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Original Research Article

Estimation of Age from the Rib by Phase Analysis - An Autopsy Study in Population of Central India

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Abstract

Introduction: The rib technique has certain advantages over the pubic symphyseal method. Metamorphosis in the rib is detectable well beyond the maximum age that can be estimated reliably from the pubic symphysis. Aim of this study is to test the reliability and accuracy of Iscan's phase analysis method in estimation of skeletal age at death from fourth rib & to find out mean age for each phase in male & female population of central India. **Material & Methods:** As per defined inclusion & exclusion criteria the dead bodies brought for autopsy were selected & the costochondral junction of right & left fourth rib of 128 dead bodies of known age and sex were removed, processed and examined by phase analysis depending upon component stages. Findings were graded using phase analysis method originally used by Yasar Iscan & Padmakumar et.al. Each rib was placed in one of the phases extending from '1' to '8'. **Results:** Out of total 128 samples, 90 were of male (70.31%) and 38 were of female (29.69%) gender. Maximum number of male sample fall in phase 6 followed by phase 2, however most of female samples fall in phase 2 and 3. The morphological changes initiate earlier in female than male. No significant inter costal phase variation seen between right ribs and their left side counterpart. The most rapid changes were seen in phases 1 to 4 with an interval of 5 - 8 years. Statistical analysis inferred that phage analysis method of age estimation is safe and reliable in population of Central India. **Conclusion:** The age of unknown dead bodies can be estimated within range of ± 2 to 5 years up to 45 years of age by phase analysis. The phase analysis method was found to be useful in Central Indian population in both sexes, except in phase 6 to 8 where age can be determined only within a wide range.

1. Introduction

Identification of a person is important as it may pose many problems from medico legal point of view, especially in unknown dead bodies, decomposed/mutilated bodies and of skeletal remains. Accurate identification is mandatory for the establishment of corpus Delecti after

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homicide since unclaimed bodies, portion of dead body or bones are routinely brought to medical experts for examination. Determining age at death has always been considered the most challenging of the four main identifiers, especially in the adult skeleton.[1] After the age of 25 years, estimation of age becomes more uncertain, whether in living or dead. Microscopic method of age estimation is expensive and requires more time, equipment and skill. It is therefore not practicable. The macroscopic methods are faster and do not involve destruction of the specimen. The principle macroscopic changes found are closure of cranial sutures, metamorphosis of the pubic symphysis and degenerative changes in vertebral bodies and joints.

Iscan and associate developed two techniques (component and phase analysis) to determine age by direct examination of sternal extremity of the rib. Phase analysis was based on nine metamorphic stages (phases) observed in bones of both sexes of Whites.[2] The rib technique has certain advantages over the pubic symphyseal method. Metamorphosis in the rib is detectable well beyond the maximum age that can be estimated reliably from the pubic symphysis. Another factor is that rib is not directly affected by the stress of pregnancy and parturition as in the pelvic region. Histologically the most important factor underlying the observed changes is the continuous periosteal deposition of new bone, possibly accompanied by perichondral ossification. Thus, the “deepening” of the pit seen with increasing age is actually a build-up of periosteally produced walls of bone surrounding the sternal extremity of the rib and extending over the costal cartilage. Another factor, endosteal resorption, must also be considered. Following ossification of the growth cartilage, the sternal extremity of the rib has no active growth zone. However, endosteal resorption continues at an even greater rate than periosteal deposition, thinning, and in some cases eventually eroding through the floor of the junction.[3]

Aim of this study is to test the reliability and accuracy of Iscan’s phase analysis method in estimation of skeletal age at death from fourth rib & to find out mean age for each phase in male & female population of central India.

2. Material & methods:

The present observational study was carried out in the Morgue and the Department of Forensic Medicine and Toxicology, of Government Medical College from Maharashtra, a post graduate and undergraduate training institute. As per defined inclusion & exclusion criteria the dead bodies brought for autopsy were selected during the study period. Right and left 4th ribs of 128 dead bodies of known age and sex were removed, processed and examined by phase analysis depending upon component stages. Information about case including age was recorded in a prepared Performa, based on documents presented for conduction of post mortem & details as narrated by family member.

