



Determination of Safe Apnoea Period in Relation to Haemoglobin Concentration at Komfo Anokye Teaching Hospital

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Abstract

The induced cessation of ventilation or suspension of external breathing is a common occurrence in anaesthetic practice. During the period of induced apnoea, there is no movement of the muscles of inhalation and lung volumes initially remain unchanged. Maintaining haemoglobin saturation during airway management is critical to guarantee the safety of patients. However, there has been little quantitative study of apnoea especially on the rate of change in oxygen saturation in relation to haemoglobin concentration in the apnoeic period of induction of anaesthesia. The study sought to determine the safe apnoea period in relation to haemoglobin concentration. The study showed that patients with low haemoglobin concentration took less time to reach 100% oxygen saturation, while those with high haemoglobin concentration took more time to reach 100% oxygen saturation during pre-oxygenation. The study found that the average safe apnoea period for patients in relation to haemoglobin concentrations to desaturation (from 100% - 92%) following the administration of suxamethonium was 7.0 - 9.9 g/dl was 3:15.07 minutes, 10.0 - 12.9 g/dl was 3:43.05 minutes and 13.0 - 16 g/dl was 4:23.04 minutes. And the overall safe apnoea time for the patients was 3:27.02 minutes. All the patients who attained 100% oxygen saturation without inhaling 100% oxygen were living in mountainous areas. The mean induction period (6:52.26 ms) of the patients was higher than the mean safe apnoea period (3:27.02 ms). The study recommends that anaemia should be corrected before induction of anaesthesia and people living at high altitude should be advised to occasionally move down to areas of low altitude to improve tissue oxygenation.

Subject Areas

Anaesthesiology, Pain Management

Keywords

Apnoea, Hemoglobin Concentration, Anaesthesia, KATH

1. Introduction

The induced cessation of ventilation or suspension of external breathing is a common occurrence in anaesthetic practice. According to Wills and Sirian (2009) [1], safe apnoea period is the duration of time following cessation of breathing or ventilation until critical arterial desaturation occurs (SaO₂ 88% to 90%). Patients undergoing general anaesthesia go through a period of apnoea during laryngoscopy and tracheal intubation, which makes it necessary to ensure safe apnoea period [2].

Health report for 2000-2002 by American Society of Anaesthesiologist Task Force in United Kingdom on Confidential Enquiry into Maternal and Child health associated three out of seven maternal anaesthetic deaths to apnoea as a result of failed endotracheal intubation [3] [4]. A further study in the Netherland for more than two years revealed that 10% out of 87,000 clients' death associated with anaesthesia was as a result of improper management of apnoea [5]. In addition, most studies in apnoea took place in the settings of advanced and emerging countries with highly sophisticated medical technology. However, there has been little quantitative understanding of apnoea, on the rate of change in oxygen saturation in relation to haemoglobin concentration in the apnoeic period of induction of anaesthesia due to its clinical research limitations, hence the need to study that during suxamethonium apnoea at Komfo Anokye Teaching Hospital.

2. Materials and Method

It's an observational research design with independent variables of haemoglobin concentration and administration of induction agent, whereas the dependent variable was oxygen saturation. The population of the study comprised all surgery patients admitted to department of Surgery at Komfo Anokye Teaching Hospital from 1st April to 31st July 2016.

A sample size of Ninety-four was used, Kirkwood and Sterne (2003) [6], this ensured statistical representation of the sample size in relation to the study population. Purposive sampling was used to sample patients from the population, aimed at obtaining clients eligible for the study.

An approved guideline and ethical clearance from Kwame Nkrumah University of Science and Technology Ethics Committee and Committee on Human Resource Publication and Ethics (CHPRE) of Komfo Anokye Teaching Hospital was obtained. Consent was sought from patients and their relatives before the study in which the consent form was read and explained in the local dialect for patients who could not read.

