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ORIGINAL RESEARCH

Respiratory Muscle Strength in Healthy Indian Children of Age 7–17 Years: A Cross-Sectional Study

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Purpose: As the values of respiratory muscle strength vary according to race, ethnicity, and geographical area, there is a wide-ranging difference among different populations. Thus, the available reference values may not have an application for use in the Indian paediatric population, creating a need for generating values which will be appropriate for the Indian paediatric context.

Materials and Methods: Assessment of respiratory muscle strength was carried out by assessing maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) and synthesising predictive formulas using anthropometric variables like height, gender and age, which will be suitable for Indian children.

Results: We calculated MIP and MEP of 320 (boys=160 and girls= 160) children in the age range of 7 years to 17 years of Mangaluru city, India. Results stated that mean MIP and MEP for boys were 72.5±32.8 cm H_2O and 73±33.2 cm H_2O, while for the girls it was 67±30.2 cm H_2O and 68±30.1 cm H_2O, respectively.

Conclusion: This study concluded that there is a difference in respiratory pressure values of Indian children with respect to those of other countries. Age, gender, height and BMI have a significant role in determining respiratory muscle strength. Boys demonstrated higher MIP and MEP. As age, height, weight and BMI increases, so does MIP and MEP.

Keywords: maximal expiratory pressure, maximal inspiratory pressure, respiratory muscle pressure, respiratory muscle strength

Introduction

The origin and insertions of respiratory muscles being multifarious, it is challenging to study their functions. Pressure generated within the thoracic cavity depends on the coordinated action of many muscles of the thoracic wall.[1] The diaphragm is the primary muscle for inspiration along with the external intercostal and the scalene muscles, whereas the abdominals and internal intercostal are the major muscles for expiration.[2]

Respiratory muscle strength is reflected by maximal inspiratory pressure (MIP) and maximum expiratory pressure (MEP), produced at the mouth during a static maximal inspiratory and a maximal expiratory effort.[3] The clinical measurements of these variables are quick, simple and non-invasive procedures, used to determine the index of respiratory muscle strength and capacity to perform daily activities.[4–6]

In children, the assessment of respiratory muscles are used as a diagnostic and a prognostic tool.[7,8] It can be used to quantify the severity and for follow-up of

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various neuromuscular and respiratory conditions.[4,9,10] Association between respiratory muscle weaknesses is observed with recurrent respiratory complications like infections and failure. This further compromises the ventilatory capacity, thus leading to the onset of more severe morbidities. Therefore, to evaluate different clinical conditions, measurement of respiratory muscle strength can be a helpful tool.[11]

There is a difference in the measurements of respiratory functions in different ethnic groups due to differences in the lung recoil, chest wall, respiratory muscle strength, compliance and dimensions.[12] Moreover, respiratory muscle strength varies significantly with anthropomorphic and other factors, ie gender, age, nationality.[13] However, previous studies show a significant difference between ethnic groups in various anthropomorphic factors like sitting and standing height and weight. Thus, the reference values should ideally be derived from a geographically related and specific population to improve both predictive abilities and the accuracy of the generated reference values.[14]

Other factors that could add on to the variation seen in the available reference values can be technical factors like the type of mouthpiece used, presence of intentional leaks in the mouthpiece, no of trials taken into consideration before concluding the final value and motivation of the subject to carry out the procedure and leak in the air from the nose and mouth.[1]

A systematic review on respiratory muscle strength among children of Brazil, Canada, Australia, Spain, Germany, Mexico United Kingdom, U.S.A, Poland and Switzerland shows significant individual variability in the values among subjects within the same group and marked differences in normative data generated among different groups of the population.[15]

Because of these variations in the reference values, the generated values of respiratory muscle strength of children from these populations may not be suitable for Indian children. To the best of our knowledge, no retrievable data is available for use in clinical practices on Indian children. Therefore, this study aims to synthesise normative values of respiratory muscle strength, which are specific for use in Indian paediatric population based on age, gender, height, weight and BMI; and generate a predictive equation for MIP and MEP for its application in routine clinical practices.