Case selection criteria*:*

Inclusion criteria:

1. Individuals above 17 yrs.
2. Individuals of known age and sex.

Exclusion criteria:

1. Individuals with deformed or diseased ribs.
2. Cases of Road traffic accident with chest trauma.
3. Cases whose age not surely known.
4. Unknown bodies

The specimens were separated from body during autopsy by cutting at two points i.e., 3 cm inner to and 5 cm outer to costochondral junction using a rib cutter without damaging the costochondral junction. Scissors were utilized for cutting the muscles attached to the ribs. The portion of ribs was labeled and kept in water containers for three to four weeks. The remaining soft tissue and cartilages were removed by keeping the bones in boiling water for ten to fifteen minutes. Bones were thoroughly dried at room temperature and each rib was examined to ascertain the stage of morphological change. Based on metamorphosis of the features the specimens were separated into eight groups- phases 1 to phases 8. Phase 0 was excluded from study as all samples were of age above 17 years as changes in rib morphology are observed only after age of 17 years. These phases will be developed on changes noted in the form, shape, texture and overall quality of costochondral junction of ribs.

For determination of phase of each rib pit depth (component I), pit shape (component II), and rim and wall configuration (component III) were observed.

Component I: Pit depth- The sternal end of the rib shows one of the most apparent age-related changes detected which is the formation and deepening of a cavity (pit). The maximum depth of this pit is measured with a Vernier caliper depth calibrated to

0.1 mm. The site for taking measurement is place of maximum distance between the base of the pit and the adjacent anterior or posterior wall. The caliper is held perpendicular to the base of the pit.

Component II: Pit shape-Component II deals with changes in the shape of the pit. Initially, the pit is only a slight, amorphous indentation, which in about 1 year from its first appearance, develops into a structure which is V-shaped. The posterior and anterior walls of the rib form this V-shape. Over the next few years, the base of the V widens to become U-shaped. As age increases the walls of the pit grow thinner forming a progressively wider U.

Component III: Rim and wall configurations-Component III analyzes changes in the configurations of the rim and walls of the pit. The rim begins with even, consistent border around the pit that quickly adopts a scalloped but still fairly regular shape. Eventually, with advancing age the rim and walls become increasingly irregular, thin and sharp.[4]

Component Division: Each component was divided into six stages as follow:

Component I: Pit depth that is the maximum depth of the pit is measured with the Vernier depth caliper calibrated to 0.1mm.

It is divided into following six stages (**Fig. 1**):

0. Flat to slight billowy extremity with no indentation (pit) greater than 1.1 mm.
1. Definite pit formation with a depth ranging from 1.1 to 2.5mm.
2. Pit depth ranging from 2.6 to 4.5mm.
3. Pit depth ranging from 4.6 to 7mm.
4. Pit depth ranging from 7.1 to 10mm.
5. Pit depth of 10.1mm or more

Component II: Pit shape. It is divided into the following 6 stages(**Fig. 2**):

1. No pit formation at the flat and billowy articular surface.
2. A shallow amorphous indentation.
3. Formation of V shape pit with thick walls.
4. The pit assumes a narrow U shape with fairly thick walls.
5. Wide U shape pit with thin walls.
6. Pit is still wide U shaped, yet deeper, more brittle and poore texture with some disintegration of bone.