The patients were grouped into three based on their haemoglobin concentration. Group one for those with haemoglobin concentration 7.0 to 9.9 g/dl, Group two 10.0 to 12.9 g/dl and Group three 13.0 to 16.0 g/dl. They then had their oxygen saturation with room air checked with Life Box pulse oximeter. Selected patients had their noninvasive blood pressure checked with automated dash 4000 monitor; manual respiratory rate count with breast watch; axillary temperature checked with mercury thermometer; height and weight with body mass index taken with a tape meter and standing scale respectively. Those with haemoglobin concentration checked more than 24 hours before the study, had venous blood sample taken during intravenous set up for haemoglobin concentration and blood group. The clients were then positioned on operating table. The Life Box pulse oximeter was applied to the thumb finger for continuous measure of oxygen saturation before pre-oxygenation and during induction of anaesthesia. The rate of increase in oxygen saturation during pre-oxygenation was recorded against the time as T_i . Each participant underwent routine anaesthesia induction as follows: inhalation of 100% oxygen for three minutes, administration of an anaesthetic agent of choice by anaesthetist, followed by suxamethonium 1 mg/kg. The rate of increase in oxygen saturation during pre-oxygenation was recorded against the time as T_i . The face mask was removed for laryngoscopy and intubation after fasciculation. The time between the administrations of induction agent until the end of endotracheal intubation during the apnoeic period was recorded against the time for each participant for each group as **Table 1**.

In cases where patient desaturated to 92% before a successful intubation, then patient was ventilated to raise the oxygen saturation to 100% before the next attempt of intubation and the values for the repeated time was recorded as T_1 per table a with respect to the changes in oxygen saturation. The induction agent used, duration of induction, diagnosis, type of surgery and premedication used were recorded.

Table 1. Times for recording apnoeic period for the participants.

Oxygen saturation %	Time period in milliseconds T	Time period in milliseconds T_1 (second attempt)
100	T100	$T_{1,100}$
99	T99	$T_{1,99}$
98	T98	$T_{1,98}$
97	T97	$T_{1,97}$
96	T96	$T_{1,96}$
95	T95	$T_{1,95}$
94	T94	$T_{1,94}$
93	T93	$T_{1,93}$
92	T92	$T_{1,92}$

The data were processed using Microsoft Excel 2010 Professional Edition.

Descriptive statistics such as means, standard deviation as well as inferential statistics such as correlation and one-way analysis of variance were used to analyse data collected. An error margin of 0.05 was used for all inferential analyses.

3. Results

A little over half (52.1%) of the patients were females, while 47.9% were males.

Patients presented for surgery based on the classification of American Society of Anaesthesiology (ASA), with 55.3% being emergency and 44.7% being elective.

Table 2 represents perioperative haemoglobin level groupings with 66% classified as anaemia and 34% being normal.

7.0 - 9.9 g/dl - Moderate anaemia

10.0 - 12.9 g/dl - Mild anaemia

13.0 - 16.0 g/dl - Normal blood level

Figure 1 presents result of preoperative fitness of patients presented for surgery based on the classification of American Society of Anaesthesiology (ASA), with 55.3% (ASAIE + ASAII) being emergency and 44.7% being elective.

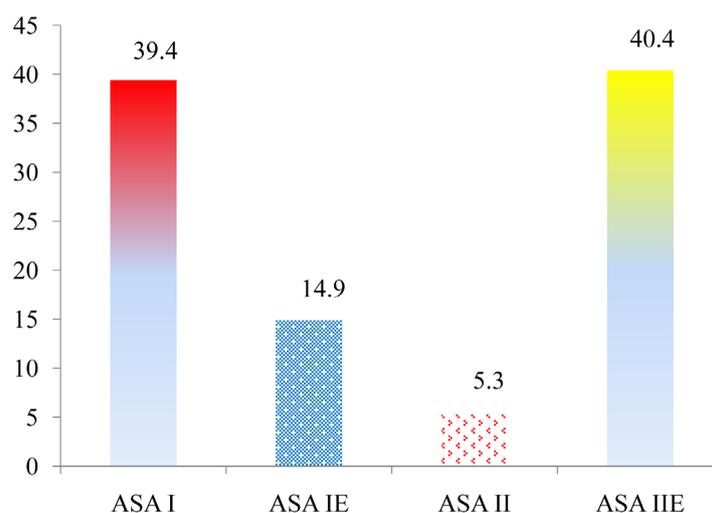


Figure 1. Percentages of preoperative physical status of the patients presented for surgery. Source: Field data (2016).

Table 2. Preoperative haemoglobin concentration levels of sampled patients.

Hb level (g/dl)	Frequency	Percentage
7.0 - 9.9	29	30.9
10.0 - 12.9	33	35.1
13.0 - 16.0	32	34.0
Total	94	100.0

Source: Field data (2016).

ASA I—Normal healthy patient: 39.4%

ASA IE—Normal healthy patient who required emergency surgery: 14.9%

ASA II—Patient with mild systemic disease; no functional limitation: 5.3%

ASA IIE—Patient with mild systemic disease who required emergency surgery: 40.4%

Table 3 shows BMI data on sample size with 68% (normal), 27.7% (overweight) and 4.3% (obese).

