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LONDON JOURNAL OF MEDICAL AND HEALTH RESEARCH

Volume 1 | Issue 1 | Compilation 1.0

COMPILED IN UNITED KINGDOM

ENGLISH

LONDON JOURNAL OF MEDICAL AND HEALTH RESEARCH

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PUBLISHER

Great Britain Journals Press
Journals Press Limited
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Phone: +447700176688 (11:30 AM to 5:30 PM BST)

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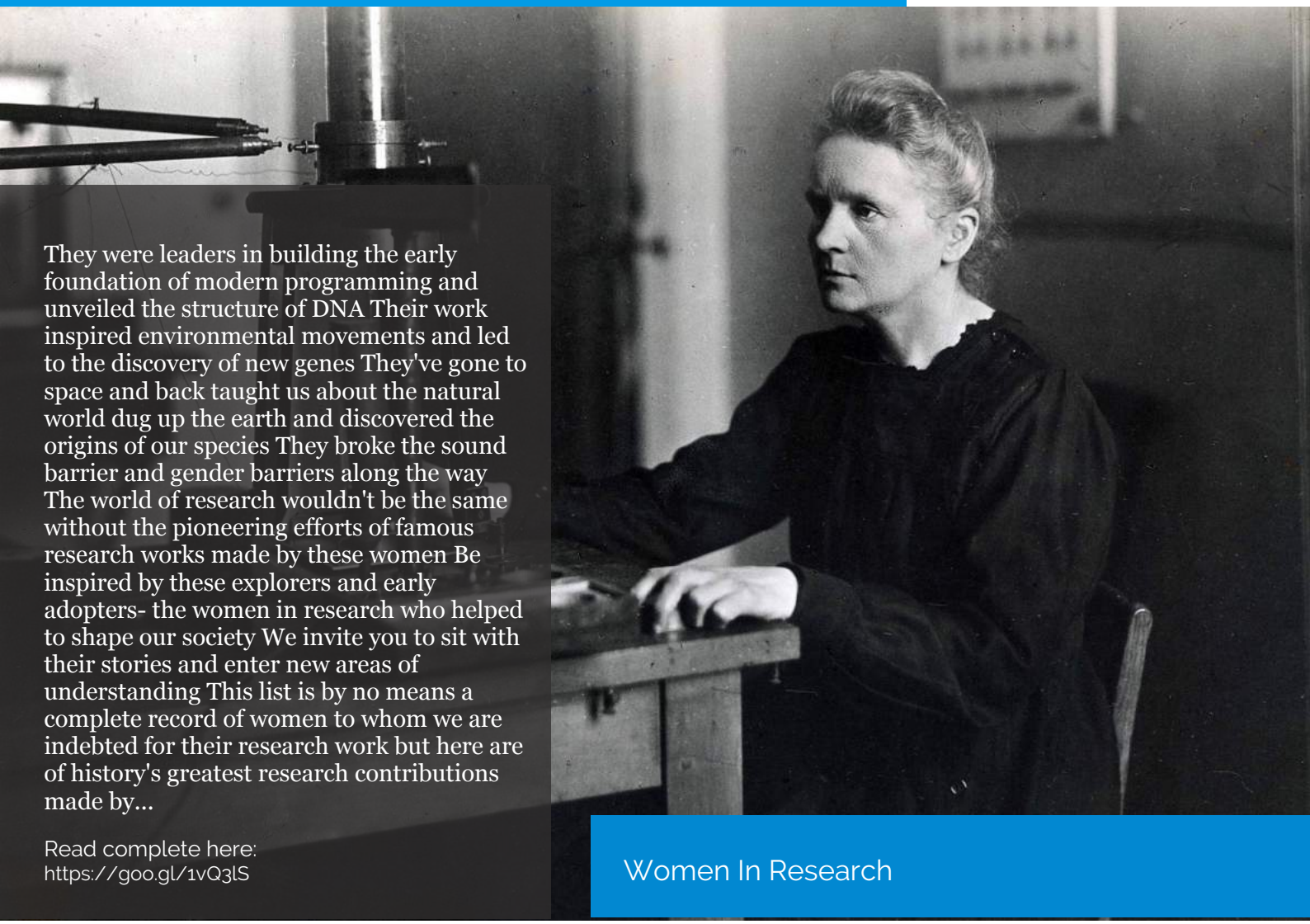

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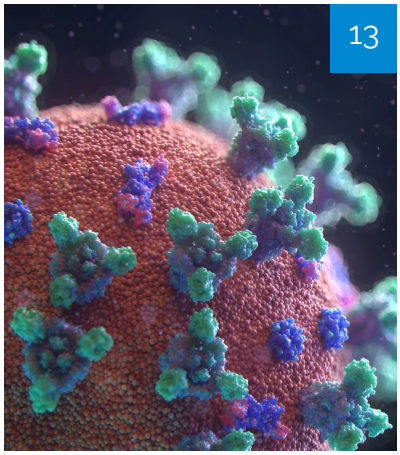

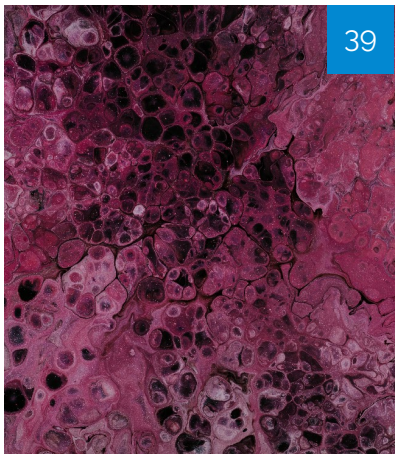

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The Clinical Utility of Electrocardiographic Holter Monitoring for Detecting Sleep Apnea

Marcelo Lapa Kruse, José Cláudio Lupi Kruse, Tiago Luiz Luz Leiria, Geraldo Rizzo, Leonardo M. Pires, Catarine Lopes, Alexandre Kreling Medeiros, Raphael Guimarães, Daiane Silvello, Marco Aurélio Lumertz Saffi & Gustavo Glotz de Lima

Fundação Universitária de Cardiologia

ABSTRACT

Objectives: Obstructive sleep apnea (OSA) is an emerging risk factor for cardiovascular disease and is mostly undiagnosed. Sudden changes in heart rate mediated by the autonomic nervous system are observed during sleep apnea episodes. It is not clear whether the presence of cyclic variation of heart rate (CVHR) is useful in predicting OSA. The purpose of the present study was to estimate the diagnostic accuracy of electrocardiographic Holter monitoring to identify patients with significant OSA in a selected population compared to polysomnogram.

Methods: 136 consecutive patients underwent polysomnography (PSG) and electrocardiogram (ECG) Holter monitoring simultaneously for eight hours during sleep-time. All data from the PSG, the ECG Holter recordings and the automated sleep apnea software were evaluated to compare patients with regard to cyclic variation of heart rate, apnea and hypopnea index (AHI) and sleep parameters.

Keywords: sleep apnea, obstructive; electrocar- diography, ambulatory; heart rate; sensitivity and specificity; polysomnography.

Classification: NLMC CODE: WM 188

Language: English

Great Britain Journals Press

LJP Copyright ID: 392811

London Journal of Medical and Health Research

Volume 1 | Issue 1 | Compilation 1.0

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The Clinical Utility of Electrocardiographic Holter Monitoring for Detecting Sleep Apnea

Marcelo Lapa Kruse[α], José Cláudio Lupi Kruse[σ], Tiago Luiz Luz Leiria[ρ], Geraldo Rizzo[ↀ], Leonardo M. Pires[¥], Catarine Lopes[§], Alexandre Kreling Medeiros[χ], Raphael Guimarães[ν], Daiane Silvello[Θ], Marco Aurélio Lumertz Saffi[ζ] & Gustavo Glotz de Lima[£]

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Results: Patients diagnosed as severe OSA had longer duration of CVHR measured by the Holter monitoring. The likelihood of having significant OSA was directly proportional to the presence and duration of the CVHR. There was a moderate correlation between the duration of the CVHR episodes and the AHI (r = 0.50; P<0.0001; 95% CI; r=0.36 to 0.62). The diagnostic utility of CVHR detected on Holter monitoring in the detection of severe OSA was determined using receiver operating characteristic (ROC) curves, assuming AHI > 15 as references for severe OSA. We also described the likelihood ratios for OSA stratified by different ranks of CVHR duration.

Conclusion: Electrocardiographic Holter monitoring has good accuracy for the detection of significant OAS in a selected population and therefore can be considered as a valuable simplified technique.

Keywords: sleep apnea, obstructive; electrocardiography, ambulatory; heart rate; sensitivity and specificity; polysomnography.

Author α ρ ¥ § χ £: Instituto de Cardiologia – Fundação Universitária de Cardiologia (IC-FUC), Porto Alegre, Rio Grande do Sul, Brazil. Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS). Cardiology, Department of Internal Medicine, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA).

σ ↀ ν: SONOLAB - Sleep Lab at Hospital Moinhos de Vento Porto Alegre, Rio Grande do Sul, Brazil.

Θ: PhD, Post - Graduate Program in Pulmonary Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

ζ: Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil.

I. INTRODUCTION

There is a large amount of recent evidence supporting that obstructive sleep apnea (OSA) is an independent risk factor for cardiovascular disease [1]. The prevalence of OSA appears to be increasing due to epidemic rising rates of obesity. The current prevalence of moderate to severe OSA in the adult population are 10% to 15% in males and 3% to 9% in females when defined as an apnea-hypopnea index (AHI) ≥15 events per hour as measured by a polysomnogram accompanied by at least one symptom or sign of OSA [2,3].

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Individuals with OSA are at greater risk of stroke [4], hypertension [5,6], atrial and ventricular arrhythmias [7-9], impaired quality of life [10] and has been linked to adverse cardiovascular outcomes including coronary artery disease and congestive heart failure [11,12]. Severe untreated OSA has been associated with increased all-cause and cardiovascular mortality [13-16].

The OSA syndrome is a treatable form of disordered breathing in which the upper airway closes repeatedly during sleep. Evidence suggests that a large proportion of individuals with OSA remain undiagnosed [17]. Effective diagnosis and treatment may reduce the morbidity and mortality associated with OSA [18-20]. The gold standard diagnostic test for OSA is an overnight polysomnography (PSG), but it has several limitations including a prolonged wait time depending on available local resources, inconvenience of an overnight sleep study and expense, especially for lower socioeconomic areas [21]. Portable monitors can be used as an alternative tool to PSG for OSA diagnosis in patients with high pre test probability of having moderate to severe OSA. Portable monitors also can be indicated when critical illness or limited mobility is an issue [22].

Sudden changes in heart rate mediated by the autonomic nervous system are observed during sleep apnea episodes [23]. There is a specific pattern of cyclical variation of heart rate (CVHR) which consists of bradycardia during the apnea followed by an abrupt tachycardia upon its cessation.

The diagnosis of OSA based on electrocardiogram (ECG) recordings during PSG has been evaluated by previous researchers [24-26]. This study proposes an alternative tool to detect sleep apnea using a specific Holter software to detect CVHR.

II. METHOD AND MATERIALS

2.1 Population and study protocol

We conducted a cross-sectional study to evaluate patients with OSA through the presence of CVHR. Consecutive patients who were specifically referred to PSG for evaluation of suspected sleep-disordered breathing were invited to participate. A full PSG study and ECG Holter monitoring were recorded simultaneously during sleep - time. Two independent physicians, each one blinded to the results of the other, performed heart rate variability analysis and synchronized polygraphic scoring. They completed a questionnaire to provide a measurement of the subject's general level of daytime sleepiness (Epworth sleepiness scale) [27].

Exclusion criteria were permanent or paroxysmal atrial fibrillation during the electrocardiographic Holter monitoring or permanent pacemaker. Participants gave written informed consent before starting the PSG test. The study protocol was approved by the Research Ethics Committee of the *Instituto de Cardiologia – Fundação Universitária de Cardiologia* (protocol number UP4785/12). We followed the STARD statement for studies of diagnostic accuracy [28].

2.2 Polysomnographic Data

A full-night PSG was performed using a digital system *(BMW III PSG Neurovirtual, Brazil)* at *Sonolab* (the sleep laboratory *Hospital Mãe de Deus*, in *Porto Alegre*) during the subject's habitual sleep time. The following physiological variables were monitored simultaneously and continuously: four channels for the Electro-encephalogram (EEG); two channels for the electrooculogram (EOG); two channels for the surface electromyogram (EMG) (submentonian region and anterior tibialis muscle); one channel for an ECG; airflow detection via two channels through a thermocouple (one channel) and nasal pressure (one channel); respiratory effort of the thorax (one channel) and of the abdomen (one channel) using inductance plethysmography; snoring (one channel) and body position (one channel); oxy- hemoglobin saturation (SpO2); and pulse rate. EEG arousals and leg movements were scored according to the criteria established by the AASM Manual for Scoring Sleep and Associated Events [29]. Apneas and hypopneas were scored and classified following the recommended respiratory rules for adults

suggested by the AASM Manual. Sleep staging was performed using *Rechtschaffen* and *Kales* criteria [30]. One trained technician visually scored all PSGs according to standardized criteria for investigating sleep and an experienced blind examiner reviewed and reported all polysomnogram tests. Severity of OSA was categorized as follows: mild (AHI > 5 to 15 events/h); moderate (AHI > 15 to 30 events/h) and severe (AHI > 30 events/h).

2.3 Electrocardiographic Holter Analysis

The software used for the analysis of Holter tracings was a software designed for detecting sleep apnea through analysis of the RR tachogram (graphs of heart rate vs time) and CVHR *(Cardio Sistemas® Comercial e Industrial LTDA, São Paulo, Brazil)*. An experienced blind examiner reviewed and reported all Holter tests in order to identify the presence of sleep apnea and CVHR. The Holter ECG signals were scanned on a personal computer using a customized beat- detection and beat-classification algorithm that identified all QRS complexes and labeled the beats as normal, ventricular ectopic, suprave- ntricular ectopic, and artifact. The results were reviewed, and all errors in beat detection and classification were corrected interactively on the computer screen by expert technicians of Holter ECG, who were blinded to the subjects' polysom- nographic diagnosis and other characteristics. For the purpose of CVHR detection, the R-R interval time series were interpolated with a horizontal-step function using only N-N intervals and resampled at 2 Hz. The detection of CVHR was performed by a cardiologist with arrhythmias expertise who was blind to both subject characteristics and other polysomnographic data. Duration of CVHR on tachograms was determined. CVHR patterns were characterized as high amplitude (HR changes > or = 20 beats/min per cycle) versus lower amplitude (6-19 beats/min per cycle). Tachograms were classified as having visible HR changes versus not visible (flat) as seen in Figure 1.

III. STATISTICAL ANALYSIS

Continuous variables are expressed as mean ± standard deviation or as median (with 25^{th}, 75^{th} percentiles), as appropriate. Categorical variables are presented as counts and percentages. Univariate comparisons were made with the χ^2 or the two-sample *t* test, as appropriate [31]. The correlation between total duration of CVHR episodes and the apnea-hypopnea index was assessed with the correlation coefficient analysis. The CVHR on Holter monitoring for the detection of OSA was determined using receiver operating characteristic (ROC) curves, assuming AHI $\geq$ 15 as references for significant OSA. We also described the likelihood ratios for OSA stratified by different ranks of CVHR duration [31]. All statistical analyses were performed using the MEDCALC statistical software version 7.3 *(MedCalc Software, Ostend, Belgium)* and SPSS v.22 *(IBM, Chicago IL, USA)*.

IV. RESULTS

Two hundred and eight patients were invited to participate in the study during one year of follow-up. One hundred and thirty-six consented. Three patients (2%) were excluded from our analysis. Two due to atrial fibrillation that makes impossible to measure the CVHR and one had less than 3 hours of Holter recording (figure 2).

The main characteristics of the patients are listed in Table 1. Two thirds were male. The OSA group had a body mass index (BMI) that was greater than the group without significant OSA (33.4±8.1 vs. 28.1±5.2; P<0.001). As it was expected the minimum oxygen saturation was lower in the OSA group (73±11.7 vs. 85.3±7.1; P<0.001). There was no significant difference in sleep duration, heart rate or autonomic function (SDNN).

There was a moderate correlation between the duration of the CVHR episodes and the AHI (r=0.50; P<0.0001; 95% CI for r=0.36 to 0.62) (Figure 3). Patients diagnosed as severe OSA had longer duration of CVHR measured by the Holter monitoring (Figure 4).

When groups were stratified by the duration of CVHR as shown in table 2, there was a much higher probability of having severe OSA when this duration exceeded 200 minutes of CVHR (+LR=16.28 for severe OSA; CVHR >100-199 min +LR=3.10 for severe OSA).