Component III: Rim and wall configuration. It is divided into following six stages(**Fig. 3**):

0. Smooth regular rim with no wall formation.
1. Beginning walls with a thick, smooth regular rim.
2. Definite visible walls that is thick and smooth with a scalloped or slightly wavy rim.
3. The scalloped edges are disappearing wall are thinning yet the walls are fairly without significant deterioration in the texture.
4. The rim is becoming sharper and increasingly irregular with more frequent bony projection, often more pronounced at the cranial and caudal margins. The walls Shows further thinning cranial and are less sturdy with noticeable deterioration in texture.
5. Texture shows extreme friability and porosity, rim is very sharp, brittle and highly irregular with long bony projection. Occasionally windows are formed in areas where the walls are incomplete.[5]

Figure 1: Component I Pit Depth Stages

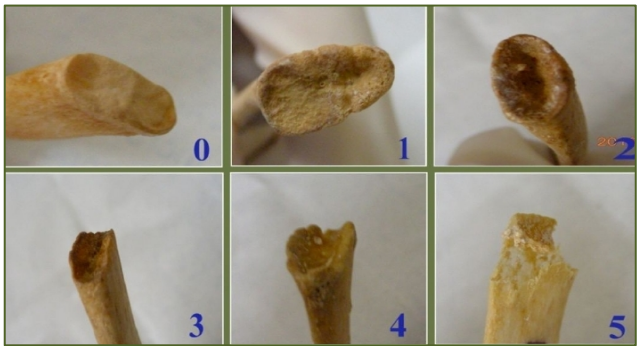


Figure 2: Component II Pit Shape Stages

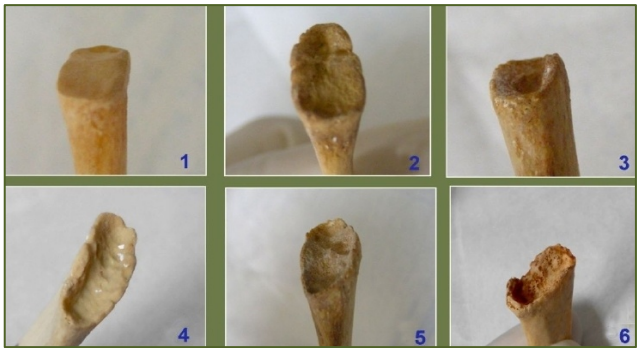
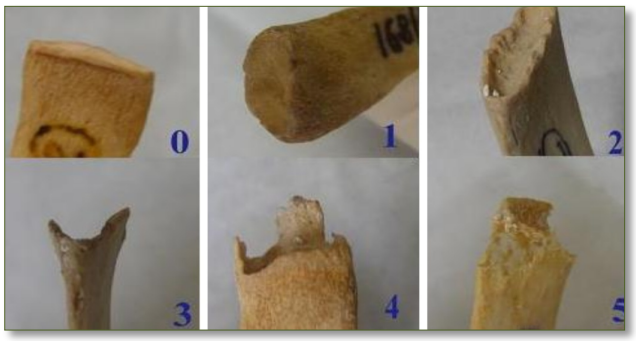


Figure 3: Component III Rim and wall configuration Stages



Component I: (0) nearly flat, billowy surface. (1) There is no pit formation pit has formed to a maximum depth of 1.2mm. (2) pit depth is 2.2mm. (3) pit depth is 3.7mm. (4) pit depth is 3.7mm. The bony projection on the superior border of the rib in not included in the measurement. (5) Pit depth has reached 11.1mm[5] (**Figure 1**). **Component II:** (0) billowy with no pit formation. (1) Amorphous pit between the anterior and posterior walls. (2)V-shaped pit is seen. (3)U shaped pit with thick walls. (4) U shaped pit fairly thinning walls. (5) U-shape pit is shallow, badly deteriorated and sharp, irregular rim with very thin wall, badly deteriorated[5] (**Figure 2**).

Component III: (0) the rim rounded, regular and no wall formation. (1) The rim still smooth, rounded, with incipient wall formation defining the shallow pit. (2) The rounded, wavy, rim shows some scallops forming at the edge and thick, smooth walls. (3) no regular scalloping remains, sharpening edge of the increasingly irregular rim but still fairly dense and smooth. (4)The sharp rim which is starting to show irregular long bony projections , thinning walls along with porosity and deterioration of bone. (5) Rim very sharp, badly deteriorated, porous walls with "window" formation[5] (**Figure 3**).