Below 25 kg/m²—Normal BMI

25 - 29.9 kg/m²—Overweight

30 and plus kg/m²—Obese

Table 4 represents mean oxygen saturation of 97% in relation to different haemoglobin concentration groupings of patient's breathing room air during anaesthetic assessment. And those with haemoglobin concentration of 7.0 - 9.9 none attained 100% saturation.

Table 5 shows no statistical significance since significant value of 0.974 was more than acceptable margin of error 0.05. Imply that there were similar oxygen saturations with room air across the three haemoglobin concentrations.

Table 3. Frequency and percentage of body mass index of patients presented for surgery.

BMI (kg per m ²)	Frequency	Percentage
Below 25	64	68.0
25 - 29.9	26	27.7
30 and plus	4	4.3
Total	94	100.0

Source: Field data (2016).

Table 4. Oxygen saturation of patients' breathing room air with difference in haemoglobin concentration levels during anaesthetic assessment.

Hb levels (g/dl)	N	Mean SpO ₂	Min	Max
7.0 - 9.9	29	97.24	94.0	99.0
10.0 - 12.9	33	97.21	93.0	100.0
13.0 - 16.0	32	97.16	94.0	100.0
Total	94	97.20	93.0	100.0

Source: Field data (2016).

Table 5. One-way analysis of variance on SpO₂ of patient's breathing room air across haemoglobin concentration levels to show statistical significance.

Groups	Sum of squares	F	Sig.
Between groups	0.115	0.026	0.974
Within groups	203.044		
Total	203.160		

Source: Field survey (2016). Sig. value for Levene's test = 0.341.

Sig. value = p-value (probability value)—it is the actual error associated with the test

df = degree of freedom (number of sub-categories minus one)

F-statistic = Anova value

Data comparing oxygen saturation before induction among the three haemoglobin concentration levels of the sampled patients with mean SpO₂ of 97% across the three different haemoglobin level groups and those with haemoglobin of 7.0 - 9.9 none attained 100% Oxygen saturation with room air (**Table 6**).

From the **Table 7**, a Sig value for the Levene's test of 0.297 shows that the analysis did not violate the assumption of homogeneity of variance. This was because the Sig. value for Levene's test of 0.297 was greater than the accepted margin of error of 0.05.

Sig. value = p-value (probability value)—it is the actual error associated with the test

df = degree of freedom (number of sub-categories minus one)

F-statistic = Anova value

The results (**Table 8**) showing that patients with low haemoglobin concentration took less time to reach maximum oxygen saturation, while those with high haemoglobin concentration took more time to reach oxygen saturation during pre-oxygenation. The results further show a positive relationship between haemoglobin concentration levels and time taken by patients to reach oxygen saturation during pre-oxygenation.

As shown in **Table 9**, there is statistical significant association among the different haemoglobin concentration levels in relation to the rate of increase in oxygen saturation during pre-oxygenation.

Table 6. Oxygen saturation of patients' breathing room air with difference in haemoglobin concentration levels before induction of anaesthesia.

Hb levels (g/dl)	N	Mean of SpO ₂	Min	Max
7.0 - 9.9	29	97.55	94.0	99.0
10.0 - 12.9	33	97.24	93.0	100.0
13.0 - 16.0	32	97.09	92.0	100.0
Total	94	97.29	92.0	100.0

Source: Field data (2016).

Table 7. One-way analysis of variance on SpO₂ before induction across haemoglobin concentration levels to assess statistical significance.

Groups	Sum of squares	F	Sig.
Between groups	3.293	0.539	0.585
Within groups	277.952		
Total	281.245		

Source: Field survey (2016). Sig. value for Levene's test = 0.297.

Table 8. Rate of increase in oxygen saturation (in milliseconds) in relation to haemoglobin concentration during pre-oxygenation at induction of anaesthesia.

Hb levels (g/dl)	N	Mean	Min	Max
7.0 - 9.9	29	0:53.46	0:19.09	4:49.06
10.0 - 12.9	33	1:18.01	0:24.04	3:00.00
13.0 - 16.0	32	2:11.32	0:00.00	3:34.05
Total	94	1:28.45	0:00.00	4:49.06

Source: Field data (2016).

Table 9. One-way analysis of variance on rate of increase in oxygen saturation in relation to haemoglobin concentration during pre-oxygenation to assess statistical significance.