Additionally, considering a CVHR duration of more than 76 minutes as the diagnostic cutoff point for moderate to severe OSA, we observed that the area under the ROC curve was 0.77 (CI 95% AUR 0.69 to 0.84). These data evidence that CVHF as a good diagnostic discriminant for OSA (figure 5).

V. DISCUSSION

In this study, Holter ECG monitoring was tested for its ability to diagnose OSA and was simultaneously validated against PSG in patients with clinically suspected sleep disordered breathing. The results show that patients with severe OSA have longer duration of CVHR on Holter monitoring. Our results are slightly different to those reported by other authors in studies using similar or alternative methods [23-26].

In the mid 80´s *Guilleminault et al.* [23] were the first authors who have demonstrated the presence of CVHR in patients with OSA. At onset of a sleep apnea episode all OSA patients showed progressive bradycardia, followed by abrupt tachycardia on resumption of breathing. *Stein et al.* [24] used heart rate tachogram derived from ECG monitoring during overnight PSG to identify CVHR revealing OSA diagnosis. *Hayano et al.* [26] published an important study to examine the ability of ECG-based automated CVHR detection to estimate the presence and severity of OSA in a large-scale clinical setting. They have observed that the CVHR index closely correlated with the AHI (r=0.84) and showed a good performance in identifying patients with moderate-to-severe OSA.

In the last few years, several authors have developed algorithms for the automated ECG detection of OSA. *Khandoker et al.* [32] used a machine learning technique for automated recognition of OSA from wavelet analysis of R-R intervals and ECG-derived respiratory signal. They developed a classification algorithm using 83 training sets of sleep studies and applied it to 42 test studies selected from the Physionet Apnea-ECG database. The algorithm correctly recognized 24 of 26 OSA subjects and 15 of 16 non - OSA subjects. *Mendez et al.* [33] compared the empirical mode decomposition and the wavelet analysis for detecting OSA from the ECG signal. Using 25 training sets and 25 test sets of sleep studies generated from the Physionet Apnea-ECG database, they reported 85% accuracy for the empirical mode decomposition and 89% accuracy for wavelet analysis in classifying minute-by-minute apnea/non-apnea periods; both methods perfectly discriminated OSA patients from normal subjects. In the present study, we observed comparable performances for the independently optimized ACAT algorithm in the Physionet Apnea-ECG database. Only a few studies, however, have been performed in clinical settings. *Roche et al.* [34] proposed an increase in the relative power of very - low-frequency component (0.01 to 0.05 Hz) of interbeat interval increment (%VLFI) as a marker for OSA. Among a sample of 150 patients referred to a university hospital for clinically suspected OSA, the authors reported an AUC of 0.70 for identifying the patients with an AHI ≥15 per hour (n=100) and 64% sensitivity and 69% specificity with using %VLFI >4% as the cutoff threshold.

There is a need to improve access to diagnostic and effective treatment strategies for patients with OSA. Although portable monitoring may emerge as a new diagnostic tool, clinical studies to address careful interpretation are needed to continue to advance the field [35].

Patients with OSA are prone to the development of abrupt changes in heart rate. The mechanisms related to this phenomena are not fully understood, but changes in autonomic tone and the coexistence of common shared risk factors are the probable causes [23]. Additionally, the circadian distribution of sudden cardiac death among OSA patients has a peak during the sleeping hours [16]. Holter monitoring is a useful

tool to record such phenomena. There is some evidence suggesting that the occurrence of CVHR indicates the presence of apnea episodes in patients with OSA [24]. In our study we could better understand the association of the CVHR and the presence of severe OSA.

VI. STRENGTHS AND LIMITATIONS

The strengths of the present study include the use of the Holter equipment to demonstrate the presence of moderate to severe OSA. This can change the indications of ECG Holter monitoring.

Some limitations of the present study should be noted. First, subjects with atrial fibrillation or implanted pacemakers were excluded because we could not determine the CVHRS in these cases. Second, it is not possible to exclude the effect of periodic leg movements, which may affect CVHR. This is a specific population with high pretest probability of OSA. It is difficult to extrapolate this data to all patients.

6.1 Clinical Implications and Future Research Directions

The results of this study have potentially important clinical implications, because they suggest that many patients submitted to ECG Holter monitoring can be identified as having significant OAS and perhaps could be treated properly.

There is a need to test the sleep apnea software in different populations and perceive its clinical utility in these settings.

VII. CONCLUSION

The high prevalence of untreated sleep apnea and links to serious morbidity and mortality underscore the population burden of this condition and the need for greater clinical recognition and strategies to reduce prevalence.

Electrocardiographic Holter monitoring has good accuracy for the detection of significant OAS in a selected population and therefore can be considered as a valuable simplified technique.

Sources of support

This work was supported by Cardio Sistemas® *(Cardio Sistemas Comercial e Industrial LTDA, São Paulo, Brazil)*; *Scientific Initiation* Scholarships given by *FAPICC* (Support Fund of the *Instituto de Cardiologia/FUC*) and a research incentive fund from the National Counsel of Technological and Scientific Development *(Cn PQ)*.

Conflicts of interest: None

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Table 1: Clinical and demographic characteristics

Variables	All (n=133)	OSA (+) (n=40)	OSA (-) (n=93)	*P*-value
Age*	49±17	55±15	47±17	0.013
Male gender†	88 (66)	30 (75)	58 (62)	0.169
BMI*	29.7±6.7	33.4±8.1	28.1±5.2	<0.001
Epworth sleepiness scale*	10±5.4	13.4±4.3	8.6±4.6	<0.001
AHI‡	8 (1.5 – 21.8)	37.8±3.4	41.3±5.8	<0.001
Minimum O_2 Saturation during sleep	81.5±10.4	73±11.7	85.3±7.1	<0.001
Sleep Duration in minutes*	381.3±150	370.80±75	386.26±76	0.281
Heart Rate*				
Minimum	51.9±7.8	52±8	52±8	0.979
Mean	68.8±9.2	71±11	68±9	0.245
Maximum	111.4±19	111±17	112±20	0.861
SDNN*	90.5±37.8	91.1±21.1	90.3±29.6	0.859
CVHR ‡	21 (5.5 −62.5)	58	15	<0.001

*Mean and Standard Deviation (SD); †Data are n (%); ‡Median (25th to 75th percentile); OSA: Obstructive sleep apnea; BMI: body mass index; AHI: apnea - hypopnea index event/hour; SDNN: standard deviation of NN intervals on Holter; CVHR: cyclic variation of the heart rate

Table 2: Cyclic variation of the heart rate data

CVHR	OSA (+) (n=40)	OSA(–) (n=93)	L.R.
≥ 200	7	1	16.28
100 to 199	8	6	3.10
31 to 99	1 2	25	1.17
0 to 30	1 3	61	0.69

CVHR: cyclic variation of the heart rate in minutes; OSA: obstructive sleep apnea; L.R.: likelihood ratio

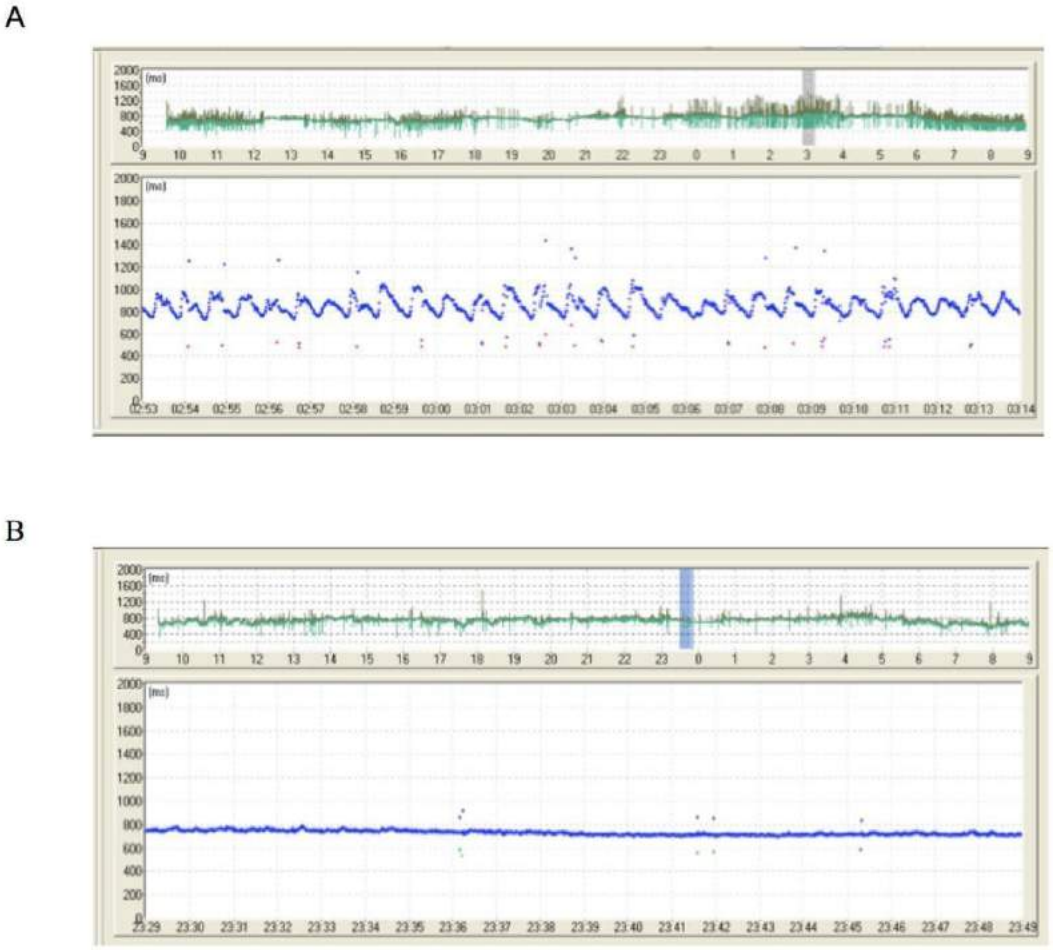

Figure 1: **A)** The Holter tachogram shows high amplitude CVHR during a severe sleep apnea episode. **B)** The Holter tachogram shows usual RR pattern in patient without sleep apnea

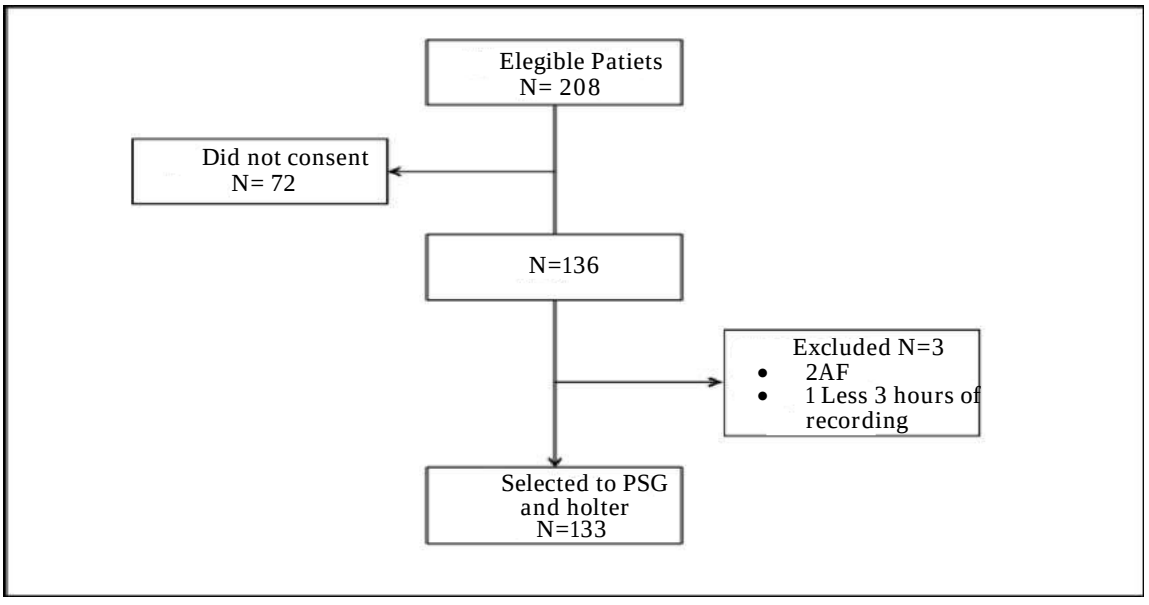

Figure 2: Flow diagram of patients included in present study

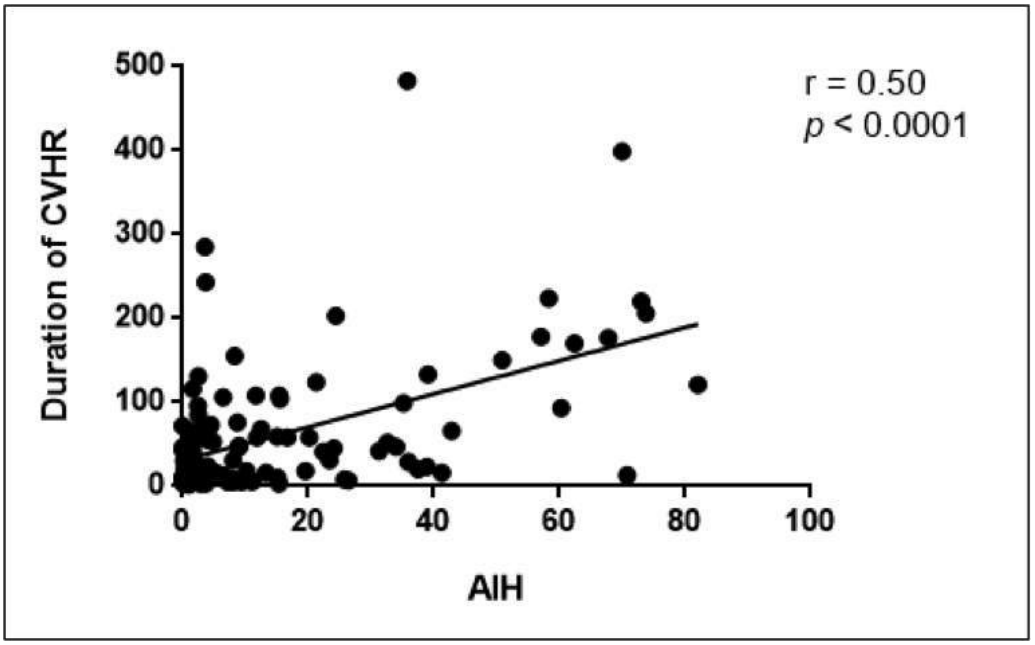

Figure 3: Relationship between the AHI and the duration of CVHR in minutes

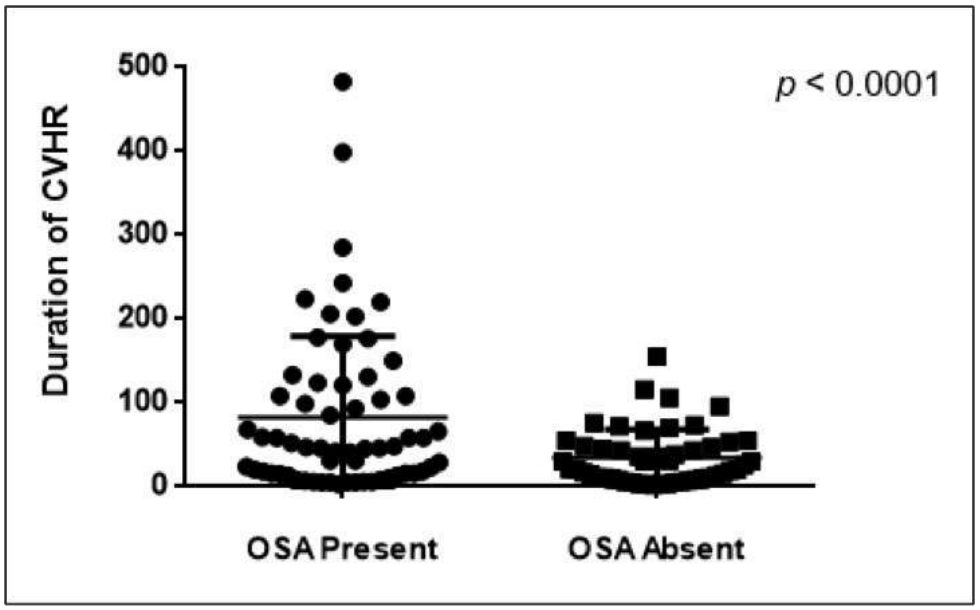

Figure 4: Duration of CVHR in minutes in patients with OSA present and OSA absent