Observed Phases: On observation of different component stages each rib was placed in particular phase (**Figure 4)** as follows:

Phase 0: The articular surface is flat or billowy with a regular rim and rounded edges. The bone itself is smooth, firm and very solid.

Phase 1: There is a beginning amorphous indentation in the articular surface, but billowing may also still be present. The rim is rounded and regular. In some cases, scallops may start to appear at the edges. The bone is still firm, smooth and solid.

Phase 2: The pit is now deeper and has assumed V-shaped appearance formed by the anterior and posterior walls. The walls are thick and smooth with a scalloped of slightly wavy rim with rounded edges. The bone is firm and solid.

Phase 3: The deepening pit has taken on a narrow to moderately U - shape. Walls are still fairly thick with rounded edges. Some scalloping may still be present but the rim is becoming more irregular. The bone is still quite firm and solid.

Phase 4: Pit depth is increasing but the shape is still a narrow to moderately wide U. The walls are thinner, but the edges remain rounded. The rim is more irregular with no uniform scalloping pattern remaining. There is some decrease in the weight and firmness of the bone; however, the overall quality of the bone is still good.

Phase 5: There is little change in pit depth but the shape in this phase is predominantly a moderately wide U. Walls show further thinning and the edges are becoming sharp. Irregularity is increasing in the rim. Scalloping pattern is completely gone and has been replaced with irregular bony projections. The condition of the bone is fairly good; however, there are some signs of deterioration with evidence of porosity and loss of density.

Phase 6: The pit is noticeably deep with a wide U-shape. The walls are thin with sharp edges. The rim is irregular and exhibits some rather long bony projections that are frequently more pronounced at the superior and inferior border. The bone is noticeably lighter in weight, thinner and more porous, especially inside the pit.

Phase 7: The pit is deep with a wide to very wide U - shape. The walls are thin and fragile with sharp, irregular edges and bony projections. The bone is lighter in weight and brittle with significant deterioration in quality and obvious porosity.

Phase 8: In this final phase the pit is very deep and widely U-shaped. In some cases, the floor of the pit is absent or filled with bony projections. The walls are extremely thin, fragile and brittle with sharp, highly irregular edges and bony projections. The bone is very light weight, thin, brittle, friable and porous. 'Window' formation is sometimes seen in the walls.[6]

Figure 4: Fourth sternal rib phase 0 to 8

Statistical analysis: The statistical analyses of the data were carried out using the software 'statistical package for social sciences (SPSS 16.0). The CROSSTABS PROCEDURE was done for finding out statistical significance of the distribution of samples.

They were analyzed according to the age and phase distribution. With the help of chi-square test p-value calculated. Mean age and 95% confidence interval of mean age for each phase were calculated using one way ANOVA.

3. Results

Out of total 128 samples, 90 were of male (70.31%) and 38 wereof female (29.69%). Maximum number of cases 39 (30.5%) were from age group of 20-29 years followed by age group 30-39 years with 25 cases, 40-49 years with 24 cases, 50-59 years with 20 cases, 60-69 years with 11 cases. Only 3 cases were in age group of 17-19 years while 6 cases were above 70 years of age. Youngest case was of 18 years old while oldest was of 85 years. Though right and left 4th rib of all samples differ in some component stages, all rib of both sides falls in their same respective phase. No significant inter costal phase variation found in right and left 4th rib. Large number of ribs fall in phase 2 i.e., 28 (21.76%) followed by phase 3 i.e.,25 (19.53%). Maximum number of male sample fall in phase 6 followed by phase 2, however most of female samples fall in phase 2 and 3. There are only one female sample which falls in phase 5, phase 6 and phase 8.