Materials and Methods

This cross-sectional study recruited healthy Indian children aged 7–17 years from primary and secondary schools in and around Mangalore. The study was conducted in accordance with the Declaration of Helsinki, following approval by the Institutional Ethical Committee, Kasturba Medical College, Mangalore, Karnataka INDIA (IEC/KMC/MLR/11-18/416) and block education officer, Mangalore, Karnataka INDIA. The consent (used only for children of 12 years or above age groups) and assent forms printed in English, Kannada and Malayalam languages were sent to parents. After obtaining parental consent, the primary investigator recruited healthy children of either gender, aged 7–17 years with a BMI of 18–29.5 kg/m^2 for the study. Children with any history of cardiovascular, neuromuscular, haematological or musculoskeletal condition, recent hospital admission and high BMI were excluded from the study. Eligibility details were obtained from the respective parents and verified by the primary investigator prior to the procedure.

Children recruited for the study were, grouped into 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16–17 years. A quiet room of the school was selected, and children of a specific age were assessed during each visit. Height, weight and BMI assessed before the measurement of MIP and MEP. Height was measured using a vertical stadiometer mounted on the wall, and weight was measured using a standard calibrated weighing scale. A hand-held respiratory pressure meter (MicroRPM CareFusion 234 GmbH Germany: 2018-04-06) was used by an experienced primary investigator to measure MIP and MEP. In children who are ten years and below, the procedure was demonstrated along with verbal instruction to minimise procedural error. Children were instructed in their vernacular language and allowed to practice the procedure for ten minutes before the actual measurement.

Children were made to sit upright on a chair with hip and knee at 90° of flexion with back support. Child was instructed not lean forward or laterally during procedure. Undue movement were controlled by the child itself. A nose clip was worn and a rigid type of mouthpiece was used. According to ATS/ERS statements,[22,23] to measure MIP the children were asked to exhale until RV then perform a "Mueller" manoeuvre, a forced inhalation against the MicroRPM with as much effort as possible for as long as possible while maintaining pressure up to 1.5 seconds. Similarly, to measure MEP, the children were asked to inhale up to TLC, then perform a "Valsalva" manoeuvre, a forced exhalation against the MicroRPM with as much effort as possible for as long as possible while maintaining pressure for no more than 1.5 seconds.

After every manoeuvre the respiratory pressure meter displayed maximum average inspiratory/expiratory pressure sustained over a 1 second period of the test, in cmH_2O. Measurements were taken three times with one minute of rest between the efforts, and the maximum values that varied by less than 10–20% is considered.

Results

The study included 320 children (B-160; G-160) having ages ranging between 7 and 17 years. The anthropometric data of the subjects, the MIP and MEP values expressed as mean and standard deviations are in Table 1. The values of MIP and MEP shown to be progressively increasing as age increases in both genders (Figure 1).

Karl Pearson's correlation coefficient was used to study the correlation between age, height, weight, BMI with MIP and MEP. In both boys and girls, the average MIP and MEP, correlated positively with age, height, weight and BMI (Figures 2–5). A strong positive correlation of respiratory pressure was found with age followed by height and weight, whereas a moderate positive correlation was seen with BMI (Table 2).

Multiple regression analysis with paired *t*-test was used to form a predictive equation for MIP and MEP in boys and girls. For MIP, regression analysis showed significance for height, gender and age; whereas for MEP, age, gender, height and BMI showed statistical significance ($p<0.05$). Weight was not significant for both MIP and MEP. A predictive equation was synthesised based on these analyses, for boys and girls, respectively (Tables 3–6).

Based on a linear regression model, variables height, gender, age, and BMI considered in the synthesis of predictive equations for MIP and MEP as variable weight did not have any notable significance.

a. The predictive equation for MIP using height, gender and age:

-3.646 −0.380*Height + 4.672*Gender + 10.653 * Age

(Gender: if boys substitute 1 if girls substitute 0)

b. The predictive equation for MIP using BMI, gender and age:

Table 1 Anthropometric Data, MIP and MEP Values for Boys and Girls

Age	Gender	Height (Mean ± SD)	Weight (Mean ± SD)	BMI (Mean ± SD)	MIP (Mean ± SD)	MEP (Mean ± SD)
7	B	125.5± 5.7	24.6 ± 5.8	17. ± 1.0	−32.85±4.67	32.38±4.72
	G	125.6 ± 5.1	21.4 ± 4.0	15± 3.9	−31.69±8.33	30.13±9.67
8	B	127.3 ± 4.0	26.2 ± 3.5	15 ± 4.3	−40.00±6.52	39.24±9.10
	G	126.6 ± 3.0	24.8 ± 2.8	15.6 ± 1.31	−47.92±5.73	49.92±6.73
9	B	133.8 ± 6.0	30.1 ± 9.6	17.6± 4.1	−52.57±6.68	54.50±7.67
	G	130.3 ± 3.0	29.1 ± 6.5	16.6 ± 2.13	−52.57±11.13	53.81±11.73
10	B	135.7 ± 5.4	35.1 ± 10.7	20.6 ± 8.6	−48.84±4.36	49.47±5.35
	G	132.2 ± 6.4	35.8 ± 7.4	20.5 ± 0.9	−45.09±6.42	46.09±6.74
11	B	137.8 ± 2.4	38.3 ± 9.2	20.8 ± 7.5	−50.76±6.50	51.71±7.82
	G	139.8 ± 9.5	38.6 ± 4.5	19.5 ± 1.1	−47.69±10.02	48.15±9.78
12	B	147.3 ± 7.4	43.7 ± 4.6	19.5± 1.13	−54.06±8.82	54.88±9.30
	G	143.3 ± 6.5	43.1 ± 6.7	19.3 ± 1.0	−54.14±4.02	54.87±4.83
13	B	154.7 ± 9.7	48.2 ± 4.2	19.8± 1.9	−76.14±7.60	74.57±9.57
	G	154.4 ± 3.5	46.6± 3.2	20.2 ± 0.3	−57.31±5.05	57.63±5.71
14	B	157.7 ± 6.7	46.7 ± 3.5	20.1 ± 3.7	−91.63±8.38	92.13±8.63
	G	157.3 ± 7.5	49.9 ± 4.2	20.8 ± 0.8	−77.21±11.64	77.29±10.34
15	B	160.9 ± 2.8	53.3 ± 3.5	20.1 ± 2.4	−110.36±11.35	110.57±10.70
	G	159.1 ± 5.6	53.6 ± 2.1	20.8 ± 1.1	−97.88±7.35	98.63±7.56
16–17	B	168.2 ± 7.0	60.4 ± 7.9	21.3± 5.3	−111.47±19.24	114.87±18.16
	G	167.4 ± 2.1	61.3 ± 7.6	21.5± 2.8	−118.8±13.07	118.13±13.89

Abbreviations: BMI, body mass index; MIP, maximal inspiratory pressure; MEP, maximal expiratory; B, boys; G, girls.

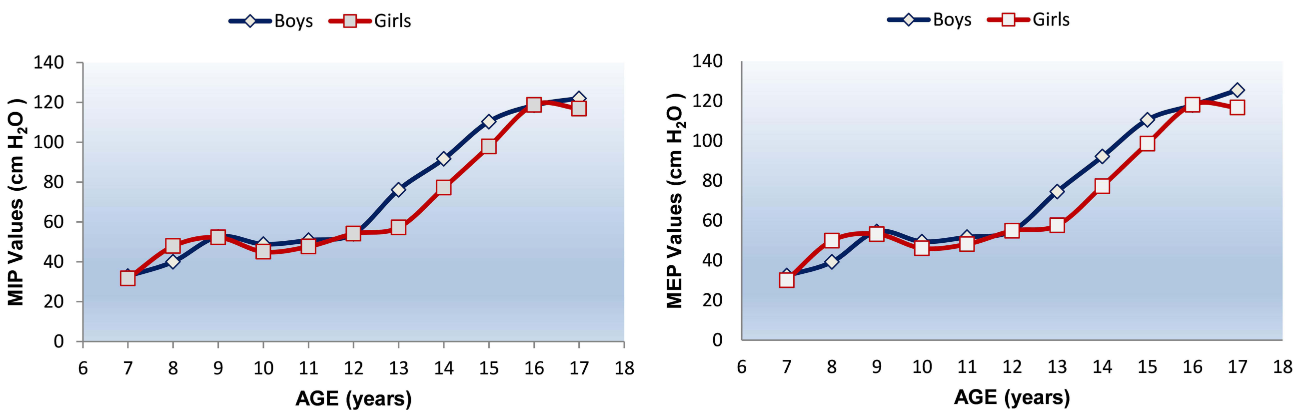

Figure 1 Average MIP & MEP for boys and girls.