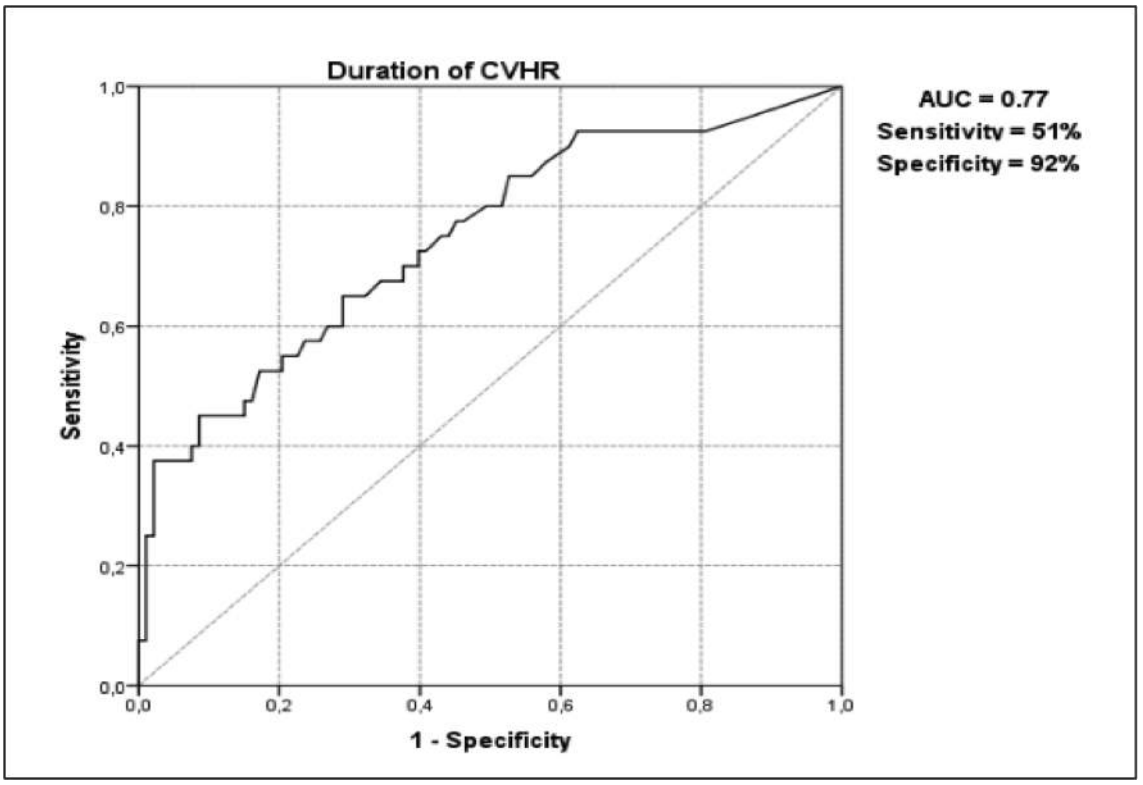

Figure 5: The ROC curve of the CVHR duration in minutes for detecting subjects with moderate-to-severe OSA had an AUC of 0.77 (standard error, 0.050). The ROC curve analysis also indicated that the highest average of sensitivity (51%) and specificity (92%) was obtained with a cutoff threshold of CVHR duration ≥ 76 minutes (95% CI=0.68 to 0.84)

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Normal Tissue Stroma Colonization and Stromatogenesis: Another Hallmark of Tumors

Michael Chukwudi Ezeani
Nnamdi Azikiwe University Awka

ABSTRACT

Understanding the biology of cancer is one major advanced achievement in approach for cancer remission. Although discovery of 10 hallmarks of cancer has played a beneficial role towards this, guiding the understanding of cancer development, metastasis and drive to rational drug design, hallmarks of cancer may not have ended. Besides, there is little or no due consideration to the substratum of cancer development. Sequel to some novel observations emanating from current understanding of cancer development, I reviewed the existing 10 hallmarks of cancer considering new biological trademarks that would augment further the understanding of tumor/cancer biology. I looked into the bedrock of tumor cells, considering that in line with the definition of current hallmarks of cancer, cancer cells may acquire all the functional capabilities to enforce its independent capacities and achieve autonomous potentials for proliferation, unending replication and subsequent metastasis, but without this adequate substratum obtained by colonization and stromatogenesis of the stroma, the development may not progress. I found that cancer development is highly based on the ability of the overwhelmingly mutated cells to colonize the normal tissue stroma in other to form their own stroma.

Keywords: tissue stroma, colonization, stroma- togenesis, hallmarks, cancer, tumor-+.

Classification: NLMC CODE: QZ 200

Language: English

Great Britain Journals Press

LJP Copyright ID: 392812

London Journal of Medical and Health Research

Volume 1 | Issue 1 | Compilation 1.0

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Understanding the biology of cancer is one major advanced achievement in approach for cancer remission. Although discovery of 10 hallmarks of cancer has played a beneficial role towards this, guiding the understanding of cancer development, metastasis and drive to rational drug design, hallmarks of cancer may not have ended. Besides, there is little or no due consideration to the substratum of cancer development. Sequel to some novel observations emanating from current understanding of cancer development, I reviewed the existing 10 hallmarks of cancer considering new biological trademarks that would augment further the understanding of tumor/cancer biology. I looked into the bedrock of tumor cells, considering that in line with the definition of current hallmarks of cancer, cancer cells may acquire all the functional capabilities to enforce its independent capacities and achieve autonomous potentials for proliferation, unending replication and subsequent metastasis, but without this adequate substratum obtained by colonization and stromatogenesis of the stroma, the development may not progress. I found that cancer development is highly based on the ability of the overwhelmingly mutated cells to colonize the normal tissue stroma in other to form their own stroma. I highlighted on the strong defensive and accommodating framework of the normal stroma and its originality in body defence and revealed the: strong dependency of the aggressive mutated cells on the stromal framework; ability of the mutated cells to reverse the stroma activities for their independent development and subsequent invasiveness and metastasis, without which tumor microenvironment may not be formed. I suggested that stromal colonization and stromatogenesis is an acquired functional capability from mutational changes or genetic alterations that induced abnormal and aggressive behavior on the transformed cells, aggressive enough to overwhelm and polarize the defensive functional nature of the stroma cells to suit its autonomous progressive tendencies.

Questionable Issues at the back of this Review

- *Considering the importance of tumour microenvironment, could establishment of tumour microenvironment be made possible without being aided and abetted by tissue stroma framework and the stroma cells sustainable activities?*
- *Hallmarks, in other words are trademarks of cancer cell which are inevitably identified with them. Considering the complexity of cancer cells and their biology of development, could there be further hallmarks of cancer in addition to the existing ones?*
- *Hallmarks of cancer has been described as fundamental functional capability of the cancer cells, can cancer cells gain these capabilities and utilize them without adequate tissue stroma as bedrock and engagement of stroma cells as aids?*
- *Research has shown that normal cells will not seed or survive an inappropriate micro-environment because they lack necessary cell autonomous survival signals. However, cancer cells undermine this local tissue hostility and evade anoikis during development and progression. The fact remains that this could be made possible because the tumor that could turn to cancer starts by interacting with the surrounding extracellular matrix (ECM), initiating a bidirectional relationship with its surrounding stroma.*
- *Why does it take accumulation but not singular cellular and gene mutational changes for cancer to develop in the*

London Journal of Medical and Health Research

surrounding tissues? Could it be that in the process of trying to colonize and exploit the stroma, it takes multiple or accumulated mutational changes to overwhelm the stroma due to its resistant nature and its functional ability to reestablish the homeostatic balance?.

Keywords: tissue stroma, colonization, stroma-togenesis, hallmarks, cancer, tumor-+.

Aurhor: Immunology Department, Faculty of Medicine Nnamdi Azikiwe University Awka Anambra State Nigeria.

I. INTRODUCTION

Sequel to the tremendous efforts and the huge success trailing the explorations in the field of cancer which have resulted to wider under-standing of its biology and developments, the hallmarks of cancer became manifest. The wonderful scientific work of Dauglas Hanahan and Bob Weinberg is highly appreciated in this direction. No doubt, the existing 10 hallmarks of cancer ealier proposed by [1, 2], currently are guiding the understanding of cancer development, metastasis and drive to rational drug design.

However, hallmarks of cancer may not have ended. Leaning on the advancements and clearer understanding of different aspects of carcinogenesis in the recent years, doors are still open in search of more features to aid understanding of the disease and enabling approach toward diagnosis, prevention and treatment. In line with this, this review was embarked on, to develop further ideas towards clearer understanding of cancer/tumor development, invasiness and metastasis.

Inevitably, more efforts are required in other to achieve remarkable progress in cancer research; couple with some novel observations emanating from current understanding of cancer development, research is stirred to consider new biological trademarks that would augment the understanding of tumor/cancer biology, thus, I looked into the framework or the bedrock of tumor cells, with much focus on the foundational ground of tumor development considering that cancer cells may acquire all the capabilities to enforce its independent capacities and achieve autonomous potentials for proliferation, unending replicative potentials and subsequent metastasis, but without this adequate substratum, the development may be stalled. Cancer establishment is like someone who wants to have a progressive sleep; in this case, it is important to first secure a bed space before bringing in mat or bed. Sequel to the aforementioned axiom, formation of a clinically significant tumor may not be possible, without first engaging or colonizing a formidable surrounding normal tissue stroma irrespective of other capabilities possessed by cancer/tumor cells. It was emphasized in [3] that appreciation of tumor stroma is essential to an understanding of the biology of tumor growth.

Besides, all solid tumors, regardless of their site of origin, require stroma if they are to grow beyond a minimal size of 1 to 2mm[4"] [3].

Hallmark of cancer was defined in [1], "as acquired functional capabilities that allow cancer cells to survive, proliferate and disseminate; these functions are acquired in different tumor types via distinct mechanisms and at various times during the course of multistep tumorigenesis" [2].

Subsequent to the initial six (6) hallmarks of cancer, many outstanding developments in cancer/tumor research have opened further observations, thus raising questions and ideas which encouraged revisit to the original hallmarks (The first Six Hallmarks) and ponder on others that are equally biologically involving. Therefore in 2011, Hanahan and Weinberg updated their hallmarks and came with four more hallmarks of cancer. This is evident that more hallmarks of cancer are inevitable and could continue to emanate considering the complexity of cancer.

Hanahan and Weinberg publications on Hallmark of Cancer were influential reviews and highly relevant in the field of oncology. They compressed the developmental and metastatic knowledge of cancer biology into six major hallmarks and subsequently ten hallmarks including: "self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless

replicative potential, sustained angiogenesis, tissue invasion and metastasis" [2]; two enabling traits: "genome instability and mutation, and tumor-promoting inflammation" [2] and two emerging hallmarks: "reprogramming energy metabolism and evading immune response" [2].

Their reviews have become the backbone of cancer biology and understanding of developmental and cancer metastatic traits.

However, this great achievement by these two prominent great researchers is but not without criticism and challenges. Example, in [4], it was argued that cancer is a sequalae to malignant tumors and a hallmark, being a distinguishing feature, may not be peculiar to cancer but could be used to desdcribe tumor as well [4]. They alluded this to the fact that five of the six initial hallmarks (all except invasion and metastasis) are inherent in both benign and malignant growths and thus shows no distinctive value over non-malignant tumors [4]. Besides, nonmalignant conditions, such as endometriosis, have been reported to have their cells migrate to extra-anatomical sites and invade new tissues, yet maintain benign histological appearance [5].

Attempt to draw a more organized and updated picture of the cancer hallmarks was made in [6], when they defined seven hallmarks of cancer including: "selective growth and proliferative advantage, altered stress response favoring overall survival, vascularization, invasion and metastasis, metabolic rewiring, an abetting micro-environment and immune modulation". Their principle was based on "evolutionary perspective on the mutation theory in which carcinogenesis is a dynamic process that might initiate (and terminate) within cells, life-spans, with manifesting cancer hallmarks emerging throughout such a journey" [6]. The above were dusts reaised due to emergence of the work of Hanahan and Weinberg. Having said these, I found solace in the hallmark definition by Hanahan and Weinberg because I believe that no emerging unique hallmark of cancer would evolve without crossing or overlapping with the principles of their work. Trying to create entirely new basic hallmark principle, may lead to repetitions. However, I strongly uphold that there may not be a limit to derivation of new hallmark(s) of tumor/cancer or reviewing the existing ones until the disease is suppressed to a mere physiological headache that can easily be removed with paracetamol. This is based on the complexity, twisted and high levels of molecular interactions involved in the disease complex and considering the fact that environmental influences (exogenous and endogenous) could continue to add in manipulations of the disease process with regards to its complexity and kinks. The interest in this review however is to propose new hallmark in expansion of our knowledge about cancer, in line with its complexity and evolutionary trends, then augment the preventive and diagnostic measures and process of therapeutic drives.

Before moving further, it is important to understand the principles at the back of the mind of Hanahan and Weinberg that spurred the creative review that has brought progress in the field of research today. First, they viewed cancer research development as a rational scientific study, where the disease complications, described in the laboratory and clinic, will become understandable in terms fundamental values.

They perceived these values as fundamental problems or changes that would in the first place induce or redirect normal cells transformation into cancer/tumor cells. Secondly, long termed research observations showed that virtually all types of human cancers have in common small number of molecular, biochemical, and cellular traits-acquired-capabilities [2]. They held strongly to an interpretation owing to the knowledge from the teachings of cell biology "that virtually all mammalian cells carry similar molecular machinery regulating their proliferation, differentiation, and death"[2]. Such similar molecular or cellular machineries constitute the bases of hallmarks of cancer. Thirdly: their reviews proved to them that the process of tumor/cancer development in human has multisteps echoing genetic mutations that initiate the progressive transformation of normal cells into highly malignant spinoffs [2]. Fourthly: "Many types of cancers are diagnosed in the human population with an age-dependent

incidence implicating four to seven rate-limiting stochastic events" [2]. Fifthly: "Pathological analyses of a number of organ sites reveal lesions that appear to represent the intermediate steps in a process through which cells evolve progressively from normalcy via a series of pre-malignant states into invasive cancers" [2]. "Moreover these observations were concretized in [7], with indications that the genomes of tumor cells are invariably altered at multiple site, having suffered disruption through lesions as subtle as point mutations and as obvious as changes in chromosome complement. With these at the back of Hanahan and Weinberg mind, it is evident that whatever could qualify as a hallmark of cancer, must be common traits in all cancers. Example, "virtually all mammalian cells carry similar molecular machinery regulating their proliferation, differentiation, and death" [1]. With regards to numerous distinct cancers and tumors that develop in a particular organ, sequel to abnormal cell regulatory circuits, the following questions also guarded their discovery of hallmarks of cancer: "How many distinct regulatory circuits within each type of target cell must be disrupted in order for such a cell to become cancerous"? "Does the same set of cellular regulatory circuits suffer disruption in the cells of the disparate neoplasms arising in the human body"? "Which of these circuits operate on a cell-autonomous basis, and which are coupled to the signals that cells receive from their surrounding microenvironment within a tissue"? "Can the large and diverse collection of cancer-associated genes be tied to the operations of a small group of regulatory circuits"? [1]. Based on the above considerations, it was suggested in [1] that the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth, resulting to the six hallmarks of cancer development. "Each of these physiologic changes and novel capabilities acquired during tumor development represent the successful breaching of an anticancer defense mechanism hardwired into cells and tissues" [2]. Proposal that these six capabilities are shared in common by most and perhaps all types of human tumor was made in [1].

Other distinct attributes of tumor cells, proposed to be functionally important for the development of cancer and considered to be emerging hallmarks of cancer where reported by [8, 9, 10] and they include (1) major reprogramming of cellular energy metabolism in order to support continuous cell growth and proliferation, replacing the metabolic program that operates in most normal tissues and fuels the physiological operations of the associated cells and (2) active evasion by cancer cells from attack and elimination by immune cells; this capability highlights the dichotomous roles of an immune system that both antagonizes and enhances tumor development and progression. It is therefore critically evident that considering the complexity of cancer biology and the quest to untwist the foldings, more attributes that could be used to understudy tumor towards cancer development and subsequent metastasis must have emerged, thus the essence of this review. It is under these guides and guise that I propose Tissue Stroma Colonization and Stromatogenesis as additional hallmarks of cancer.