The study male sample age ranges from 18 to 85 years while female sample age ranges from 18 to 80 years. The initial morphological changes in rib (phase 1) started at 18 years in both sexes. It can be stated that initial morphological changes start early in female however thereafter delayed maturation in female rib observed in all phases except in phase 4. Age range was found to increase as phase progresses and it is 5 years in male and 4 years in female in phase 1. Age range is between 5-8 years for each phase till phase 5 and thereafter age range shows very high variability. In male it is 18 years in phase 6 and as high as 25 years in phase 8. Highest age range found in female in phase 3 and 7 and it is of 10 years. Out of all 38 female samples only one sample fall in phase 5, 6 & 8 because of which age range for these phases could not be calculated (**Table 1 & 2**).

Table No. 1: Phase wise 95% confidence interval of mean age in male

					95% ConfidenceInterval		Age	
Phase	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1	11	20.73	1.421	0.428	19.77	21.68	18	23
2	16	25.19	1.974	0.493	24.14	26.24	21	29
3	15	32.33	1.952	0.504	31.25	33.41	30	35
4	7	42.57	1.902	0.719	40.81	44.33	40	45
5	9	45.67	4.416	1.472	42.27	49.06	40	55
6	17	52.00	4.243	1.029	49.82	54.18	42	60
7	8	58.88	3.227	1.141	56.18	61.57	55	65
8	7	69.86	8.591	3.247	61.91	77.80	60	85
Total	90	40.77	15.511	1.635	37.52	44.02	18	85

Table No. 2: Phase wise 95% confidence interval of mean age in female

					95% ConfidenceInterval		Age	
Phase	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1	4	20.25	2.062	1.031	16.97	23.53	18	22
2	12	25.58	2.353	0.679	24.09	27.08	22	30
3	10	33.80	3.676	1.162	31.17	36.43	30	40
4	6	42.50	2.739	1.118	39.63	45.37	40	45
5	1	50.00	-	-	-	-	50	50
6	1	60.00	-	-	-	-	60	60
7	3	65.00	5	2.887	52.58	77.42	60	70
8	1	80.00	-	-	-	-	80	80
Total	38	35.95	14.805	2.402	31.08	40.81	18	80

Table 03: Frequency distribution of phase by age intervals

		Age							
Phase		17 to 19	20 to 29	30 to 39	40 to 49	50 to 59	60 to 69	70 to 100	Total

1	Count	3	12						15
	% ROW	20.0%	80.0%						100.00%
	% COLUMN	100.0%	30.8%						11.70%
2	Count		27	1					28
	% ROW		96.4%	3.6%					100.00%
	% COLUMN		69.2%	4.0%					21.90%
3	Count			24	1				25
	% ROW			96.0%	4.0%				100.00%
	% COLUMN			96.0%	4.2%				19.50%
4	Count				13				13
	% ROW				100.0%				100.00%
	% COLUMN				54.2%				10.20%
5	Count				7	3			10
	% ROW				70.0%	30.0%			100.00%
	% COLUMN				29.2%	15.0%			7.80%
6	Count				3	13	2		18
	% ROW				16.7%	72.2%	11.1%		100.00%
	% COLUMN				12.5%	65.0%	18.2%		14.10%
7	Count					4	6	1	11
	% ROW					36.4%	54.5%	9.1%	100.00%
	% COLUMN					20.0%	54.5%	16.7%	8.60%
8	Count						3	5	8
	% ROW						37.5%	62.5%	100.00%
	% COLUMN						27.3%	83.3%	6.20%
	Count	3	39	25	24	20	11	6	128
	% ROW	2.30%	30.50%	19.50%	18.80%	15.60%	8.60%	4.70%	100.00%
Total	% COLUMN	100%	100%	100%	100%	100%	100%	100%	100%

The CROSSTABS PROCEDURE was done for finding out statistical significance of the distribution of samples. They were analyzed according to the age and phase distribution and observation are shown in above **table 03**. As shown in above **table 03**, in phase 4 all specimens belonged to a single decade. The age groups twenty to twenty-nine, thirty to thirty-nine, forty to forty-nine & fifty to fifty-nine, sixty to sixty-nine and above seventy showed changes belonging to adjacent phases.