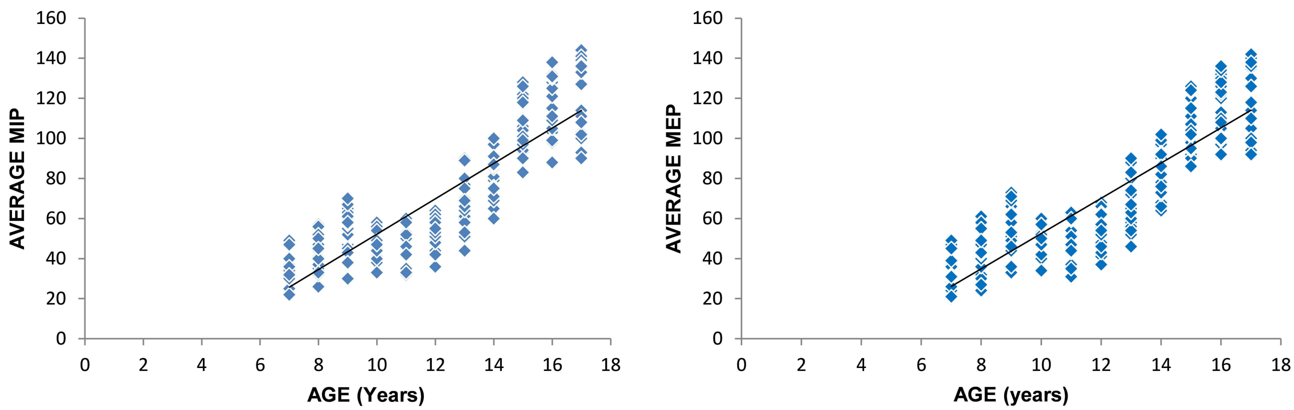

Figure 2 Correlation between MIP, MEP and age.

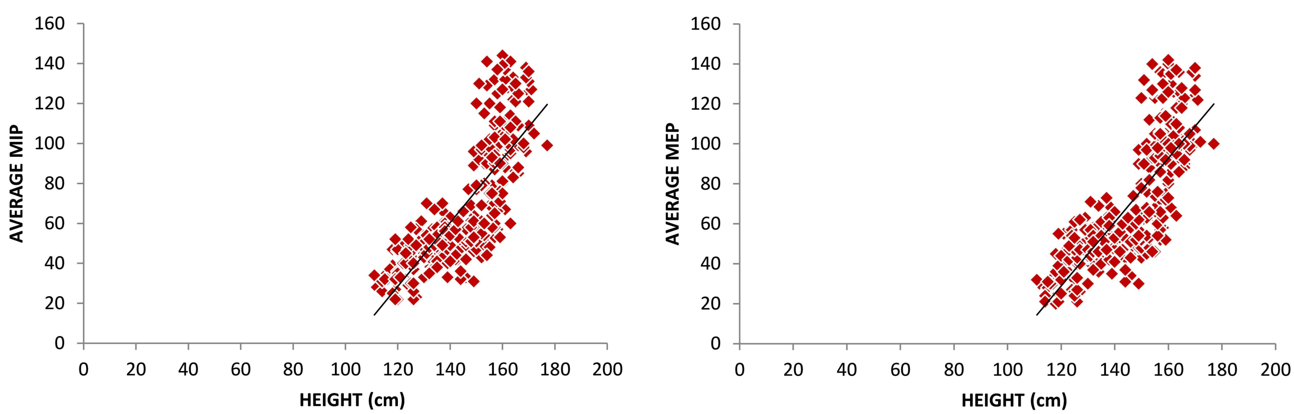

Figure 3 Correlation between MIP, MEP and height.