We consider tumor/cancer cells to be highly trickish, smart, sensitive, and dynamic with pragmatic evolutionary tendencies. Research has shown that under normal circumstances, developmental and survival specificity exist in environmental niches, under the biological control of microenvironment [11], indicating that normal cells do not venture or thrive in another environment else, the cell will encounter the hostility of tissue in form of detachment-induced cell death (anoikis) in that environment [12]. This simply means that normal cells will not seed or survive an inappropriate microenvironment because they lack necessary cell autonomous survival signals [11]. However, cancer cells undermine this local tissue hostility and evade anoikis during development and progression. This could be made possible because the tumor emanating to cancer starts by interacting with the surrounding extracellular matrix (ECM) [13], initiating a bidirectional relationship with its surrounding stroma [14, 15]. The tumor cells finally carve a niche from the stroma, different from the well organized and physiologically

controlled normal tissue stroma. This is evident in reports from [16,15,17] emphasizing on initial engagement of the normal stroma by tumor cells, to enable further processes in development such as invasion and subsequent metastasis. This introduces the importance of cancer cells to first colonize and carve a niche in form of framework to avoid anoikis and to abet initiation and progression. This is referred to in this review as tissue stromal colonization. Some of the cellular activities recorded during this stromal colonization, sequel to creation of tumor microenvironment (stromatogenesis), to sustain tumor progression include modifications of the extracellular matrix composition, activation of fibroblasts, myoepithelial cells and the recruitment of pericytes or smooth muscle cells, immune and inflammatory cells as indicated in [18].

As stated in [2], tumors exhibit another dimension of complexity as they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the tumor microenvironment.

The importance of this characteristic was initially recognized as enabling characteristic to generate the hallmarks traits, but the importance and process of acquisition of the normal tissue stroma as framework or bed of cancer/tumour cells to form the tumor microenvironment (tumor stroma) was not recommended amongst the existing hallmarks of cancer. Engagement or colonization of the stroma as a bed rock of the functional cells (the parenchyma cells), is therefore proposed here as the chief of the acquired functional capabilities by cancer cells to abet initiation and progression. Besides, development of tumor microenvironment depends on the initial engagement of the normal stroma (without which development of tumor microenvoronment could be stalled) which is made up of original standard cells functional in stromal tissue and subsequent tumor microenvironment [18].

So it is suggested here that tumor cells first hijack or colonize and devastate the normal stroma to create tumor microenvironment. This unique dynamic environment called tumor micro-environment could not have emerged without tumor interaction with the host [19] basically the host stroma. The interaction with the host stroma could be orchestrated by accumulated cellular and gene mutational changes taking place in surrounding tissues. The importance of tissue stroma and subsequent tumor stroma is so intriguing for its colonization efforts by tumor/cancer cells to be ignored as a hallmark of cancer. The development between stroma tissue and tumor microenvironment (tumor stroma) is like engagement of farmland and various nutrients in farm land to develop a fertile garden.

The farm land is the stroma; the nutrients are the stroma cells while the garden is the tumor microenvironment. On the same farmland, the nutrients on the farmland could be used to create an orange orchard (the garden) thus tumor microenvironment is like a garden created out of a farmland and nourished by the nutrients of the farmland. This hypothesis is supported by the earlier pronouncement of [20], that “the constant bidirectional interaction of epithelial cancer cells with the surrounding microenvironment allows damaged stromal cells usage as a source of nutrients for cancer cells, maintains the stroma renewal thus resembling a wound that does not heal, and affects the characteristics of tumor mesenchymal stem/stromal cells (MSCs)”. The normal stroma is the farm land with nutrients (stroma cells) upon which tumor micro-environment (garden) was made. The garden could not have been created without the farmland as the framework. The farmland could be engaged for creation of different types of garden depending on the choice of cultivation. Tumors “contain a stromal compartment (the garden), which is composed of stem cells, tumor-associated fibroblasts (TAFs), endothelial cells, immune cells, adipocytes, cytokines, and various types of macromolecules comprising the extracellular matrix (ECM)” (the nutrients) [20]. The normal stroma environment has its normal function until it is engaged by the tumor cells which have suffered accumulated genetic and epigenetic mutations and during which certain levels of cells polarization ensue. It is at this juncture that

tumor microenvironment sets in and this is completely dependent on the fact that normal stromal tissue and the stroma cells are nutrient-like enough to form such a bed for tumorigenesis. The emphasis here is that colonization of normal tissue stroma is a prerequisite for tumorigenesis/carcinogenesis, thus a hallmark for tumorigenesis/carcinogenesis.

In line with this, it was reported in [21] that "the interaction between stromal cells and tumor cells (mutated cells) is known to play a major role in cancer growth and progression". The stromal cells have also been seen in bone marrow with special role in haematopoiesis and inflammations. In the skin, the growth factors released by the stromal cells mediate cell division [22], thus, enabling background regeration support of the epidermis from the bottom layer to replenish the sloughing cells at the top layer [22]. Moreover, the inflammatory role and mediation of cells accumulation to site of inflamed tissue as indicated in [22], is highly notable. Cancer/ tumour cells do not operate in isolation, so it is a foremost character or feature of cancer/tumour cells to engage the tissue stroma and the stromal cells for oncogenic process prior to cancer development. Therefore engagement or colonization of normal tissue stroma and stromatogenesis is strongly regarded here as a forgotten, ignored and initially or previously unrecognized hallmark of cancer.

I consider the use of stroma by tumor/cancer cells to form their bed, as the chief hallmarks or strong trademarks of tumors/cancer because without the rocks on its bed, the stream may not have any song or noise. Suffice it to say that cancer/tumour without stroma is like garden without land or stream without ground. By the definition of hallmarks of cancer according to [1], the ability to engage/colonize and devastate normal stroma is a strong acquired functional capability for initiation, progression and even metastasis of cancer and therefore should be regarded as a hallmark of cancer. The subtopics presented below are analyzed with the intention to expose the possible oncogenic potential factors as envisiaged in their normal physiological functions.

II. THE TISSUE STROMA

Even though tissue epithelium is made up of highly specialized cells that orchestrate specific activities, the proper development and function of the cells are not without contextual signals from the stroma [23, 24]. The stroma referred to as layer or bed, "is the part of a tissue or organ with a structural or connective role". As indicated in [25], it is made up of all the parts without specific functions of the organ - for example, connective tissue, blood vessels, nerves, ducts etc, while the other part, the parenchyma, consists of the cells that perform the function of the tissue or organ.

Furthermore, stroma is composed of stromal cells which may be indirectly involved in hematopoiesis, providing a microenvironment that influences the function and differentiation of hematopoeietic cells enabled by colony stimulating factors, which they generate [25]. Cell types that constitute the bone marrow stroma include: fibroblasts (reticular connective tissue) macrophages, adipocytes (fat cells) osteoblasts (synthesize bone) osteoclasts (resorb bone) endothelial cells, which form the sinusoids [26].

Crosstalk between the emanating tumor cells and the creation of tumor stroma, highly responsible for the progression of tumors and their metastasis has been increasingly unveiled [27, 28]. As such, a lot of importance has been attached to tissue stroma. Crosstalk between the host stroma involvement in carcinogenesis should be seen as a strong panacea or enabling link to important signaling pathways promoting growth, considering that cancer is a disease of signaling malfunction in which case signals to growth-inhibiting genes (tumor suppressor) are disabled [29] and rather abets the activation of a growth-promoting (oncogene) pathway by genetic mutation which could lead to novel therapeutic interventions targeting tumor stroma receptors [29]. "Stromal connective tissues are found in the stroma and belong to the group of connective tissue proper". "The function of connective tissue proper is to secure the parenchymal tissue, including blood vessels and nerves of the stroma, and to construct organs and spread mechanical tension to reduce localized stress" [30].

Furthermore, “stromal tissue is primarily made of extracellular matrix containing connective tissue cells and extracellular matrix is primarily composed of ground substance-a porous, hydrated gel, made mainly from proteoglycan aggregates - and connective tissue fibers” [30].

The stromal cells are endowed with the capacity of becoming the connective tissue cells of many organs of the body. Basically, in [31], normal stroma was reffered to as a “Native Micro-environment (NME) of live tissue and described it as a “mechano-physiological space provided to tissue cells, which in turn contribute to the overall appearance and function of the tissue”, indicating that “NME is rather specified on the basis of physical, physiological, metabolic and other functions of particular tissues or organs” [31]. It has been demonstrated in [32, 31] that, the bone microenvironment is necessary for normal growth of bone tissues while the heart microenvironment is essential for cardiomyocytes, other heart cells and blood vessels are to maintain the heart muscle kinetic functions [32, 31]. Normal stroma “ therefore plays vital roles in maintaining the integrity and functionality of tissues ranging from growth and static to kinetic activities, with an exception in regenerative microenvironment (RME), where a reprogrammed tissue growth is involved” [31]. This is evident to the capital reason why developing tumor cells colonize tissue stroma to enable progression. This is also evident to say that tumor stroma (tumor microenvironment) is not a normal physiological niche like the normal tissue stroma but a pathological niche influentially created by persistent genetic and epigenetic instabilities. The current dominant paradigm with the impression that multiple genetic and epigenetic “lesions provide both the impetus for and the Achilles heel of cancer development, might be inadequate to understand cancer as a disease process” [33], without the enabling framework, the tissue stroma. Therefore the undertakens between tumor cells and the tissue stroma needs to be well understood. In support of this, reports earlier described “Tumor microenvironment (TME) as an abnormal native physiological condition, where tumor cells and their associated stromal cells undergo uncontrolled growth, proliferation, migration, excessive deposition of certain extracellular proteins and other cancerous cellular activities that result in irregular ECM networks and tissue growth” [34, 2]. Therefore, I uphold here and in line with the report of [35], that the accumulated genetic alteration would lead to abnormal cell growth and subsequent malignant cells.

Persistence of these genetic changes consequently changes the normal defensive stucture of the host stroma compartment to enable a permissive and supportive environment for invasive tumor/cancer cells. This is the engagement and activation of the normal tissue stroma which could be regarded as the first line of the process after multiple genetic alterations and development of malignant cells. The evolutionary trend is established by accumulation of genetic and epigenetic irrationalities which impact negatively on their surrounding environment, recruiting and corrupting non-malignant cells in the area thus form what is known as a tumor microenvironment (TME) [36, 31, 37]. What forms or induces tumor microenvironment is naturally, normally and functionally in existence in the body, yet the body is devoid of tumour microenvironment until the normal and functional stroma is adversely engaged and devastated to create tumor microenvironment.

Suffice it to say that tumor microenvironment is an oncophysiological tissue space initiated by tumorigenesis with enabling framework the stroma. Tumor microenvronment is a trademark of both solid and liquid tumors, thus virtually all cancer development are initiated by engagement of normal tissue stroma and “require stroma for nutritional support and for the removal of waste products” [38]. “In the case of leukemias, blood plasma serves as stroma” [38], “although an additional stromal response, angiogenesis, may develop in the bone marrow” [39]. Tumors growing in the body cavities have plasma exudates (ascites) as their stroma [40]. Where solid tumors are involved, connective tissues, blood vessels, inflammatory cells, serve as stroma, interposing between the malignant cells and normal host tissues. In all tumors, stroma is largely a product or an averse donation by the host and is induced

as a result of tumor cell-host interactions, while in tumors of epithelial cell origin (carcinomas) a basement membrane is often interposed between the tumor cells and the stroma, but in other types of tumors, malignant cells directly abut on or intermingle with stromal elements" [41]. These reports backup my indications that where there is no capability for stroma colonization, no tumorigenesis or carcinogenesis.

Normal tissue stroma could also be referred to as source of maintenance of normal body homeostasis which alteration by tumor cells could be seen as a hallmark of cancer development. This could as well be regarded as alteration of normal body homeostasis to generate homeostatic instability. Therefore alterations of the normal tissue stroma would generate homeostatic instability that induces cell polarity to favor tumorigenesis and even cancer metastasis, which aligns with explanation on homeostasis in [42] as a general principle that safeguards the stability of natural and artificial systems, where stability is understood in its more classical sense of robustness against external perturbations.

2.1 Stromal cells and their Bionatural Potential Oncogenic Factors

Presention of stromal cells as essential component of the stromal scaffold of the bone marrow that provides physical and functional support during hematopoiesis was made in [43]. It was further indicated in [43], that stromal cells engrafted into immunodeficient non-obese diabetic severe combined immunodeficient (NOD-SCID) mice give rise to pericytes, myofibroblasts, bone marrow (BM) stromal cells, osteocytes, osteoblasts, and various endothelial cells. Stromal cells also reffered to as Mesenchymal Stromal cells (MSCs), is one of the most studied types of stromal cells as indicated by [44] and [45] and are further shown to be a heterogeneous population of adherent cells with fibroblast morphology that proliferate in vitro and can differentiate into osteoblasts, chondrocytes, and adipocytes [43].

MSC acronym used for Mesenchymal stromal cells here should not be confused with mesenchymal stem cells. To address this inconsistency between nomenclature and biologic properties, and to clarify the terminology report in [46], it is suggested that the fibroblast-like plastic-adherent cells, regardless of the tissue from which they are isolated, be termed multipotent mesenchymal stromal cells, while the term mesenchymal stem cells is used only for cells that meet specified stem cell criteria [46].

Researchers have defined mesenchymal stromal cells (MSCs) as multipotent cells that are able to adhere to plastic surfaces, have the capacity to differentiate into osteoblasts, chondrocytes or adipocytes in culture, and express the cell surface markers CD73, CD90, and CD105, but not any leukocyte markers [47, 48, 49], while conventional stem cell properties such as plastic adherence and the expression of CD44, CD90 and CD105 are unspecific for stem cells [47, 48, 49].

Moreover, both fibroblasts and MSCs have been shown to have multipotent and immuno-modulatory functions [50]. Comparative efforts to separate the two cells (MCSs and fibroblast) and also indicate similarities that colony-forming capacity and differentiation potential are specific important properties that discriminate MSCs from fibroblasts was made in [48]. Additionally, while both MSCs and fibroblasts occupy the stroma of many tissues, MSCs are capable of migrating through the body via blood vessels, whereas less evidence exists that fibroblasts migrate via circulation [51]. Relative to fibroblasts and other stromal cell types, the primary functions of MSCs are to regulate the immune response and to promote tissue regeneration and are also the sources of osteoblasts and chondrocytes in bones and joints respectively [52, 53]. In the cause of this review, the acronym MSC is used for mesenchymal stromal cells. The International Society for Cellular Therapy (ISCT) encourages that the scientific community or investigators must clearly define the more scientifically correct designation in their reports adopt this uniform nomenclature in all written and oral communications [54].

Research has indicated that mesenchymal stromal cells (MSCs) possess self-renewal capacity and multilineage differentiation potential, indicating

their prospects as cellular therapeutic agents for regenerative medicine [55]. These cells have also been shown to be suitable for use in clinical applications because of their various properties, such as: low immunogenicity, immunomodulatory effects [56]; and migration potential to sites of injury and regenerative potential [57]. The Mesenchyme which is the source of mesenchymal stromal cells is the first embryonic mesenchymal tissue to emerge, and it is produced from epithelial mesenchymal transmission (EMT) in epiblast cells [57]. It is an unspecialized cell which occurs during gastrulation which is the process in which the three primary germ layers, endoderm, mesoderm, and ectoderm develop during the embryonic development of an animal" [58]. "It is induced by the primitive streak through Wnt signaling, and produces endoderm and mesoderm from a transitory tissue called mesendoderm during the process of the gastrulation [58].

III. STROMA COLONIZATION AND STROMATOGENESIS

Ealier research has shown that cells in the nonmalignant stroma (normal tissue stroma) are usually in a quiescent state and maintain homeostasis in the ECM and epithelial compartment, in part by negatively regulating the proliferation, motility, and invasion of cells in the epithelial layer [49, 31]. As early as 1989, it was reported in [59] that the majority of neoplasms, as well as healthy viscera, are composed of the parenchyma and nourishing or supporting tissues, comprising the stroma [59]. It was indicated that tissue stroma generally do not take part in neoplastic transformation and are comparable to other components of the host which are not neoplastic, however, the tissue stroma is directed by the neoplasms, at least partially during the neoplastic disease process [59]. Sequel to this, I suggest that the tumor stroma and the accompanying cells could not have been contracted ex-vivo but from within the developmental niche. Therefore, I uphold that the molecular composition of the host stroma is different from that of the tumor stroma as reported by [60, 49]. However it is important to note that this difference could be as a result of the persistence or chronic inflammatory engagements on the host stroma which must have activated lots of molecular activities such as cytokines, chemokine and fibroblast to mediate cellular and molecular polarization in the host stroma, this polarization therefore, enables acquisition of protumor potentials by the cells and thus lose their host defensive potentials. This phenomenon is capable of imposing distinctive molecular expression status to tumor engaged stroma. This submeission, is in line with the emphasis laid by many research works in [61, 62, 60], that the MSCs and cancer associated fibroblasts (CAFs) localized in the tumor stroma have a different phenotype compared to MSCs and fibroblasts isolated from normal tissues, which may be as a result of the constant exposure of these cells to inflammatory and cancer cell-secreted cytokines inducing procancerous characteristics. Before now, transformation of the stroma to desmoplastic loosely vascularized dense connective tissue and the remodeling of the stromal extracellular matrix (ECM), inducing local cancer cell migration and metastasis have been indicated in [63, 64, 65 and 66]. Other reported gradual evolution process of the tumor microenvironment include: increased local vascular permeability; the extravasation of plasma and macromolecules, such as fibrinogen and plasminogen; the activation of coagulation mechanisms in the developing tumor microenvironment; the formation of fibrin gel deposits; the formation of a provisional stroma comprising cancer cells, fibroblasts, and immune cells; the initiation of angiogenesis in the provisional stroma; the degradation and replacement of the provisional stroma fibrin with highly vascularized granulation connective tissue as described in [63, 64, 65 and 66]. It has earlier been reported in [67, 68, and 20] that tumors are organ-like structures, composed of numerous cell types whose interactions are required to drive and promote their growth and metastasis.