Groups	Sum of squares	F	Sig.
Between groups	352,325,739.3	14.783	0.001
Within groups	1,084,427,878		
Total	1,436,753,617		

Source: Field survey (2016). Sig. value of 0.001 (F-statistic = 14.783).

Sig. value for Levene's test = 0.392 (Levene statistic = 0.947)

Eta squared = 0.25

Sig. value = p-value (probability value) – it is the actual error associated with the test

df = degree of freedom (number of sub-categories minus one)

F-statistic = Anova value

Eta squared = statistical tool for measuring the size of an effect

Multiple comparison of mean rate of increase in SpO₂ during preoxygenation (**Table 10**), showing the mean score for patients with haemoglobin concentration of 13.0 - 16.0 g/dl (mean = 2:11.32; stdv. = 0:57.06) was significantly different from that of patients with haemoglobin concentration of 7.0 - 9.9 g/dl (mean = 0:53.46; stdv. = 1:03.24), and patients with haemoglobin concentration of 10.0 - 12.9 (mean = 1:18.01; stdv. = 0:52.18) in relation to the rate of increase in oxygen saturation during pre-oxygenation.

Data on mean rate of oxygen desaturation from 100% - 92% following suxamethonium administration of sampled patients showing increased mean rate as haemoglobin concentration increases (safe apnoea period in relation to haemoglobin concentration) as follows: 7.0 - 9.9, 10.0 - 12.9 and 13.0 - 16.0 to 3:15.07, 3:43.05 and 4:23.04 minutes respectively. Plus overall mean rate of desaturation from 100% to 92% being 3:27.02 minutes (**Table 11**).

The results of a one-way analysis of variance with post hoc test showing statistical significance of the observed differences in the rate of oxygen desaturation in relation to haemoglobin concentration following the administration of suxamethonium as significant value of 0.001 is within an acceptable error margin of 0.05 (**Table 12**).

Table 10. Multiple comparison for rate of increase in SpO₂ during pre-oxygenation.

Multiple Comparisons						
Dependent Variable: Ti = rate of increase in SpO ₂ during preoxygenation						
Tukey HSD						
(I) haemoglobin concentration categories	(J) haemoglobin concentration categories	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
7.0 - 9.9 g/dl	10 - 12.9 g/dl	-0:24:15	0:14:38	0.227	-0:59:09	0:10:37
	13 - 16 g/dl	-1:17:46*	0:14:45	0.000	-1:52:55	-0:42:37
10.0 - 12.9 g/dl	7 - 9.9 g/dl	0:24:15	0:14:38	0.227	-0:10:37	0:59:09
	13 - 16 g/dl	-0:53:30*	0:14:16	0.001	-1:27:31	-0:19:29
13.0- 16.0 g/dl	7 - 9.9 g/dl	1:17:46*	0:14:45	0.000	0:42:37	1:52:55
	10 - 12.9 g/dl	0:53:30*	0:14:16	0.001	0:19:29	1:27:31

*The mean difference is significant at the 0.05 level. Source: Field survey (2016).

Table 11. Mean rates of change (minutes) in oxygen desaturation in relation to haemoglobin concentration following suxamethonium administration from 100% to 92%.

Time of desaturation	N	7 - 9.9 g/dl	10 - 12.9 g/dl	13 - 16 g/dl	Total
No change in SpO ₂	94	2:33.35	3:31.16	3:39.26	3:15.44
Time for SpO ₂ at 99%	48	2:56.57	3:43.56	3:22.37	3:16.15
Time for SpO ₂ at 98%	35	3:05.24	3:50.43	3:30.42	3:16.46
Time for SpO ₂ at 97%	27	3:12.31	3:26.26	3:49.06	3:19.14
Time for SpO ₂ at 96%	27	3:20.25	3:34.44	4:02.02	3:25.06
Time for SpO ₂ at 95%	26	3:26.26	3:39.21	4:06.03	3:30.58
Time for SpO ₂ at 94%	26	3:33.18	3:49.03	4:07.33	3:37.45
Time for SpO ₂ at 93%	24	3:34.45	3:52.23	4:39.06	3:39.38
Time for SpO ₂ at 92%	20	3:39.35	4:05.07	4:41.03	3:45.12
Total	94	3:15.07	3:43.05	4:23.04	3:27.02

Source: Field data (2016).

Table 12. One-way analysis of variance on rate of desaturation of oxygen in relation to haemoglobin concentration following suxamethonium administration.