-32.9+4.113*Gender+9.22*age-0.521*BMI

(Gender: if boys substitute 1 if girls substitute 0)

c. The predictive equation for MEP using height, gender and age:

-9.243–0.328*Height+4.83*Gender+10.686*Age

(Gender: if boys substitute 1 if girls substitute 0)

d. The predictive equation for MEP using BMI, gender and age:

-31.622+4.198*Gender+9.262*Age-0.602*BMI

(Gender: if boys substitute 1 if girls substitute 0)

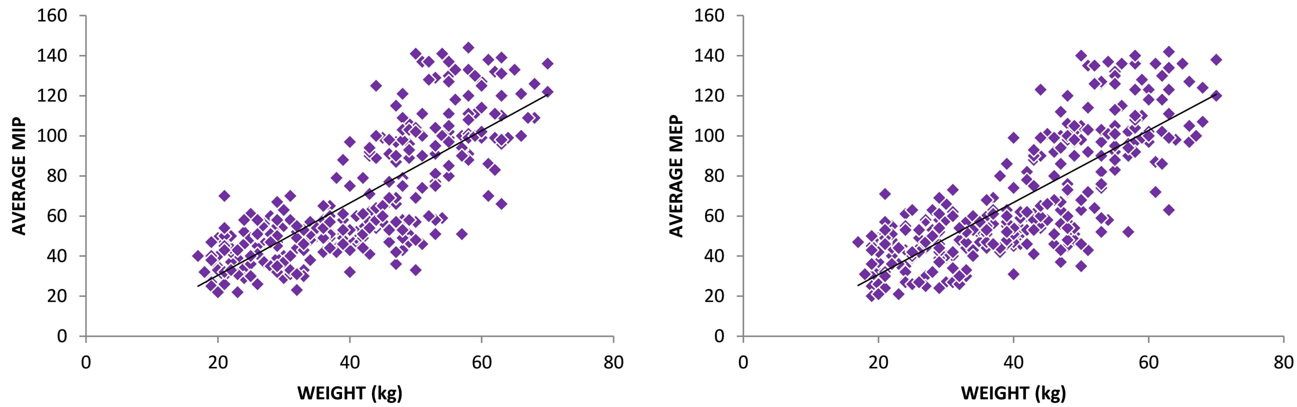

Figure 4 Correlation between MIP, MEP and weight.

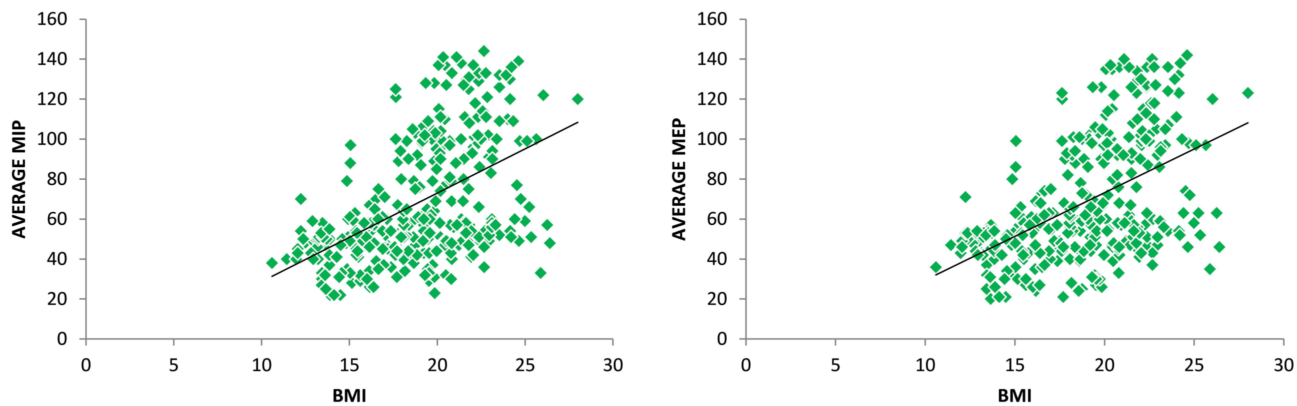

Figure 5 Correlation between MIP, MEP and BMI.

Discussion

The present study attempted to generate the normative value of respiratory muscle strength in healthy Indian children of Mangalore city aged between 7 and 17 years. The results found that mean MIP and MEP for boys were 72.5±32.8 cm H_2O and 73±33.2 cm H_2O, while the mean MIP and MEP for girls were 67±30.2 cm H_2O and 68 ±30.1 cm H_2O respectively.