To substantiate the claim on stromal colonization by transformed or highly mutated cells and strongly support its importance as a highly significant omen or event in tumorigenesis or

carcinogenesis, I lay credence to the reports from the work in [68], titled "changes in the tissue surrounding a growing tumor and the significance of the precancerous state". Here, the results of the experimental investigations on the titled above were summarized under the indication that the changes so frequently observed in tissues surrounding a growing tumor may be caused by different conditions: (1) the development and growth of a malignant tumor depends upon a local interaction between tumor cells and organ cells, when the cells of a normal organ are capable of inhibiting tumor growth, then an impairment of the normal state of the parenchymatous cells of this particular organ is essential" [68]. He posited that this precancerous state does not consist primarily of an inflammatory change in the adjacent connective tissue, as indicated in [67]. (2) in a degeneration of the parenchymatous cells of the host organ, if, in another instance, the cells of the normal organs are unable to inhibit the proliferation of the tumor cells, then no preparation of the cells of the organ is necessary for the tumor to grow, i.e., no precancerous state is needed to enable the tumor to grow. On the other hand, the proliferating tumor cells injure normal cells, either mechanically or chemically, producing a condition that appears on superficial examination like that described as the precancerous state. In reality, however, this condition is the resultant effect of the tumor growth and may be more correctly designated the postcancerous state. Still, of greater importance is the submission in [68] that the general condition of resistance or immunity to cancer growth exerts a greater influence on the organism of the animal than any of the local conditions described above.

The local resistance of a testicle to tumor growth in a generally susceptible animal may be overcome, but if an animal is made generally immune to the growth of cancer, neither the animal as a whole nor a single organ or tissue in it can be made susceptible to the growth of the tumor [68]. With this, it shows that the importance of tissue stromal colonization by cancer cells has been on debate long time ago.

My evocation on this is that looking at the Levin's submissions in [68] emanating from original research works performed to determine the actual event taking place at the incipient of cancer establishment on a tissue or organ, from the current perspective, this is turned to mean that inflammation does not just occur without irritants and as far as the defensive mechanism of the organ can surmount the irritants, the ensuing inflammation runs on acute state and resolves after the irritants have been removed. But if the host is short of defensive efforts and the irritants persist, it will eventually overwhelm the host. Now in the case of cancer cells, these cells experience multiple genetic and epigenetic alterations. This is in line with the report of [69], that advances in gene expression, genetic, and epigenetic profiling of stromal cells have improved our understanding of how mesenchymal-epithelial cell interactions may create a permissive microenvironment for malignancy and identified potential targets for cancer prevention and treatment including chemokine and cytokine networks. Furthermore, it was determined in [70], that extensive gene expression changes occur in all cell types during cancer progression and that a significant fraction of altered genes encode secreted proteins and receptors. Importtantly, it should be noted that despite the dramatic gene expression changes in all cell types, genetic alterations were detected only in cancer epithelial cells by [69], indicating that tumor only manifests when genetic alterations must have overwhelmed the stroma and the cells. Many works have emphasized that reciprocal interactions between responding normal cells, their mediators, structural components of extracellular matrix (ECM) and genetically altered neoplastic cells, regulate all aspects of tumorgenicity. Furthermore, CXCL14 and CXCL12 chemokines overexpressed in tumor myoepithelial cells and myofibroblasts respectively, bind to receptors on epithelial cells and enhance their proliferation, migration, and invasion [71, 72, 73, 18]. These informed the source of the initial changes in the stroma followed by the gradual overwhelming suppressive activities being mediated by the mutated transformed cells. Possibly, at the initial stage of the alterations, may be at the stage of only one mutation, the host immune defense continues to resist the neoantigens and at this stage, cancer

or tumor growth may not develop unless there are external influences against the tissue, like chemical toxins to suppress the tissue parenchymatous cells (considering levin's first submission). But as the cells continue to experience gene mutations, even the immune stability in the host could lose defense due to persistent generation of the neo antigens resulting from multiple and overwhelming gene alterations. Suffice it to say that it may not necessarily be that the host is originally immuno-compromised but that the surrounding cells have suffered numerous gene mutations that could resist the immune response. So when this happens, the transformed cancer cells can override the activities of the parenchymal cells without additional aid (as indicated in Levin's second submission). Yes, inflammation may not play a passive role at the stage when only one mutation has occurred instead it will play an acute tissue defensive role to protect the environment. But at the stage of multiple overwhelming mutations, the transformed cancer cells will injure the normal cells by biochemical secretions and damages thus suppressing the surrounding or connective tissue cells function, activating the usual quiescent cells and subsequent emanation of their phenotypes, attracting and polarizing immune cell and subsequent secretion of cytokines and chemokines such as cytokine release symdrome (CRS), as well as overwhelming level of immune complexes to mediate protumor effects. Suffice it to say that a lot of unnoticed or asymptomatic events have been going on in the cancer possessed tissue possibly years long before the tissue is overcomed by the cancer cells and exhibit symptoms. At the stage in which the cancer cells have overcome the tissue cells, Levin indicated that no precancerous state needs to be induced. In line with the submissions of Levin, inflammation may not induce or aid cancer growth when the cells have not suffered enough mutation to sustain chronic inflammation as against the claims in [67], but when the smoldering inflammation is sustained by multiple mutations to persist and develop into chronic inflammation, tumor growth may ensue if no counter measures are taken. So this is the initial step in cancer development meaning that it takes multiple genetic and epigenetic mutations to colonize the tissue stroma and redirect the formal homeostatic, tissue defensive nature of the stroma to favor tumor growth. By doing this, the cancer cells carve a niche from the stroma to avoid anoikis as stated above which thereafter known now as tumor stroma. It is not therefore scientifically and clinically proper to jump or ignore this initial capability of cancer cells, the basic effort by the cancer cells to initiate and progress amongst the halls of characteristics or trademarks of cancer, referred to as hallmarks of cancer. Meanwhile this understanding, may reveal the possibility of stopping cancer at the earliest stage of development but however demands for regular check up and adequate laboratory analysis to achieve this. Research has shown in [74] that most transformed cell lines are not able to survive after transplantation and are therefore considered to be cells continuously growing without enough tumorigenic potency (more acquired capabilities needed), even among highly carcinogenic cell lines, only a small subset harboring stem cell-like characteristics are able to initiate tumor growth *in vivo* [74]. This indicates that, either the cell line could not engage adequate stroma or that the host tissue stroma is immune-competent enough to resist the initiation of the cancer, which is reffered to as 'elimination' in tumor immune response interaction. In other words creation of tumor microenvironment is not innate or constitutive, but requires functional capability of the cancer development process to colonize and devastate the host stroma prior to progression.

It is obvious that the transformed (carcinogenic) cells, recruit nontumorigenic cells both locally from the neighboring tissues as well as from the circulation to enable the tumor micro-environment, sequel to this, it is understandable from the submissions in [75, 76], that through reciprocal cancer-stroma interactions, tumor microenvironment co-evolves to promote cancer progression through paracrine signaling and physical interactions. It is evident in [61, 60, 49], that when cancer develops, the stroma undergoes vast changes to become fibrotic and activated, the ECM becomes denser and more rigid, and is composed of alternative forms of connective

fibres, such as tenascin and fibronectin, which cancer cells can invade. Fibroblasts and MSCs change shape and expression profiles and become more proliferative and secrete higher levels of growth factors, cytokines, and chemokines. These changes or polarizations result to stromal phenotypic cells operating in the tumor stroma.

Evident is on the fact that the normal tissue cellular composition is the same with the tumor stroma cellular composition as indicated overleaf, but differ in molecular composition. It is also in evident [68], that the tumor microenvironment also contains cancer-associated fibroblasts (CAFs), endothelial cells [77, 78], immune cells [79, 80], adipocytes [81], cancer stem cells (CSC) that differentiate into metastatic epithelial cells [82, 83], mesenchymal stromal cells (MSCs) that can differentiate into fibroblasts and other types of cells representing mesenchymal lineages [84] and various types of extracellular matrix (ECM) proteins [68], needed for reciprocal messaging and the stimulation of tumor growth. Research has earlier shown in [75], that the stroma and stromal cells originating from MSCs, have been recognized as players in carcinogenesis, affecting tumor growth, development, and progression beginning at the early steps of tumorigenesis, influencing the construction of the micro-environment, epithelial mesenchymal transition, and metastasis, that is, functions that are essential for tumor maintenance and metastasis to other tissues [85, 86].

Tissue stroma is regarded in this study as a bed or farmland or ranch, framework, "a fundamental feature of the architecture and functional design of vertebrate animals is a stroma, composed of extracellular matrix and mesenchymal cells, which provides a structural scaffold and conduit for blood and lymphatic vessels, nerves, and leukocytes [87]. The stroma cells, embedded on the stroma include the mesenchymal cells and epithelial cells as mentioned above [87].

Colonization or engagement of these structural scaffold and conduit and its cellular components by the cancer cells initiates strotomagenesis (tumor stroma). Stromatogenesis therefore is the formation of new, specific type stroma at sites of active tumor cell invasion as an integral part of the invading process [88], just like a garden carved out from the farmland as described above.

During tumorigenesis, reciprocal changes in stromal fibroblasts and tumor cells induce changes to the neoplastic microenvironmental landscape [89]. In stromagenesis, both the complex network of bi-directional stromal fibroblastic signaling pathways and the stromal extracellular matrix are modified [89]. The parenchyma of a carcinoma is composed of those cells from either an epithelial lining membrane or a gland, which underwent malignant transformation. In contrast, the stroma of such a neoplasm, composed of the original stroma of the affected organ or of the newly initiated stroma of the tumor, is non-neoplastic in character [89].

This is supported by the findings in [90], that the stroma as colonized by the cancer cells has no mutational changes unlike the cancer cells which have suffered lots of mutational changes to enable transformation. It is also evident that the unmutated host tissue stroma was colonized by the mutated cancer cells to gain a soft landing, with emphasis on the different mutational status [90]. "Enzymatically it relates to the control of the neoplastic parenchyma to a greater or lesser degree. The stroma plays a definite role in different stages of tumor development beginning with secretion of angiogenetic factors and other starting tumor developments of which the progression of the tumor depends on the stromal reaction"[59].

The newly formed tumor stroma was mentioned by [88] as engaging a void space, i.e. at the free surfaces, whether internal or external (extramural stromatogenesis for tumors) and that the new stroma formation is generated and governed by the invading tumor cells with the tolerance and complicity of the adjacent activated fibroblasts [88]. The "seed and soil" hypothesis was presented more than a century ago by [91, 92], but until recently, we began to understand the complex crosstalk between the tumor cells ("the seeds") and the tumor-growing microenvironment ("the soil"). We opined that this soil occupies the farmland (the tissue stroma) which tumor cells

have smartly spotted, fertilized and colonized to form a bed for growth and progression. This is an indication that the tumor microenvironment (the soil emanated from the farmland (the stroma), both serving as base required for tumor growth, enabling framework for proper nurturing. Soil should not be mistaken as land, soil is rather embaded on the land and could be washed off by any enabling force such wind or flood while the land remains on point. Lands are different from soils, this while soils on lands may differ. By this illustration, tumour microenvironment (soil) could be separated from the stroma (land). The tumor growth or development is highly dependent on these compartments. This is sequel to the report that interactions between cancer cells and the stromal compartment have major impacts on cancer growth and progression [93]. Earlier, on this issue of seed and soil concept in [94], it was indicated that cancer cells, called seeds, survive in a highly complex microenvironment of the surrounding stroma, called soil. Describtion in [88] showed that the new stroma (tumor stroma) is neostroma and "that the neostroma is complementary to cancer cell metabolic activity, important for buffering of cancer cell waste products and for the prevention of cancer cell acidic death. Thus, cancer cells and neostroma should not be seen as a mixture of heterogeneous uncoordinated cells but rather as a unified morphologic and metabolic domain with a harmonious collaboration between aerobic (myofibroblasts, endothelial cells) and anaerobic compartments (cancer cells) as indicated in [88].

In line with these, it is hypothesized in this research that Cancer without stroma is like faetus without womb/placenta or chick embryo without egg sac. Tumor inducing genes are normally and functionally in existence in the body but the body is devoid of tumor until the genes are mutated and tumor evolutionary trend established. This means that tissue stroma is normally in existence with its normal physiological functions until it is colonized by transformed tumor cells and subsequently activated. Probably, these above avowals would drive home the importance of this forgotten and deemphasized hallmark of cancer being proposed in this article. This informs the reason why [95], indicated that "a tumor cannot develop without the parallel expansion of a tumor stroma". Meanwhile, one of the histological cornerstones of cancer development is the formation of a dense fibrotic stromal matrix comprising ECM and activated fibroblasts (myofibroblasts). In this last phase of stromal development, the granulation tissue transforms into desmoplastic dense connective tissue characterized by poor vascularization [95], additionally in [96], it was mentioned that the expansion of the tumor stroma with a proliferation of fibroblasts and dense deposition of ECM is termed a desmoplastic reaction, morphologically termed desmoplasia and initially intended for defense against tumor growth. This is secondary to malignant growth and can be separated from alveolar collapse, which shows neither activated fibroblasts nor the dense collagen/ECM [96, 97, 98]. On the other hand, data have shown that in established tumors, this processes are seen in several aspects of tumor progression, such as angiogenesis, migration, invasion, and metastasis [99]. It could be said that the activation of the stroma, desmoplasia, is an attempt by the tumor tissue to heal the injury produced by the infiltrative and destructive growth of cancer cells, indicating the invasive and malignant (colonization/stromatogenesis) charac-characteristics of the tumor. However, it has been suggested that the increased collagen synthesis in desmoplasia, together with myofibroblast-induced tissue retraction, may paradoxically constitute a protective mechanism with invasive character-istics [78].

Another important illustration is the similarity between stroma from wound and that from tumors. Though active angiogenesis and numerous proliferating fibroblasts secreting a complex ECM are seen in both stromas with ensuing background of fibrin deposition, the tumor stroma was notably referred to as activated or reactive stroma [3]. Evident of normal stroma colonization, indicating that "aggressive malignant cells are clever at exploiting the stromal environment, example, tumor cells can reside in the stroma and transform it" [100] and "modify the metabolism of resident cells" [101], thus

tumor yield a stroma that is permissive rather than defensive (Normal tissue stroma) [27].

Cancer target therapies would be more successful if the crosstalk between normal stroma and tumor are adequately deduced, in line with understanding cancer genetics per se [27]. The stroma is determinant for the tumor progression and therefore is an important therapeutic target [102], thus its colonization is an omitted chief hallmark of cancer.

IV. STROMA TISSUE/CELLS IN CANCER DEVELOPMENT

As stated above, Tumor or Cancer microenvironment is not simply made of self-sufficient neoplastic cells but also composed of fibroblasts, immune cells, endothelial cells, and specialized mesenchymal cells [27]. Originally, the cell types exhibit defensive function with their natural tumor-suppressive abilities and homeostatic responses in tissues before being unfortunately colonized, undergo alteration and transformed by the tumorigenesis or stromatogenesis and thus activated to support tumor growth and facilitate metastatic dissemination [27]. It should be noted as in [99, 49], that in tissue homeostasis, normal fibroblasts are in an inactive quiescent state, embedded within the fibrillar ECM primarily of collagen type 1, laminin, fibronectin, and proteoglucan and interact with their surroundings through cell receptors called integrins. “Fibroblasts become activated in wound healing and fibrosis”, to give rise to these cells called myofibroblast [103]. It has been indicated that myofibroblast differ morphologically and functionally from quiescent fibroblasts and on activation, these cells are capable of producing relevant signal mediators, such as growth factors, cytokines, chemokines and other immune modulators [104, 27]. But as soon as the wound healing is completed, most of these activated fibroblasts are removed from the granulation tissue by apoptosis [105, 106]. This is evident that recruited tumor fibroblasts are originally in isolation with tumor micro- environment and that tumor microenvironment forms only when the tumor engages the stromal tissue and activates the stromal cells. Sequel to this, cancer has been considered a wound, however reffered to a wound that never heals because the activated fibroblasts are not removed by apoptosis as in normal wound healing [107, 108], thus indicating that cancer is a continued or persistent lesion that should have been eliminated by the acute stage of inflammation but rather the inflammatory response was overwhelmed by more radically transformed cancer cells with features that mediate polarization of or retention of activated stroma fibroblast, thus giving rise to a phenotype called cancer associated fibroblast (CAF), secretion of cytokines and chemokines inducing cytokine release symdrome (CRS) and migration of immune modulators to maintain the wound like environment and thus enabling immune evasion. So instead of the wound healing, these cells are rather prominent contributors in carcinogenesis [109], engaging the original mesenchymal and epithelial reciprocal interactions in morphogenesis of tissues and organs [87]. This is a tumorigenic ability. “The spindle cells of the ‘neostroma’ (Tumor stroma) are intensely proliferating myofibroblasts, which are characterized by the frequent expression of α-smooth muscle actin and the particularly frequent expression of thymidine phosphorylase, PDGF-receptors and SPARC (secreted protein acidic rich in cysteine)” [88]. This also is a copy of normal tissue stromal fibroblast.

