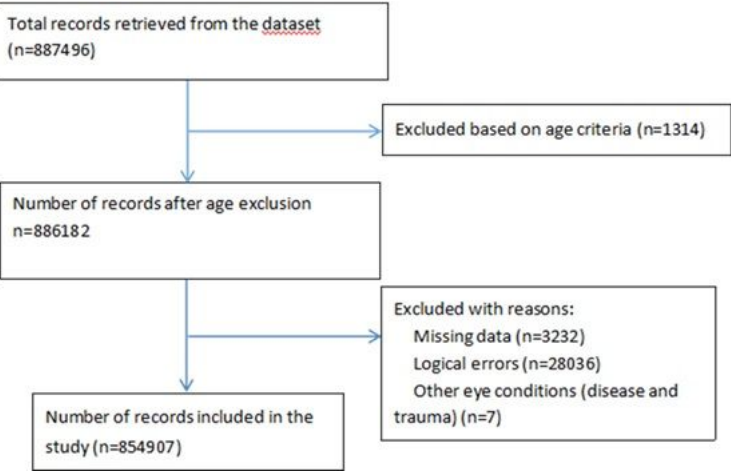


Figure 1
Flow chart of participant’s inclusion and exclusion

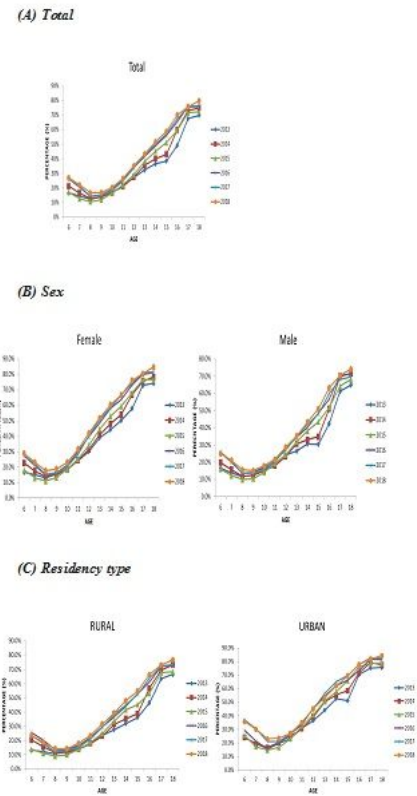


Figure 2
Myopia Prevalence from 2013 -2018

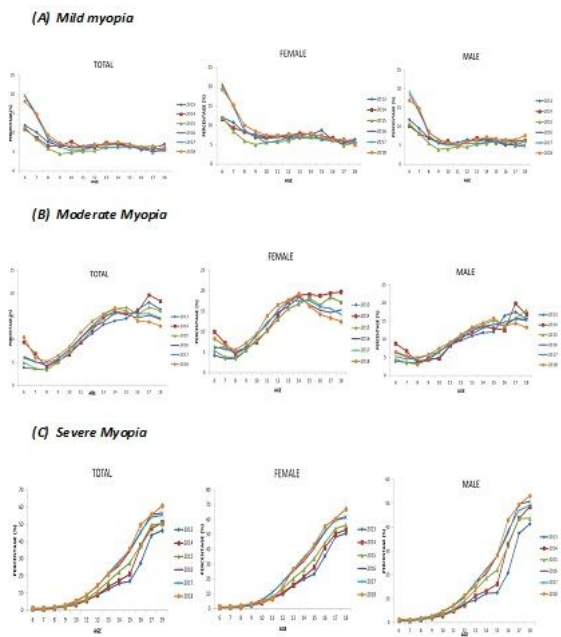


Figure 3

Levels of Myopia Prevalence among Students

Supplementary Files

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