The normal respiratory pressure values found for healthy Indian children in the present study was found to be different when compared to the studies published previously in other countries. It was noted that a study by Choi et al reported respiratory pressure values in 8 to 12 years children where the mean MIP values ranged from 42.08±15.13 cm H_2O to 104.12±19.92 cm H_2O in boys and 45.85±13.13 to 94.94±115.93 cm H_2O in girls. Similarly the mean MEP values ranged from 48.67 ±18.41 cm H_2O to 119.96±25.28 cm H_2O and 47.85 ±16.70 cm H_2O to 112.18±24.08 cm H_2O for boys and girls respectively.[6] A systematic review which studied normative values of various groups of the population age ranging from 4 to 12 years, reported pooled mean values for MIP which ranged from 60.45 cm H_2O to 102.29 cm H_2O in boys and 52.29 cm H_2O to 87.55 cm H_2O in girls. The pooled MEP values ranged from 72.87 cm H_2O to 124.03 cm H_2O and 57.12 cm H_2O to 103.22 cm H_2O in boys and girls respectively.[15] When these reference values are compared with current study results, there exists a greater difference which varies by 5 to 10 cm H_2O. The most probable factors for these differences are associated with reduced motivation among the subjects and variation in geographical locations. Moreover, technical factors like the ability to follow commands and leaks in the mouthpiece of the apparatus or air leaks at the mouth and nose during forced expiration could have impacted findings.[1,15] Factors such as the speed of manoeuvre, differences among assessors to consider either peak or sustained value of MIP and MEP and the minimum number of repetitions may have influenced the measurement of MIP and MEP values.[17]

Table 2 Correlation of Average Respiratory Pressure Values with Variables

		Age	Height	Weight	BMI
Average MIP	**Pearson correlation (r)**	0.885**	0.793**	0.785**	0.500**
Average MEP	**Pearson correlation (r)**	0.883**	0.794**	0.782**	0.494**

Note: **Correlation is significant at the 0.01 level.

There was an increase in MIP and MEP with age for boys and girls, and these were similar to findings of Arnall et al[16] and Tomalak et al[10] who reported that the increase in MIP and MEP values are directly proportional to an increase in age. Earlier studies reported difference in respiratory pressure values, where the mean MIP and MEP values were higher in boys than girls.[6,10,16] Similar changes in MIP and MEP values observed in the present study, and boys demonstrated relatively higher values compared to girls. An increase in the MIP and MEP values in boys with increasing age, can be attributed to greater skeletal and muscle mass in boys compared to girls. This might be influenced by the release of the hormone testosterone which is secreted in larger quantities in boys with the increase in age along with body maturation and neural influences.[15]

The current study demonstrates a strong positive correlation between height and respiratory pressure changes which can be due to a change in the body size with the increase in age, even though the mean height of boys (145.5±33.32) and girls (139.5±30.3) in current study was lesser compared to the study done by Hulzebos et al.[18] The present study demonstrates a positive correlation between height and respiratory pressure. These changes might be due to an exponential increase in the TLC with a change in weight (and especially height) wherein TLC directly influences the respiratory muscle strength.[19]

The present study shows a positive correlation between weight and respiratory muscle strength. A study done by Domènech-Clar et al[20] showed results similar to that of the present study, which stated that weight is substantial in

Table 3 Correlation and Linear Regression of MIP with Height Variables for Boys and Girls

Age	F Value	r Value	Regression Equation	Results
		Boys		
7	0.088	0.089	41.65–0.073X	0.772 p>0.05, NS
8	0.331	0.147	19.158+0.165X	0.573 p>0.05, NS
9	6.718	0.599	−62.84+0.866X	0.024 p>0.05, NS
10	0.111	0.081	41.342+0.54X	0.743 p>0.05, NS
11	5.39	0.514	135.46–0.594X	0.035 p>0.05, NS
12	2.409	0.365	41.161+0.644X	0.149 p>0.05, NS
13	0.024	0.045	61.368+0.070X	0.879 p>0.05, NS
14	0.004	0.016	87.577+0.025X	0.952 p>0.05, NS
15	0.473	0.145	160.271–0.308X	0.621 p>0.05, NS
16–17	0.773	0.147	213.822–0.565X	0.549 p>0.05, NS
		Girls		
7	0.038	0.052	18.254+0.112X	0.848 p>0.05, NS
8	0.003	0.017	44.890+0.024X	0.959 p>0.05, NS
9	0.805	0.226	−16.754+0.530X	0.384 p>0.05, NS
10	0.758	0.279	100.184–0.397X	0.407 p>0.05, NS
11	0.001	0.002	47.334+0.003X	0.995 p>0.05, NS
12	0.011	0.031	56.931–0.019X	0.917 p>0.05, NS
13	0.670	0.214	29.540+0.184X	0.427 p>0.05, NS
14	1.372	0.320	204.674–0.181X	0.264 p>0.05, NS
15	0.260	0.135	142.709–0281X	0.618 p>0.05, NS
16–17	0.321	0.105	190.328–0.457X	0.575 p>0.05, NS