The original defensive role of normal stroma fibroblast has been accessed with different research approaches including creation of a microenvironment that shares some of the features of tumor-associated stroma such as: regenerating myealinated fibres (RMFs) overexpressing hepatocyte growth factor (HGF) or TGFβ, alone or together, were used to humanize cleared fat pads prior to the introduction of breast organoids as stated in [110]; allowed for only normal outgrowths, the growth factor enriched RMFs allowed for the rare promotion of ductal carcinoma *in situ* (DCIS)-like lesions, adenomas and poorly differentiated tumors from ostensibly normal organoids unlike non-immortalized, normal primary human mammary fibroblasts [110]; tumors were efficiently generated from tissue recombinants when genetically modified

organoids were co-mixed with immortalized fibroblasts with or without expression of HGF, showing that tumor development was rarely observed when organoids were implanted either alone or co-mixed with normal primary fibroblasts further demonstrating that human breast cancer formation, even in the presence of oncogene-driving mutations, requires activated stoma [111]. The above results further underscore the notion that "even in the presence of robust oncogene signaling, activation of the stromal environment is an important component for malignant transformation of human breast epithelium *in vivo"* [112]. These are substantial laboratory proven facts highlighting the importance of stroma and its colonization prior to tumorigenesis/carcinogenesis. "In tumors, activated fibroblasts are termed as peritumoral fibroblasts or carcinoma-associated fibroblasts (CAFs)". "Stromal changes at the invasion front include the appearance of CAFs. Carcinoma - associated fibroblasts, constitute a major portion of the reactive tumor stroma and play a crucial role in tumor progression" [27]. In response to tumor growth, [27] showed that fibroblasts are activated mainly by TGF-β, chemokines such as monocyte chemotactic protein 1 and ECM-degrading agents such as matrix metall-oproteinases (MMPs) [27]. "Cancer associated fibtroblasts (CAFs) promote malignant growth, angiogenesis, malignant invasion and progression including metastasis" [113, 114, 115]. The roles of CAFs and their potential as targets for cancer therapy have been studied in xenograft models, and evidence from translational studies has revealed a prognostic significance of CAFs in several carcinoma types [116]. The trans-differentiation of fibroblasts to CAFs has been reported in [27] to be driven to a great extent by cancer-derived cytokines such as transforming growth factor-β (TGF-β), during which the normal stroma must have been engaged by the cancer/tumor cells. Under normal conditions, this TGF-β acts as a tumor suppressor by inhibiting proliferation, as previous studies have shown in prostate epithelial cell lines [117]. However, TGF-β has been shown to assume a protumorigenic role as cancer progresses [117]. In a study using an orthotopic xenograft model to reconstruct human mammary gland, results indicated in [111, 112], that overexpressing TGF-β in mouse fibroblasts could induce the initiation of breast cancer from normal epithelial tissue.

TGF-beta causes cancer progression through paracrine and autocrine effects. Paracrine effects of TGF-beta implicate stimulation of angiogenesis, escape from immunosurveillance and recruitment of myofibroblasts, Autocrine effects of TGF-beta in cancer cells with a functional TGF-beta receptor complex may be caused by a convergence between TGF-beta signalling and beta-catenin or activating Ras mutations as shown in [118] where experimental and clinical observations also indicated that myofibroblasts produce pro-invasive signals and that such signals may also be implicated in cancer pain [118]. Similarly, N-Cadherin which is expressed in invasive cancer cells and in host cells such as myofibroblasts, neurons, smooth muscle cells, and endothelial cells, act as invasion-promoters, this has been shown in [118] to promote matrix invasion, perineural invasion, muscular invasion, and transendothelial migration due to N-Cadherin-dependent heterotypic contacts.

The foggy controversial role of normal fibroblasts in promoting cancer, has been made clearer by the studies in [119], which provided evidence that both normal and activated cancer-associated fibroblasts (CAFs) can promote breast cancer cell growth in vitro and in animal model systems, based on constitutively secreted cytokines, such as CCL7, IL-6, and IL-8, that can activate the release of platelet-derived growth factor BB (PDGF-BB) from breast cancer cells that stimulates release of IL1-β by the fibroblasts and in turn induces breast cancer cell proliferation [119]. Interestingly, in another study, fibroblast-derived CCL2 was shown in [120] to play a key role in promoting Breast cancer stem cells (bCSC), self-renewal and tumorigenesis in a Notch1-dependent manner both in vitro and in vivo. Moreover, CAF-secreted prostaglandins have been shown to promote secretion of IL-6 that results in bCSC expansion [121, 122]. Additionally, senescent primary normal breast luminal cells activate breast stromal fibroblasts in an IL-8-STAT3 pathway-

dependent manner [123]. These activated fibroblasts displayed pro-carcinogenic features and promote a CSC-like phenotype by increasing expression of stem cell markers, such as CD44, ALDH, SOX2, OCT4, NANOG, and KLF4 and also induced epithelial mesenchymal transition (EMT) in breast cancer cells both in vitro and in vivo [123]. "Earlier study in [124], demonstrated that Sonic Hedgehog ligand secreted by TNBC cells confers and activates normal stromal fibroblasts.

These activated fibroblasts in turn secreted FGF5 and produce fibrillar collagen-rich ECM essential for maintenance of the CSC phenotype and development of chemoresistance [124]. Another study in [125] showed that breast cancer cells activate fibroblasts and induced secretion of chemokine ligand 2 (CCL2). These wide research largely elicit and implicate stromal fibroblasts activities especially their activated derivatives in stromatogenesis and tumorigenesis/carcinogenesis.

As earlier stated, the working model in the field (microenvironment) is that typically when we think about the primary tumor, the normal stroma being engaged or under devastation is primarily preventing tumor growth. This is both through normal fibroblasts, as well as through the immune system, which surveys our body to prevent the outgrowth of abnormal cells. But importantly, as indicated by [61], understanding the cross-talk between the host stroma, the stromal cells and tumor cells is highly inevitable in the quest to appreciate process of tumor growth and progression. This is because as the situation persists and progress, many of these anti-tumor components are lost owing to polarization due to chronic inflammation being fueled by persistent cytokines and chemokine activities as indicated in [61, 60]. This may possibly include accumulated immune complexes. Indicating that, there are many pro tumor components actually suppressing the activities of normal tissue cells in the in-situ formed tumor microenvironment. Due to their phenotype and functional properties, cells with clonal tumor-initiating capacity are called cancer stem cells (CSCs) or tumor-initiating cells (TICs) in [126]. The CSCs/TICs reside in specific niches in the tumor microenvironment that maintain their plasticity, protect them from immune defense mechanisms, and modify their metastatic potential [126]. In [127] MSCs was shown to interact with cancer stem cells (CSCs) or tumor-initiating cells (TICs), supporting several activities such as parenchymal cell growth and increased resistance to therapy; cancer cell dormancy and evasion from the immune system [128]. These were made possible either through paracrine secretion in [126, 129], or gap junction contact [127]. Alternatively, MSCs can affect epithelial cancer cell function by direct contact, causing increased expression of microRNAs, such as mir199a and stem cell-associated factors in the epithelial cells [130]. Mesenchymal stem cells secrete various growth-supporting cytokines, growth factors, and microRNAs that in some cases are stored inside extracellular vesicular particles (exosomes) as indicated in [131]. Exosomes are small (40–100 nm in diameter) membrane-bound organelles that function as part of an intercellular communication mechanism and characteristically include various types of molecules, including: matrix metalloproteases [132]; molecules that activate signal transduction [133], oncomiRs, bioactive lipids, and metabolites [134]. It was demonstrated in [132] that during tumorigenesis, exosome-bound factors modify the phenotype of the epithelial cancer cells or tumor stromal cells to support the aggressive phenotype and tumor progression.

Tumor stromal production exhibits similar qualities as normal wound repair such as new blood vessel formation, immune cell and fibroblast infiltration, and considerable remodeling of the extracellular matrix as indicated in [135]. Additionally, it was further stated that the recruitment of local normal host stromal cells, such as bone marrow mesenchymal stromal cells, endothelial cells, and adipocytes, help create a conspicuously heterogeneous composition and that these cells secrete an abundance of factors that help regulate tumor development [135]. Potential targets for tumor-associated stromal cell recruitment have been identified in [135] to include the following host tissue: bone marrow, connective tissue, adipose tissue, and blood vessels. Moreover,

evidence suggests that tumor-associated stroma is a prerequisite for metastasis and tumor cell invasion. These are known to arise from at least six different origins: immune cells, macrophages, adipocytes, fibroblasts, pericytes, and bone marrow mesenchymal stromal cells [135]. These are cells originally meant to defend the host tisues.

During the early stages of tumor development and invasion, the basement membrane is degraded, and the activated or reactive stroma, containing fibroblasts, inflammatory infiltrates, and newly formed capillaries, comes into direct contact with the tumor cells [136]. With this development, the newly formed stroma (activated stroma) is lodged between tissue planes of little or no resistance, thus, disrupts the continuity of normal structures and cleaving paths for the invading tumor cells–intramural stromatogenesis for endophytic tumors, with the basement membrane matrix modifying cytokine interactions between cancer cells and fibroblasts [136]. As indicated in [137], these cancer induced alterations in the stroma, will contribute to cancer invasion. In animal studies, it has been demonstrated in [27] that both wounding and activated stroma provides oncogenic signals to facilitate tumorigenesis. This is evidenced in [138] which reported that although normal stroma in most organs contains a minimal number of fibroblasts in association with physiologic ECM, the activated stroma is associated with more ECM-producing fibroblasts, enhanced vascularity, and increased ECM production. It is important to note that certain types of skin cancers (basal cell carcinomas) cannot spread throughout the body because the cancer cells require nearby stromal cells to continue their division, besides, the loss of these stromal growth factors when the cancer moves throughout the body prevents the cancer from invading other organs [138]. Stroma is made up of the non-malignant cells, but can provide an extracellular matrix on which tumor cells can grow and stromal cells may also limit T-cell proliferation via nitric oxide production, hindering immune capability [138].

V. CONCLUSION

This work succeeded in showcasing the importance of stroma in health, tumorigenesis and in cancer development and progression.

Indispensability of the stroma in nurturing epithelial tissue by effective functions of the tissue stromal cells to maintain tissue homeostatic balance was highlited. Normally, the molecular interation between epithelial cells and other cells in stromal compactment are physiologically made to be intact. So any alteration as a result of cellular perturbation would disrupt the molecular interaction and thus obstruct the whole system.

The intact of the stromal compartment, in fact is a strong hold for the whole body functional system, with stromal cells possessing anti-tumor effects.

Despite all these homeostatic and defensive integrity of the stroma and the cells, over-whelming mutational load induce changes and thus promoting neoplasia and dysplassia and subsequently cancer and metastasis. This indicates that the original natural features of tissue stroma, is protumor only by expression of loss of function and gain of function to promote tumor/cancer. This work indicated that the tumor/cancer cells being an entity, desiring autonomous growth and entire developmental pathway, sees tissue stroma as a refuge, framework and highly patho-physiologically dependable for initiation, progression and metastasis and insists on its colonization as a fertile farmland to grow on. From all indications, cancer development cannot hold without colonizing or engaging the tissue stroma.

Evidences were shown that creation of tumor stroma is not part of human development but after effect of multiple or accumulated genetic and epigenetic mutations in epithelial cells, thus stroma overwhelmed by tumorigenesis (tumor microenvironment) emanate due to genetic changes that ushered in numerous neo-antigens or foreignness into the surrounding tissues thus inducing pathophysiological activities that could overwhelm the defensive function of the stromal system and shift the functions to aid tumorigenesis. This development could be

redressed if the causal effects are resolved by the defensive and physiological/DNA repair arsenals of the body.

This work indicated that the stroma and the stromal cells are like farmland and the soil nutrients respectively. The accumulated alterations in cancer cells mediate functional changes on a framework or scaffold (the stroma) that serves for healthy physiological maintenance. This is the first beneficial thing for cancer development and/or progression, to initiate dependable bedrock and being more aggressive, hijack the multifunctional roles of the stromal cells (the nutrients) for its developmental process.

Hence it is here indicated that tumor micro-environment is like a garden or orchard formed, carved out from a farmland (stroma) and uses the farmland nutrients to grow its crops. This is what I referred to as stromal colonization and stromatogenesis for cancer progression.

I uphold the existing 10 hallmarks of cancer by [1, 2] and I key in with their definition of hallmark, thus I maintain that stromal colonization and stromatogenesis is an acquired functional capability from mutational changes or genetic alterations that induced abnormal and aggressive behavior on the transformed cells, aggressive enough to overwhelm and polarize the resisting or defensive functional nature of the stroma cells to suit its autonomous progressive tendencies.

Hence the stromal and immune cells polarization, emanate phenotypic cells types with reversed cell functions. It is important to note that prior to the clearly established pathological, radiologic and diagnostic observations, persistent inflammation en route chronic inflammation existed, indicating a prolonged engagement of the stromal tissue by the minor genetic and epigenetic cells alteration en route multiple and accumulated genetic and epigenetic cell damages which induced chronic inflammation and colonization of the tissue stroma. The enomous role of persistent immune complexes resulting from endogenous danger molecules in mediating this chronic inflammation, have earlier been indicated in [139]. My understanding of cancer development is that the development is neither automatic nor acute but trails prolonged battles with mutational developments in the surrounding tissues. In context of this study therefore, tumor microenvironment is defined as an acquired developmental niche carved out from normal tissue stroma by severely altered tumor cells due to accumulated cell mutation and epimutational alterations and colonize the surrounding environment in other to survive and metastasize.

So, it is suggested here that acquisition of a base like scaffold or framework such as stroma is an important developmental phenomenonal characteristic of cancer initiation and metastasis. Tumor microenvironment is to tumor as ranch is to cattle or garden to a plant. Many authors have enphasized on the utmost importance of the stroma dating as far back as 1889 by Paget, 1863, by Virchow, 1913, by Levin. It is important to honour these men posthumously by adopting tissue stroma colonization and stromatization as one of the trademarks of tumor/cancer.

List of abbreviations

ECM----	Extracellular matrix
MSCs----	Mesenchymal stem/stromal cells
TAFs ----	Tumor-associated fibroblasts
NME----	Native Microenvironment
RME----	Regenerative microenvironment
TME----	Tumor microenvironment
NOD-SCID	Non - obese diabetic severe combined immunodeficient
ISCT-	International Society for Cellular Therapy
CAFs----	Cancer associated fibroblasts
CRS----	Cytokine release syndrome
SPARC--	Secreted protein acidic rich in cysteine
PDGF----	Platelet derived growth factor
RMFs----	Regenerating myealinated fibres
HGF----	Hepatocyte growth factor
TGFβ----	Tumor growth factor beta
DCIS----	Ductal carcinoma *in situ*
MMPs----	Matrix metalloproteinases
PDGF-BB--	Platelet-derived growth factor BB
bCSC----	Breast cancer stem cells
ALDH----	Aldehyde Dehydrogenase
SOX2----	Sex determining region Y Box 2

OCT4----	Octamer-binding transcription factor4
NANOG	homebox protein Nanog
KLF4----	krupel like factor 4
EMT----	epithelial mesenchymal transition
TNBC----	Triple negative breast cancer
CCL2----	Chemokine ligand 2
FGF5----	Fibroblast growth factor 5
TICs----	Tumor-initiating cells

ACKNOWLEDGEMENTS

I thank Professors George Demetri, Ed Harlow and Peter Howley, Directors of High Impact Cancer Research at Harvard Medical School for their kind consideration that admitted me into the program that generated this review. I also appreciate Professor Romee Rizwan for the critical review of this article.