Table no. 4: Chi-Square Tests

	Value	Df	p-value
Pearson Chi-Square	429.2	42	0

After statistical analysis using chi square test (**Table 4**), the Pearson's Chi-square value obtained was 429.2 with degree of freedom=42. Degree of freedom= (row-1) x (column -1). From these values P value was estimated and obtained as p=0.000.

From these statistical values it can be inferred that phage analysis method of age estimation is safe and reliable in population of Central India.

4. Discussion

The costochondral junction of right & left fourth rib of 128 individuals were examined of which 90 samples were of male & 38 samples were of females. Findings were graded using phase analysis method originally used by Yasar Iscan & Padmakumar et.al. Each rib was placed in one of the phases extending from '1' to '8'. Maximum numbers of samples were from age group of 20 to 29 years i.e.,30.5%, however from age group of 17-19 years there were only 3 samples. In study of Iscan et al.[2] and that of Padmakumar et al.[6] study on Indian females also maximum numbers of cases were from age group of 20 to 29 years.

In present study, though right and left 4th rib of all samples differ in some component stages, all rib of both sides falls in their same respective phase. No significant inter costal phase variation found in right and left 4th rib in present study. Yoder C et al. (2001)[7] also found that there was no significant variation in fourth rib of both sides. Aktas et al. (2004)[8] examined intercostals variation in their study. There was some -1 or +1 variation at each rib whether left or right in

men. However, the number of cases with phase mistakes was the lowest at right 4th rib. For women, all the right and left ribs included in this study were in concordance with right 4th rib standards in all phases. Gupta P. et.al (2007)[9] found no significant changes in metamorphic developments at the sternal end of fourth rib of the two sides in Punjab population. Meena M.C. et.al (2012)[10] concluded that in male phase variation between right and left fourth ribs was not significant.

Results of present study are in accordance with various studies discussed above. In present study male sample age range from 18 to 85 years with mean age of 40.77 and standard deviation of 15.51, while female sample age ranges from 18 to 80 years with mean age of 35.95 and standard deviation of 14.80. The initial morphological changes in rib (phase 1) started at 18 years in both sexes with 20.73 mean age in male and 20.25 in female. It can be stated that initial morphological changes start early in female however thereafter delayed maturation in female rib observed except in phase 4. After calculating mean age for each phase from 1 to 8 it can be stated that as phase progresses common mean age increases. Common mean age and mean age in male and female in present study increases as phase progresses. These findings are similar to findings in study of Iscan et al.[4] in white population and Padmakumar et al.[6] in Kerala population. After comparing mean age with that of white male population studied by Iscan et al.[4] it can be stated that Indian population shows delayed changes in rib morphology.

Age range was found to increase as phase progresses and it is 5 years in male and 4 years in female in phase 1. Age range is between 5-8 years for each phase till phase 5 and thereafter age range shows very high variability. In male it is 18 years in phase 6 and as high as 25 years in phase 8. Highest age range found in female in phase 7 and it is of 10 years. Out of all 38 female samples only one sample fall in phase 5, 6 & 8 each, age range for these phases could not be calculated. One female sample in present study shows phase 8 changes in contrast to observation of Padmakumar et al.[6] in Kerala population in which no phase changes of phase 8 found in female samples of age ranging from 60 to 100 years.

Delayed maturation in present study population in comparison to Kerala population suggests that geographical factor plays role in rib maturation. In present study width of 95% confidence interval in male samples found to increase as age advances. In phase 1 width of 95% confidence interval is 2 years and gradually rises till phase 5 attaining maximum to 16 years in phase 8. In present study width of 95% confidence interval in female samples also found to increase as age advances. In phase 1 width of 95% confidence interval is 5 years and gradually rises till phase 5 attaining maximum to 25 years in phase 7. 95% confidence interval does not vary significantly in both sexes till phase 4 however afterwards differences in 95% confidence interval seen in both sexes of these two populations. About 95% confidence interval of mean age in both sexes are very much similar to Padmakumar et al.[6] study in Kerala population till phase 4 but afterwards it varies significantly.