Table 4 Correlation and Linear Regression of MEP with Height Variables for Boys and Girls

Age	F Value	r Value	Regression Equation	Results
Boys				
7	0.272	0.115	16.866+0.128X	0.612 p>0.05, NS
8	1.039	0.254	−11.127+0.399X	0.324 p>0.05, NS
9	11.04	0.692	−94.727+1.120X	0.006 p<0.05, HS
10	0.319	0.136	34.010+0.112X	0.580 p>0.05, NS
11	6.16	0.540	158.780–0.751X	0.025 p<0.05, HS
12	1.801	0.327	−35.107+0.608X	0.200 p>0.05, NS
13	0.024	0.044	61.139+0.088X	0.880 p>0.05, NS
14	0.014	0.054	78.363+0.868X	0.824 p>0.05, NS
15	0.809	0.257	194.198–0.516X	0.374 p>0.05, NS
16–17	0.289	0.131	240.490–0.508X	0.592 p>0.05, NS
Girls				
7	0.606	0.204	−30.754+0.510X	0.499 p>0.05, NS
8	0.700	0.207	55.607–0.045X	0.935 p>0.05, NS
9	0.901	0.238	−23.53+0.58X	0.395 p>0.05, NS
10	1.439	0.377	124.362–0.564X	0.253 p>0.05, NS
11	0.022	0.044	56.713–0.61X	0.886 p>0.05, NS
12	0.237	0.139	70.003–0.102X	0.635 p>0.05, NS
13	0.748	0.225	27.218+0.201X	0.402 p>0.05, NS
14	1.391	0.322	191.372–0.732X	0.261 p>0.05, NS
15	0.609	0.204	168.378–0.437X	0.488 p>0.05, NS
16–17	0.359	0.110	190.874–0.463X	0.554 p>0.05, NS

Table 5 Correlation and Linear Regression of MIP with BMI Variables for Boys and Girls

Age	F Value	r Value	Regression Equation	Results
Boys				
7	0.530	0.214	39.527–0.397X	0.482 p>0.05, NS
8	2.5	0.378	55.223–0.901X	0.135 p>0.05, NS
9	0.824	0.253	64.038–0.655X	0.382 p>0.05, NS
10	0.030	0.042	49.618–0.043X	0.864 p>0.05, NS
11	0.001	0.001	50.818–0.003X	0.996 p>0.05, NS
12	1.79	0.327	76.104–1.077X	0.201 p>0.05, NS
13	4.45	0.520	102.874–1.298X	0.056 p>0.05,NS
14	0.009	0.025	93.314–0.085X	0.927 p>0.05, NS
15	0.242	0.465	60.183+2.225X	0.094 p>0.05, NS
16–17	0.368	0.201	80.102+1.796X	0.410 p>0.05, NS
Girls				
7	0.091	0.081	36.498–0.322X	0.767 p>0.05, NS
8	2.366	0.437	28.290+1.379X	0.115 p>0.05, NS
9	0.019	0.249	54.550–0.142X	0.893 p>0.05, NS
10	0.594	0.001	35.440+0.593X	0.461 p>0.05, NS
11	0.001	0.073	47.629+0.003X	0.997 p>0.05, NS
12	0.665	0.260	52.395+0.091X	0.804 p>0.05, NS
13	1.017	0.193	67.811–0.544X	0.330 p>0.05, NS
14	0.463	0.001	53.371+1.233X	0.509 p>0.05, NS
15	0.001	0.101	97.867+0.001X	1.00 p>0.05, NS
16–17	0.301	0.035	98.287+0.913X	0.588 p>0.05, NS

Table 6 Correlation and Linear Regression of MEP with BMI Variables for Boys and Girls