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Skin Rash May be a Symptom of COVID-19 Infection: A Case Report and Further Review of Literature

Dr. Pradip Kumar Das & Dr. Eshita Das

ABSTRACT

During the pandemic of SARS-CoV-2 infection, the sudden appearance of skin rash in a patient with no other underlying cause to explain its clinical manifestation can invite the attention to the physicians to think about a possible coronavirus infection. The possibility of a skin rash being an early sign of an underlying SARS-CoV-2 infection should be considered in the context of the patient's epidemiological risk profile and the local epidemiological situation. It is relevant to co-relate that skin rashes with itching are now reported as rare signs of the coronavirus disease. Further study is awaited to augment current knowledge about epidemiology, clinical presentation, and pathogenesis of COVID-19 skin manifestations. In this backdrop, it is needed to formulate for the case of sudden appearance of an isolated skin rash can justify the SARS-CoV-2 infection especially in daily clinical practices and can indicate of home isolation and/or further testing to identify the presence or not of SARS-CoV-2 infection.

Keywords: SARS-Cov-2- severe acute respiratory distress syndrome caused by Covid-19 virus, Covid-19-novel coronavirus infection started in december 2019 at wuhan of china.

Classification: NLMC CODE: WR 140

Language: English

Great Britain Journals Press

LJP Copyright ID: 392813

London Journal of Medical and Health Research

Volume 1 | Issue 1 | Compilation 1.0

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During the pandemic of SARS-CoV-2 infection, the sudden appearance of skin rash in a patient with no other underlying cause to explain its clinical manifestation can invite the attention to the physicians to think about a possible coronavirus infection. The possibility of a skin rash being an early sign of an underlying SARS-CoV-2 infection should be considered in the context of the patient's epidemiological risk profile and the local epidemiological situation. It is relevant to co-relate that skin rashes with itching are now reported as rare signs of the coronavirus disease. Further study is awaited to augment current knowledge about epidemiology, clinical presentation, and pathogenesis of COVID-19 skin manifestations. In this backdrop, it is needed to formulate for the case of sudden appearance of an isolated skin rash can justify the SARS-CoV-2 infection especially in daily clinical practices and can indicate of home isolation and/or further testing to identify the presence or not of SARS-CoV-2infection.

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Author α: MBBS, DTM & H, Ph.D, MBA, D.Lit.
σ: MBBS, MD (PGT).

I. INTRODUCTION

Common manifestations of Covid-19 infection are fever, dry cough and shortness of breath, body ache, weakness, chest pain, loose motions, loss of taste in the tongue and loss of smell in the nose since the Covid-19 infection was declared a pandemic by the World Health Organization in March 2020, it has been more or less established in recognizing the symptoms of this viral infection. But with the advancement of study works in this field, more information about Covid-19 has come to light, new symptoms have been marked as being associated with the disease and are now helping healthcare professionals to diagnose and treat this disease in a congenial way. It has been shown by a study at King's College London and Zoe Global Ltd shows that skin rashes might be an additional symptoms and are a valuable indicator of Covid-19 infection in untested patients showing just one other classic symptom of the disease. It has been evident from a study in the *Journal of the American Academy of Dermatology* in March revealed that a COVID-19 positive patient presented with a skin rash similar to ones that appear in dengue[7, 8]. It is also established from another study from the Cleveland Clinic done in April 2020 that 20 percent of their COVID-19 patients had presented rashes of different varieties, including patchy red rashes, hives and chickenpox-like blisters in their trunk area[3]. Doctors in Thailand also observed dengue-like rashes on positive patients, while those in other countries also observed mottling.

II. CASE DESCRIPTION & DISCUSSION

A 30-year-old obese lady, residing at Panchanantala, Konnagar, Hooghly of State of West Bengal under the country of India, working as a Branch manager of a centralized bank of Paschim Midnapore District under the State of West Bengal of India presented in the private clinic on 13th May 2021 with one day history of headache but no fever with pain in the throat followed by loss of smell in the nose and loss of taste in the tongue [1, 2]. After that appearance of dot like red spots in the thighs, legs, lower abdomen and in infra-mammary regions with

dot like red spots in the thighs, legs, lower abdomen and in infra-mammary regions with itching persisting for 2-3 days then disapeared[3]. The patient had no symptoms of fever, cough, shortness of breath, or general malaise. In her personal history, she did not declare any travel abroad in the last 15 days. Due to the second wave infections highly prevailed in the state of West Bengal, swab tests for SARS-CoV-2 were recommended along with routine blood tests and chest x-ray etc. RT-PCR test was shown positive result. On her physical examination, it was noted normal Blood Pressure (130/94 mm of Hg), Pulse rate (77/m) and normal temperature (98.4Degree Farenheight), normal respiration rate (18/m). Body weight-124 kg, markedly obese. On her GI. CVS, Respiratory and Nervous system examination, did not show any abnormality or any tenderness or enlargement of the submandibular, preauricular, or cervical lymph nodes. The patient declared that she used personal protective equipment during close contact with customers while she was on duty works in bank. Her chest computed tomography and chest X-ray showed no significant parenchymal lesion in the lungs, both hila were normal, cardiac shadow was within normal, diaphragm & angles were normal. The routine blood examination showed levels of fasting glucose (112 mg/dl), C-reactive protein (09.00 mg/L), AST (26 U/L), ALT (32 U/L), LDH (194 U/L), and lymphocytes % (25%). She was started on taking systemic Ivermectine 12 mg and Azythromycin (500 mg) OD for 5 days, Levocetzine 5 mg daily for 5 days and Calamine Lotion thrice daily and instructed to self-quarantine until the complete resolution of the infection. Because of the infectious nature of COVID-19, she maintained quarantine protocols as per Government instructions.

Differential Diagnosis

Scabies, a skin infestation caused by the mite *Sarcoptes scabiei*, is a frequent cause of severe pruritus. Here cause of pruritus is due to a delayed hypersensitivity reaction to the mite proteins[10]. But in this case the patient gave no history of contracting scabies or the skin burrows could not be elicited in her skin folded areas. Thus scabies can be opted from the diagnosis. Another common cause of itching is an adverse drug reaction. It is fact that Drug-induced skin reactions may be a trouble making task to differentiate from patients of COVID-19 with skin manifestations. It is also true that, so many medicines are being used to treat COVID-19 in both hospital setup and private chambers, and many of them may be the culprit to cause itching with skin rashes[9]. So it is very difficult to identify the underlying causes between COVID-19 infection and skin rashes when managing with patients who have received these medications. In this case, an adverse drug reaction can be excluded on the fact that the patient did not consume a COVID-19–specific drug treatment. Moreover, her skin rash was the first manifestation of the disease and the patient had no history of recent or chronic drug intake. Other common causes of skin rashes with itching could be excluded by absence of history of no use of cosmetics and soaps, living in unhealthy environments or absence of chronic physical or mental stress. Patient had also no personal or family history of autoimmune illness, atopy[7].

III. CONCLUSION

In light of present studies, this case report suggests that skin manifestations, in the backdrop of consideration with other situational factors such as her profession and personal history should be taken as possible cause of SARS-CoV-2. Thus present study envisages the primary care case report of a female patient who presented with skin rash as the main clinical presentation of COVID-19. Early detection of COVID-19 is a main criteria of case identification and case isolation. To augment this activity, further study is needed to establish symptoms and signs, pathogenesis of skin manifestations in patients with COVID-19[8].

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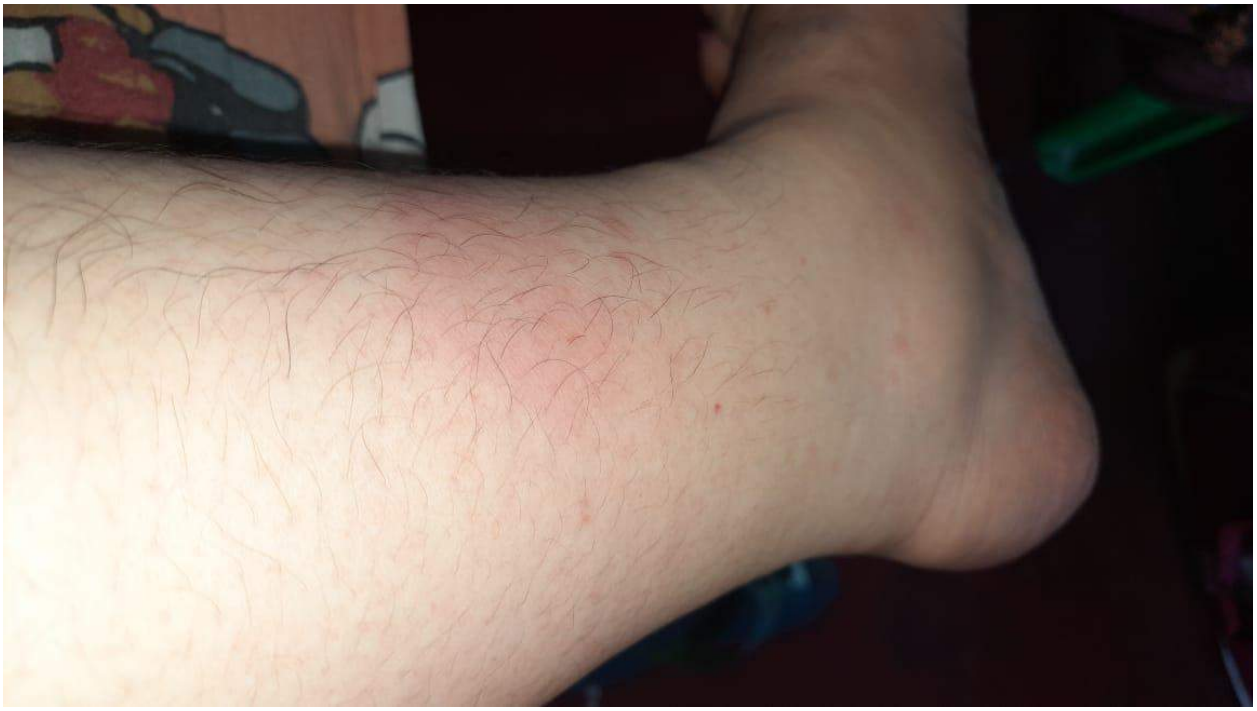
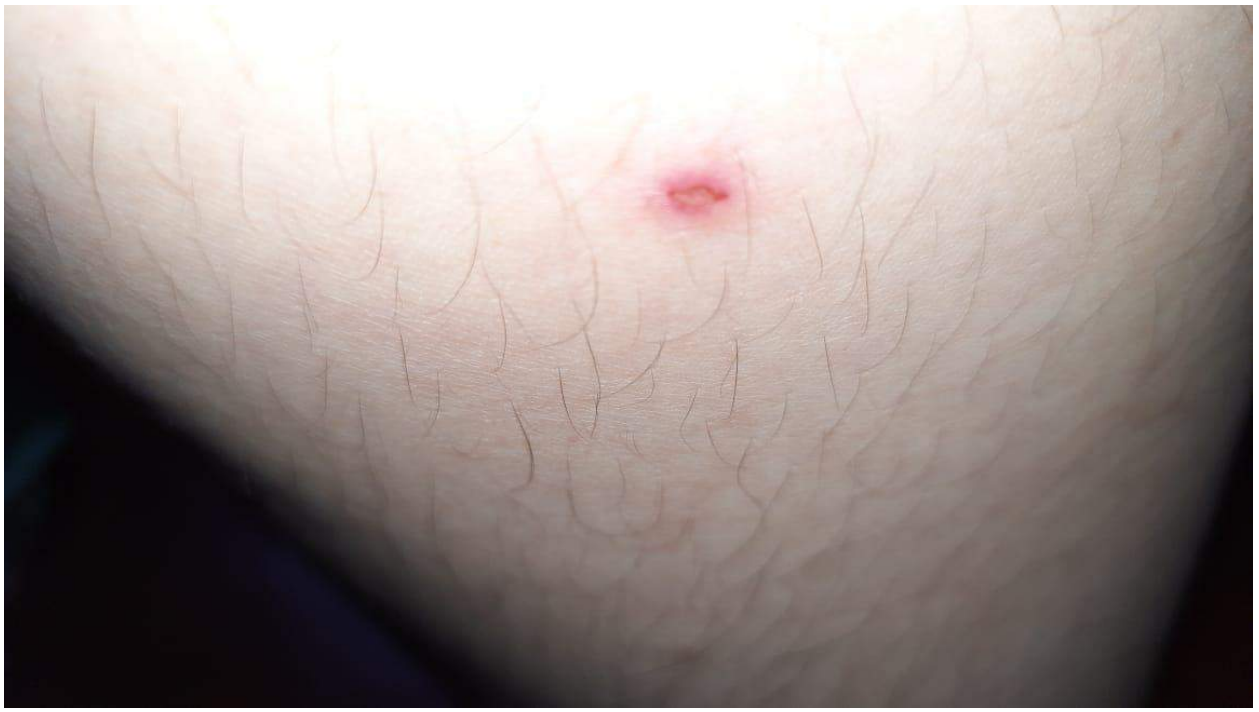
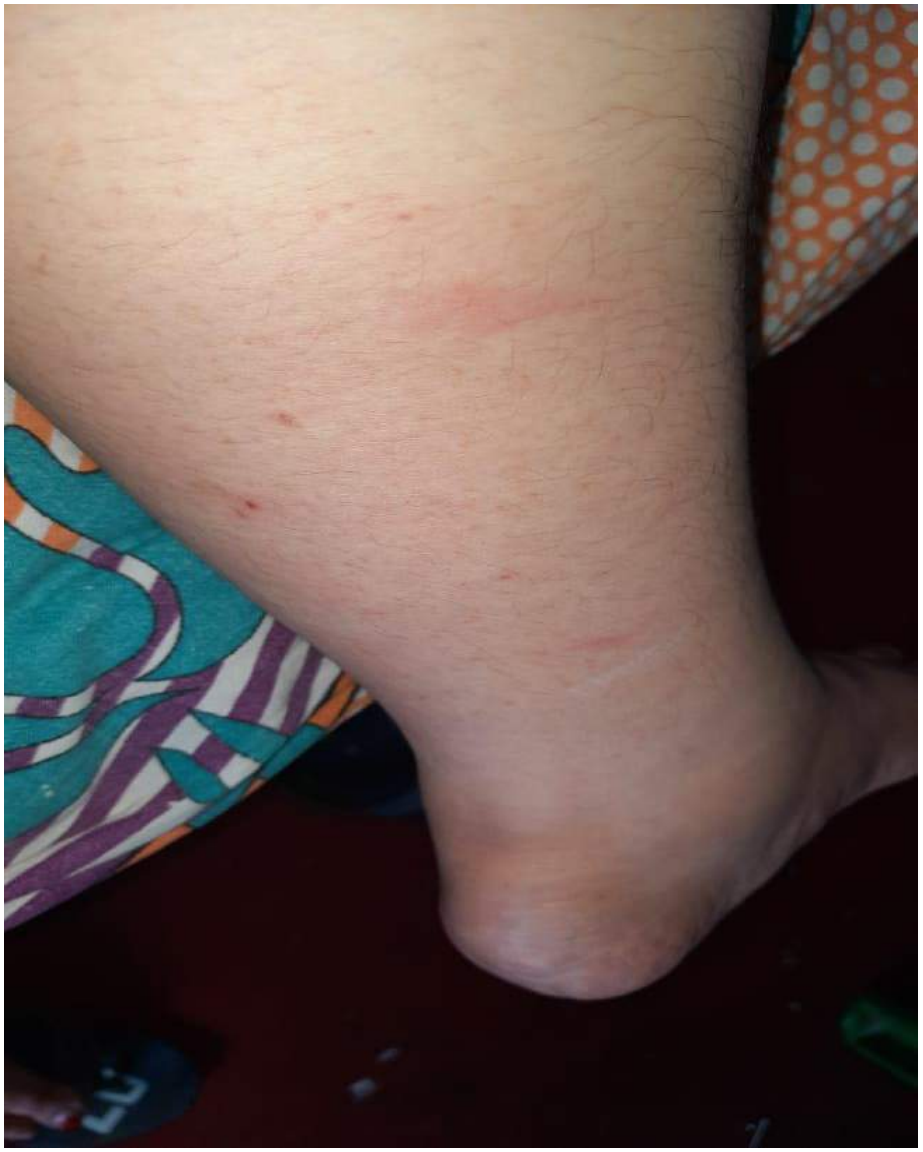


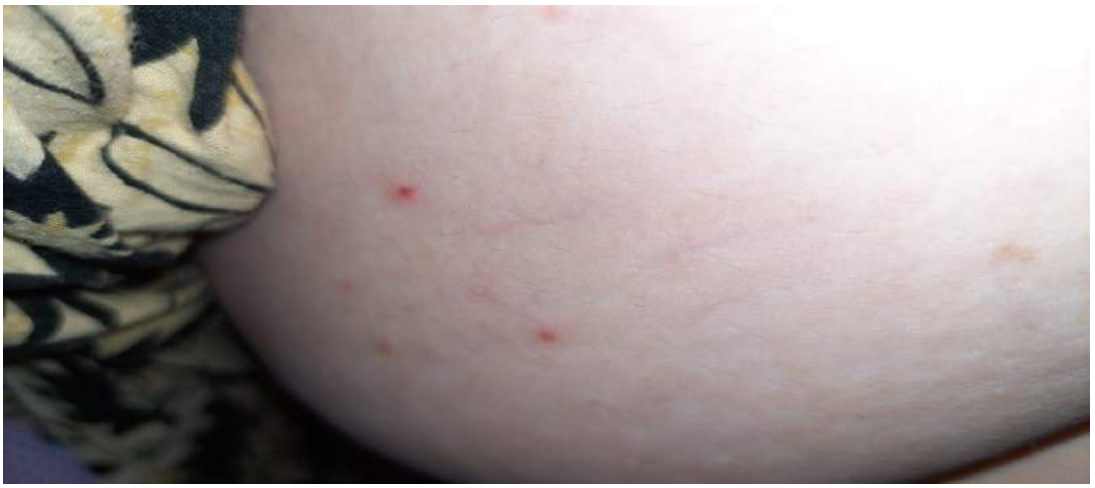
Erythomatous skin rash in lower portion of right leg



Red coloured spotted rash in upper left thig



Erythomatous papular skin rash in lower portion of left leg



Red spotted papular rash in lower abdomen

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Predictors of Attrition among HIV-Infected Youth Initiated on Antiretroviral Treatment in Rorya District Council, Tanzania

Shemmdolwa Naseeb & Switbert R. Kamazima
Muhimbili University of Health and Allied Sciences

ABSTRACT

Background: The burden of HIV infection is higher among the youth who also experience higher attrition rates compared to other age groups. Understanding the dynamics of their retention on HIV treatment and predictors of attrition is necessary for establishing streamlined interventions targeting this population.