Groups	Sum of squares	F	Sig.
Between groups	278,731,535.410	18.541	0.001
Within groups	684,026,504.644		
Total	962,758,040.053		

Source: Field survey (2016).

Sig. value for Levene's test = 0.264 (Levene statistic = 1.350)

Eta squared = 0.29

Sig. value = p-value (probability value) – it is the actual error associated with the test

df = degree of freedom (number of sub-categories minus one)

F-statistic = Anova value

Eta squared = statistical tool for measuring the size of an effect

The post-hoc analysis conducted on the areas of comparison between haemoglobin concentrations to where differences in the means of oxygen desaturation following suxamethonium administration showing statistically significant between haemoglobin concentration of 7.0 - 9.9 and other two groups and no statistical significant difference between haemoglobin concentration of 10.0 - 12.9 and 13.0 - 16.0 (**Table 13**).

Data (**Table 14**) showing normal vital signs of sampled patients who attained 100% oxygen saturation without inhaling 100% oxygen being similar to those who did not attained 100% oxygen saturation.

Table 15 represents an average induction of 6:52.26 minutes.

Data (**Table 16**) showing no statistical significant relation between induction period and safe apnoea period.

Sig. value = p-value (probability value)—it is the actual error associated with the test

Pearson correlation = Measures the strength and direction of relationship

N = sample size

Table 13. Multiple comparison for rate of oxygen desaturation (100% - 92%) following suxamethonium administration.

Multiple Comparisons						
Dependent Variable: average change in SpO ₂ (100% - 92%) following suxamethonium administration						
Tukey HSD						
(I) haemoglobin concentration categories	(J) haemoglobin concentration categories	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
7 - 9.9 g/dl	10 - 12.9 g/dl	-0:57:40*	0:11:37	0.000	-1:25:23	-0:29:58
	13 - 16 g/dl	-1:05:50*	0:11:42	0.000	-1:33:45	-0:37:55
10 - 12.9 g/dl	7 - 9.9 g/dl	0:57:40*	0:11:37	0.000	0:29:58	1:25:23
	13 - 16 g/dl	-0:08:09	0:11:20	0.752	-0:35:10	0:18:50
13 - 16 g/dl	7 - 9.9 g/dl	1:05:50*	0:11:42	0.000	0:37:55	1:33:45
	10 - 12.9 g/dl	0:08:09	0:11:20	0.752	-0:18:50	0:35:10

*The mean difference is significant at the 0.05 level. Source: Field survey (2016).

Table 14. Mean vital signs of patients attaining 100% oxygen saturation without inhaling 100% oxygen.

Vital signs	Mean	Stdv.	Range	
			Min	Max
Body mass index			20.43 kg/m ²	25.21 kg/m ²
Arterial blood pressure (before ind.)	134/75 mmHg		125/70 mmHg	148/75 mmHg
Arterial blood pressure (during ind.)	134/75 mmHg		120/63 mmHg	148/91 mmHg
Pulse rate before induction	94.4 bpm	18.47	67 bpm	112 bpm
Pulse rate during induction	105.8 bpm	105.8	92 bpm	133 bpm
Respiratory rate	14 cpm		14 cpm	14 cpm
Tidal volume	362.8 ml	64.64	280 ml	431 ml
Temperature	36.7 (°C)	0.29	36.4 (°C)	37 (°C)

Source: Field survey (2016).

Table 15. Minimum, maximum and mean Induction period of anaesthesia induction.

Mean	Range	
	Min	Max
6:52.26 ms	5:27.06 ms	14:49.02 ms

Source: Field survey (2016).

Table 16. Pearson correlation between duration of induction and safe apnoea period of patients to show the strength of the relation.

	Duration of induction	Safe apnoea period
Pearson Correlation	1	0.050
Sig. (2-tailed)		0.635
N	94	94

Source: Field survey (2016).

4. Discussion

The results show that preoperative anaemia was prevalent in the majority (66%) of the patients. This result is in agreement with the finding of Baron *et al.*, (2014) [7] that preoperative anaemia had a high prevalence in both adult men and women (31.1% and 26.5%, respectively).

From the study, the majority (55.3%) of patients were operated under emergency circumstances. This is likely to affect extensive preoperative assessment for effective airway management as described by Lane *et al.*, (2005) [8].