Age	F Value	r Value	Regression Equation	Result
Boys				
7	5.060	0.561	50.056–1.050X	0.046 p<0.05, HS
8	5.391	0.514	68.142–1.710X	0.035 p<0.05, HS
9	0.565	0.212	65.236–0.613X	0.467 p>0.05, NS
10	0.013	0.027	50.089–0.034X	0.912 p>0.05, NS
11	0.040	0.052	54.349–0.131X	0.844 p>0.05, NS
12	1.552	0.306	76.678–1.065X	0.232 p>0.05, NS
13	2.842	0.436	102.865–1.373X	0.118 p>0.05, NS
14	0.084	0.077	97.494–0.269X	0.777 p>0.05, NS
15	2.87	0.527	56.956+2.377X	0.053 p<0.05, HS
16–17	5.88	0.244	71.694+2.195X	0.314 p>0.05, NS
Girls				
7	1.62	0.323	52.493–1.495X	0.223 p>0.05, NS
8	0.675	0.252	36.646+0.932X	0.430 p>0.05, NS
9	0.072	0.069	48.569+0.294X	0.792 p>0.05, NS
10	0.116	0.113	41.495+0.282X	0.741 p>0.05, NS
11	0.039	0.059	45.368+0.146X	0.847 p>0.05, NS
12	0.055	0.068	52.916+0.102X	0.818 p>0.05, NS
13	1.25	0.287	69.664–0.624X	0.281 p>0.05, NS
14	0.520	0.204	54.862+1.160X	0.484 p>0.05, NS
15	0.001	0.006	99.037–0.019X	0.983 p>0.05, NS
16–17	0.718	0.115	88.608+1.346X	0.404 p>0.05, NS

determining respiratory muscle strength. Weight affects the mass of the diaphragm, which is a primary muscle for inspiration; thus, the influence of weight was observed while determining the strength of respiratory muscles.[1] As weight and height positively correlated with respiratory muscle strength, so does the BMI since both height and weight directly influence it.

Multiple regression analysis was done to obtain predictive equations using age, weight, height and BMI. For MIP, regression analysis showed significance for height, gender and age; whereas for MEP, age, gender, height, and BMI showed statistical significance ($p<0.05$). Weight was not found significant in both outcomes.

The sample size of the present study (n=320) is larger when compared to the previously published relevant works.[6,9,10,15,18,21] The lower limit of age chosen was seven, to ensure that the subjects recruited demonstrate co-operation and understand the test procedure, while the upper limit of age was 17. To the best of our knowledge, there is no literature available related to the reference values of MIP and MEP for Indian children.

All the subjects were assessed by the same assessor to minimise the variability of measurements. None of the subjects were a smokers or were involved in any athletic activity. We used a rigid mouthpiece and a nose clip to eliminate the underestimation of the real value of the measurement by reducing the risk of inadvertent leaks from nose and mouth.

The present study generated values that will have a significant role in practical and clinical application in the assessment and diagnosis of various respiratory conditions and for follow-up of conditions. These age-specific reference values will make it easier to remark on the strength of the respiratory muscle in children. This data will aid in evaluating the prognosis of various conditions which involve weakness of respiratory muscles. It can ostensibly also aid in designing germane therapy programs. The study's predictive equations will help in determining the values of MIP and MEP of children of specific age group using their respective height, gender and BMI. This study reflects a slice of the population located in the southern part (Mangaluru, Karnataka State) of India and ethnic differences were not analysed due to the homogeneous population and to make the values more appropriate for Indian population. The rigid mouthpiece was used in this study even though Black and Hyatt recommended the use of a large rubber mouthpiece pressed against the lips and teeth to achieve a better seal for MEP measurements.[16] Further studies can investigate the reference values needed for children with various neuromuscular and musculoskeletal conditions (ie, Scoliosis, Duchenne muscular dystrophy) and in different geographical regions to generate accurate data to plan, objective outcome-based therapeutic strategies. Studies across different geographical regions needed to generate accurate respiratory pressure data of the respective population.

Conclusion

The present study indicates a presence of difference in respiratory pressure values of Indian children compare to those of other countries. Boys had higher MIP and MEP when compared to girls. Age is a significant factor in determining respiratory muscle strength, and there is an increase in respiratory strength as age increases. The variables such as age, height, BMI and gender, play a significant role in attaining and predicting increased respiratory muscle strength values. The generated values are specific to an Indian paediatric population and we believe that these values could be used as a standard in day to day clinical practice.

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Disclosure

The authors report no conflicts of interest in this work.

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