Table no.03 shows that in present study initial metamorphotic changes took place from age of 18 years in male and females. These changes are in accordance with Kerala population but are delayed than white American population.[4,6] Oettlé A.C. & Steyn M. (2000)[11] also found tendency toward delayed maturation, as well as a diversion of the appearance of female ribs perimenopausally. Sarajlic N (2006)[12] found that the phase 7 Bosnians reach and finish each phase a little later than Americans. However, phases 7 and 8 were achieved and ended earlier in Bosnian population. Tyagi et al. (2009)[13] found that morphological changes are delayed in Indian population up to the age of 35 years. Comparison between present study male and study by Iscan et al. suggest that morphological changes are delayed overall in Indian males.[4] Present study male shows delayed morphological changes than Kerala population by Padmakumar et.al.[6] Initial rib changes seen at age of 18 in present study male in contrast to 17 years as in Iscan male and Padmakumar et al. (2011).[6] Delayed rib changes observed in present study females than Padmakumar et al. (2011)[6] Kerala female in initial years but faster rib changes than Kerala female as age progresses. In Padmakumar et al. (2011)[6] study none of rib showed phase 8 changes while in present study single female rib shows phase 8 changes. Comparison between rib changes in older female samples was not possible as phase 5, 6 and 8 had only single rib. Mean age in Kerala male and present study male vary in older age group only. However, in females of these two populations mean age varies significantly. 95% confidence interval does

not vary significantly in both sexes till phase 4 however afterwards differences in 95% confidence interval seen in both sexes of these two populations. Variation in mean age and 95% confidence interval of mean age in Kerala population and present study population are suggestive of role of geographical factors in rib maturation.

In present study as shown in **table 3**, CROSSTABS procedure was done for finding out statistical significance of the distribution of samples. They were analyzed according to the age and phase distribution. As shown in **table 3**, all specimens belonging to a single decade in phase 4. The age groups twenty to twenty-nine, thirty to thirty-nine, forty to forty-nine & fifty to fifty-nine, sixty to sixty-nine and above seventy showed changes belonging to adjacent phases. After statistical analysis using chi square test, the value obtained was 429.2 with degree of freedom=42. Degree of freedom= (row-1) x (column -1). From these values P value was estimated and obtained as p=0.000.

Results after statistical analysis were similar to that of Padmakumar et al. (2011)[6] study in Kerala population and from this observation it can inferred that phase analysis of fourth rib can safely and reliably used for age estimation in population of central India also.

5. Conclusions

1. The initial morphological changes in sternal end of ribs appeared from 18 years onwards. The early phase changes did not extend into higher age groups.
2. No significant inter costal phase variation seen between right ribs and their leftside counterpart.
3. The most rapid changes were seen in phases 1 to 4 with an interval of 5 - 8 years.
4. The age of individual can be estimated within range of ± 2 to 5 years up to 45years of age by phase analysis.
5. The phase analysis method was found to be useful in Central Indian population in both sexes, except in phase 6 to 8 where age can be determined only within a wide range.
6. Significant differences were found in mean age of Kerala population and present study population of central India.

Benefits of study:

1. The method is very simple and requires no special training.
2. Study covers subjects having wide range of age (i.e.,18 to 85 years)
3. From this study we can estimate age of deceased individual of unknown agewith variation of 2-5 years from actual age till fifth decade.
4. In females this method has upper hand over pubic symphysis as ribs are notdirectly affected by parturition or pregnancy.

Limitations of the study:

1. Possibility of observer's error cannot be ruled out.
2. Population in Central India is mixed type comprising of various socio- economic status, so this study is not applicable for another topographical region.
3. Dietary, economic, environmental factors are not studied in the present context.

Recommendations:

1. Study to correlate the finding using sternal end of 4th rib and pubic symphysis is required to minimize the age range in years.
2. As the numbers of subjects are less, for confirmation of various variations found in this study, further studies about correlation of age estimated by various findings for a large sample size of both sexes are required.

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Conflict of interest: Nil.

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