The study found that patients with low haemoglobin concentration took less time to achieve 100% oxygen saturation, while those with high haemoglobin concentration took more time to reach 100% oxygen saturation during pre-oxygenation. This disagrees with the assertion of Davis *et al.* (2008) [9] that patients with high haemoglobin concentration reach oxygen saturation faster than those with low

haemoglobin concentration. However, Baillard *et al.*, (2006) [10] posited that high haemoglobin count could occur when the body requires an increased oxygen-carrying capacity, especially for patients who smoke and those living at higher altitude.

The average time taken for patients to de-saturate from 100% oxygen to 92% in relation to haemoglobin concentrations with suxamethonium apnoea were 7.0 - 9.9 g/dl was 3:15.07 minutes, 10.0 - 12.9 g/dl was 3:43.05 minutes and 13.0 - 16 g/dl was 4:23.04 minutes, with overall the mean safe apnoea period of the sampled patients being 3:27.02 minutes. These were lower than the six to eight minutes suggested by Nielsen *et al.*, (2008) [11] as the normal time for desaturation. This could be attributed to the fact that anaesthesia reduces the functional residual capacity of patients, as well as increase oxygen consumption from suxamethonium fasciculation, which causes faster rate of desaturation following the administration of suxamethonium according to Weingart (2012) [2]. This also disagrees with Oddo *et al.*, (2006) [12] that the importance of haemoglobin is not in the storage of oxygen but in its effective transport from the lungs to the tissues.

None of the sampled patients with haemoglobin concentration level 7.0 - 9.9 attained 100% oxygen saturation compared to those with 10.0 - 12.9 and 13.0 - 16 when breathing room air during anaesthetic assessment and before induction of anaesthesia which according to Davis *et al.*, (2008) [9] indicates anaemic hypoxia.

All the patients who attained 100% oxygen saturation without inhaling 100% oxygen had the majority (80%) of such patients with haemoglobin concentration of 13 - 16 g/dl and 20% had haemoglobin concentration of 10 - 12.9 g/dl and were living in mountainous areas and within the active working age group (30 - 45 years) with respiratory rate of 14 cpm.

This was in agreement with the assertion of Palkovits *et al.*, (2014) [13] that people living at high altitudes have low arterial partial pressure of oxygen, low partial pressure of carbon dioxide resulting slight increase in arterial pH and for that matter increases their haemoglobin affinity to oxygen which makes them attain 100% saturation without releasing oxygen. However, this result disagrees with the assertion of McCahon and Hardman (2007) [14] that people of high altitude lack oxygen because of the negative relationship between oxygen saturation in air and altitude.

From the study, the mean induction period of the patients was 6:52.26 ms which was higher than the mean safe apnoea period (3:27.02). Per the argument of Davis *et al.*, (2008) [9], the implication is that the patients were generally at risk. This is because such patients have to be re-oxygenated in the process to guarantee their safety in the physiological processes before surgery.

5. Conclusions

The study found a positive relationship between haemoglobin concentration levels and time taken by patients to reach 100% oxygen saturation during pre-

oxygenation.

The time for oxygen desaturation among the patients was directly or positively related to the level of haemoglobin concentration.

All the patients who attained 100% oxygen saturation without inhaling 100% oxygen were living in mountainous areas.

The study concludes that haemoglobin concentration plays a critical role in the induction and apnoea period.

6. Limitation of the Study

Since the study was conducted in human, the critical desaturation point of 88% - 90% was set at 92%.

Another limitation of the study was difficulty of the researcher to obtain the background information about some of the patients because of pain.

7. Recommendations

The following recommendations were made to ensure safe apnoea period in patients in relation to haemoglobin concentration.

1) Anaemia must be corrected before induction of general anaesthesia in order to ensure safe apnoea period.

2) Awake anaemic hypoxia patients compensate better than during induction of anaesthesia and for that matter induction period for anaemic patient, when necessary must be done with accurate speed.

3) The study also recommend all intubators to use the apnoeic oxygenation technique for all critically ill patient during laryngoscopy and intubation

4) The study recommends that the Ghana Health Service should deploy an online database application system to store the medical and surgical records of people in the country.

5) Educational programmes to educate the public on nutrition to enable people consume balance diet that would help maintain adequate haemoglobin concentration in their bodies.

8. Suggestion for Further Study

The study suggests that further studies should be conducted into causes or reasons why patients who lived in mountainous areas attained 100% oxygen without inhaling 100% oxygen.

In such a study, the selection of patients should be based on altitude of place of stay. Regression and factor analyses could be used to determine the factors which account for the attainment of 100% oxygen without inhaling 100% oxygen.

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Conflicts of Interest

The authors declare no conflicts of interest.

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