Objective: This study examined the retention and predictors of attrition among HIV-infected youth enrolled on care and treatment in Rorya District Council (DC), Mara Region, Tanzania.

Methodology: A clinic-based cross-sectional study was conducted in Rorya DC, Tanzania. Study participants included all HIV-infected youths who were consecutively initiated on ART between October 2017 and September 2018. Records on participant's characteristics during enrolment and their clinic visits to a maximum of 12 months after ART initiation were reviewed. The data on baseline demographic and clinical characteristics and retention variables were captured by using a pre-tested structured data collection tool and Stata IC 14 was used for data entry, cleaning, and analysis.

Keywords: Youth, HIV, ART, retention, attrition, Loss to follow up, Rorya, Tanzania.

Classification: NLMC CODE: WC 140

Language: English

Great Britain Journals Press

LJP Copyright ID: 392814

London Journal of Medical and Health Research

Volume 1 | Issue 1 | Compilation 1.0

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Results: A total of 253 client's records meeting the inclusion criteria were collected and analyzed. Overall the retention at 3, 6, and 12 months were 81.4%, 69.2%, and 59.3% respectively. Independent predictors of attrition among youth were being aged 20-24 years (AOR, 5.3, CI: 2.56-10.94), being a male (AOR, 6.61, CI: 2.46-17.74), being single or never married (AOR, 4.66, CI: 2.13-10.23), having a baseline WHO clinical stage 2 or 3 (AOR, O.O2, CI: 1.09-4.63), and reporting having no treatment supporter (AOR, 9.22, CI: 2.38-35.79).

Conclusion: The overall retention of HIV-infected youth initiated on ART is still low and the independent predictors of attrition among youth are age, sex, WHO clinical stage 2 and 3, marital status, and having no treatment supporter. Urgent attention to retaining PLHIV youth initiated on treatment is required and should focus to target youths with high risks for attrition.

Keywords: Youth, HIV, ART, retention, attrition, Loss to follow up, Rorya, Tanzania.

Author α: Alumna, School of Public Health and Social Sciences, Muhimbili University of Health and Allied Sciences, P.O Box 65001, Dar-es-Salaam, Tanzania.
σ: Behavioral Sciences Department, School of Public Health and Social Sciences, Muhimbli University of Health and Allied Sciences, P. O. Box 65001, Dar-es-Salaam, Tanzania.

I. INTRODUCTION

About 36% of new HIV infections occur among the youth every day in the world.[1] Currently, over 4 million youth are HIV-infected globally, and among them, more than 60% are living in Eastern and Southern Africa. In Tanzania, about 0.2 million youth are HIV-infected comprising 11% of all People Living with HIV (PLHIV) in the country.[2] Rorya district in the Mara region, Northwestern Tanzania, has an HIV prevalence of 4%.[2]

In order to control the HIV epidemic by 2030, the United Nations (UN) has set forth 90-90-90 and 95-95-95 targets, whereby 2020 and 2030, respectively, at least 90% and 95% of people living

with HIV be aware of their status, at least 90% and 95% of those with known status be on antiretroviral treatment (ART), and at least 90% and 95% of PLHIV on ART to achieve viral suppression.[3] Diagnosis of HIV infection is an entry point into the HIV treatment cascade and linkage to care and retention are vital in achieving viral suppression which is the ultimate goal of HIV treatment and public health measures.[4-5]

Retention on HIV care and treatment has benefits on optimal clinical outcomes, prevention of further HIV transmission of HIV, and reducing healthcare costs. Through attending clinic visits, PLHIV are continuously getting access to ART, health education and psychosocial support in coping with HIV and AIDS and monitoring their treatment response.[6] Attrition which is defined as a continuous process where clients default from HIV care and treatment services may result in treatment failures, increased chances of acquiring opportunistic infections as well as further HIV transmission.[7].

Research findings show relatively lower retention rates of HIV-infected youth on ART compared to other age populations.[8-9] Furthermore, there are substantial disparities on retention among HIV-infected youth on ART in different geographical settings.[10-12] The retention of youth has also been found to decline over time with a significant decline within the first year of HIV treatment.[13-14]

Tanzania being one of the HIV epidemic countries, has been implementing preventive, treatment, and support interventions to accelerate efforts towards ending the HIV epidemic by 2030. These interventions include initiatives to increase focused HIV testing services through optimized provider-initiated testing and counseling (PITC), community-based HIV testing services (CBHS), index testing, and HIV self-testing. In 2017, the country adopted the universal test and start strategy which allowed all individuals diagnosed with HIV infection to be linked to care and initiated on lifelong ART regardless of CD4 count or WHO clinical staging. Therefore, the number of youth who are diagnosed and initiated on ART has rapidly been increasing over time and, hence, the increased demand for retaining them on HIV care and treatment services. However, there is limited knowledge on the specific dynamics of retention on ART and predictors of attrition among this age category. In this regard, interventions employed to retain PLHIV on care and treatment services may not be effective for this disproportionately affected population. In view of this, the current study reviewed the retention of HIV-infected youth over a 1-year period and examined socio-demographic and clinical characteristics associated with attrition inform programs and policy decisions for streamlining interventions to target their specific needs.

II. MATERIALS AND METHODS

2.1 Study area and period

This study was conducted in Rorya DC, northwestern Tanzania in September 2020. Rorya Council has a population of 265,241 (Census of 2012, National Bureau of Statistics) and is dominately inhabited by the *Wajaluo* , followed by the *Wakurya* and the *Wasimbiti*. It is a rural setting district with few towns, the largest being Shirati and the main economic activities in this council are farming, fishing and livestock keeping. It has the highest HIV prevalence in Mara Region and the common cultural practices fueling HIV transmission include female genital mutilation (FGM), early marriages, sexual cleansing, wife inheritance, and mourning by preparing a big feast as a sign of departure of their loved ones for a couple of weeks.

Rorya Council has 44 healthcare facilities and among them, 36 (82%) are providing HIV care and treatment services. There are 19 facilities with PLHIV-dedicated care and treatment clinics and the remaining 17 facilities have standalone reproductive and child health (RCH) clinics integrating HIV care and treatment to pregnant and breastfeeding women.

2.2 Design and participants

This study used a cross-sectional design. The study participants were the HIV-infected youth who were initiated on ART between October 1st,

2017 to Sept 30th, 2018 in all CTC and RCH clinics providing PMTC services, in Rorya DC, and whose clinical records were available on CTC2 files. The clients who were not in 15-24 years age category during the ART initiation and those who transferred to other facilities before 12 months since ART initiation were excluded. For each youth, data on socio-demographic and clinical characteristics during enrolment and follow-up clinic visit records for the period of 12 months after enrolment were collected.

2.3 Sample size

The sample size was calculated by using a single proportion formula ($n = Z^2p(100-p)/\varepsilon^2$). The proportion of attrition among youth initiated on ART within a 1 year period (p) was 19%.[9] The minimum sample size required was 237 clients with a margin error of 5%. To meet this sample size, all youth from 36 health facilities that were providing care and treatment services to PLHIV in Rorya DC, were listed to be included in the study. Out of 263 client's records retrieved, 253 met the inclusion criteria and were used in this study.

2.4 Study variables

The outcome variable was attrition throughout the 1 year period defined by the missing clinic for more than 90 days past the scheduled appointment date and not documented as a transfer out from the care and treatment clinic. Sociodemographic and clinical variables that were routinely collected during enrolment on HIV care and treatment were examined as predictors of attrition. The sociodemographic variables included age, sex, marital status, HIV status of the spouse and identification of treatment supporter. Clinical variables included the WHO clinical stage, TB co-infection, pregnancy/breastfeeding state during enrolment and the timing for ART initiation. In addition, the point for HIV diagnosis and the level and ownership of the facility delivering care and treatment services to the participants were included on the list of dependent variables.

2.5 Data collection

Data were collected from the participant's medical records in care and treatment facilities with the help of two trained research assistants (Ras) using a pre-defined data excel sheet. The primary source of information was the individual CTC 2 files. The ART registers were used to complement or validate the information. Information gathered included sociodemographic and baseline clinical characteristics and attendance visits made within a period of 12 months after ART initiation. Data collected were compiled in an excel template and then imported to Stata statistical package version 14 for cleaning and analysis.

2.6 Ethical consideration and research clearance

Ethical clearance was obtained from the MUHAS Institutional Review Board (MUHAS IRB) and permission to access data was granted by the office of Mara Regional Administration, Rorya district authority, and management of the respective facilities. Participants were identified by using unique CTC numbers or facility's file numbers for confidentiality purposes.

2.7 Data analysis

We used frequency and proportions in the descriptive analysis. Mean and standard deviation was used to summarize continuous variables. Bivariate logistic regression was used to explore the association between baseline characteristics and attrition using the Chi-square test at a 0.1 significance level. Variable categories which were significantly associated with attrition in bivariate analysis at a p-value of <0.1 were included in the single and multivariate analysis to determine those with the strongest independent association with attrition. The results are presented using the Crude and Adjusted Odds Ratios with their corresponding 95% confidence intervals.

III. RESULTS

3.1 Description of the study participants

Records for a total of 253 eligible clients who met inclusion criteria were reviewed. Among them, 85% were females, 64.8% were aged 20-24 years and 33.6% were enrolled while pregnant or breastfeeding (Table 1). The clients with identified treatment supporters were 91.3% and 1.19% screened positive for TB Co-infection. Majority of

youth started ART within 7 days of confirmed diagnosis and only 8.3% started ART after 7 days of diagnosis.

Table 1: Characteristics during enrolment (n 253)

Characteristics	Categories	Frequency, n	%
Sex	Male	38	15.0
	Female	215	85.0
Age	15-19	89	35.2
	20-24	164	64.8
Marital status	Married/Cohabiting	170	67.2
	Single	12	4.7
	Divorced/Separated/Widowed	71	28.1
Testing modality	VCT(Self referral/CBHS)	100	39.5
	PITC	153	60.5
Level of facility	DISPENSARY	76	30.1
	HEALTH CENTRE	78	30.8
	HOSPITAL	99	39.1
Facility ownership	Gov	136	53.8
	FBO	95	37.5
	Private	22	8.7
WHO stage	Stage 1	72	28.5
	Stage 2	71	28.1
	Stage 3	102	40.3
	Stage 4	8	3.2
TB screening	Screen -ve	250	98.8
	screen +ve	3	1.2
Pregnancy	Pregnant/breastfeeding	85	33.6
	Non pregnant/breastfeeding	130	51.4
ART initiation	Within 7 days	232	91.7
	Beyond 7 days	21	8.3
Treatment supporter	Yes	231	91.3
	No	22	8.7
HIV status of the spouse	Known	20	7.9
	Unknown	162	64.0

3.2 Retention of HIV infected youth initiated on ART at 3, 6 and 12 months of enrolment

Among the participants, the proportion of youth retained declined over time from 81.4%, 69.2%, and 59.3% at 3, 6 and 12 months of ART initiation respectively (Table 2).

Table 2: The proportion of HIV-infected youth retained in 3, 6 and 12 months

Months since ART initiation	n	%
3	206	81.4%
6	175	69.2%
12	150	59.3%

3.3 Bivariate analysis of the predictors for attrition

In bivariate analysis, it was found that sex, age, marital status, testing modality, level of the state, facility, facility ownership, WHO stage, pregnancy and identification of treatment supporter variables were statistically significant with attrition. (Table 3)

Table 3: Bivariate analysis of baseline characteristics with attrition (Chi^2 ; p-value)

		Attrition			
Characteristics	Categories	n	%	Chi^2	p-value
Sex	Female	102	47.4	17.525	0.000
	Male	32	84.2		
Age	15-19	33	37.1	13.908	0.000
	20-24	101	61.6		
Marital status	Single	53	74.7	19.614	0.000
	Married/Cohabiting	74	43.5		
	Divorced/Separated/Widowed	7	58.3		
Testing modality	VCT(Self referral/CBHS)	63	63.0	6.685	0.010
	PITC	71	46.4		
Level of facility	Dispensary	35	46.0	7.436	0.024
	Health centre	36	46.2		
	Hospital	63	63.6		
Facility ownership	FBO	62	65.3	10.106	0.006
	Gov	64	47.1		
	Private	8	36.4		
WHO stage	Stage 1	28	38.9	9.955	0.019
	Stage 2	37	52.1		
	Stage 3	64	62.8		
	Stage 4	5	62.5		
TB screening	Screen -ve	133	53.2	0.470	0.493
	screen +ve	1	33.3		
Pregnancy	Preg/b-feeding	30	35.3	25.852	0.000
	Non Preg/b-feeding	72	55.4		
ART initiation	Within 7 days	122	52.6	0.161	0.689
	Beyond 7 days	12	57.1		
Treatment supporter	Yes	117	50.7	5.715	0.017
	No	17	77.3		
HIV status of the spouse	Known	6	30.0	20.525	0.000
	Unknown	75	46.3		

3.4 Multivariate analysis on the predictors for attrition

Factors that were statistically significant were further analyzed by logistic regression and found that being male was associated with more than six times higher odds of attrition (AOR; 6.61, 95%CI: 2.5-17.7) compared to females. Also, being in the age group of 20-24 years was associated with five times higher odds (AOR; 5.3, 95%CI: 2.5-10.9) compared to those aged 15-19 years. More information is shown in the table below (Table 4).

Table 4: Univariate and Multivariate regression

Characteristics	Categories	LTF		Univariate regression			Multivariate regression		
		N	%	OR	p-value	95%CI	OR	p-value	95%CI
Sex	Female	102	47.4	1(ref)					
	Male	32	84.2	5.91	0.000	2.37-14.01	6.61	0.000	2.46-17.74
Age	15-19	33	37.1	1 (ref)					
	20.24	101	61.6	2.72	0.000	1.60-4.64	5.30	0.000	2.26-10.94
Martial status	Married/ Cohaboting	74	43.5	1(ref)					
	Single	53	74.7	3.80	0.000	2.07-.06	4.66	0.000	2.13-10.23
	Divorced/ Seperated/ Widowed	7	58.3	1.80	0.324	0.55-5.95	2.39	0.210	0.73-2.95
Testing modality	PITC	71	46.4	1 (ref)					
	VCT(Self referral/CBHS)	63	63.0	1.97	0.010	1.17-2.29	1.46	0.288	0.73-2.95
Level of facility	Dispensary	35	46.0	1 (ref)					
	Health centre	36	46.2	1.00	0.990	0.53-1.89	0.92	0.801	0.47-1.79
	Hospital	63	63.	2.05	0.021	1.11-3.77	2.35	0.179	0.68-8.14
Facility ownership	Private	8	36.4	1 (ref)					
	FBO	62	65.3	3.29	0.016	1.25-8.63	0.22	0.005	0.08-0.63
	Gov	64	47.1	1.56	0.353	0.61-3.94	1.13	0.824	0.39-3.27
WHO stage	Stage 1	28	38.9	1 (ref)					
	Stage 2	37	52.1	1.71	0.113	0.88-3.32	2.29	0.021	1.13-4.63
	Stage 3	64	62.8	2.65	0.002	1.42-4.92	2.14	0.025	1.09-4.17
	Stage 4	5	62.5	2.62	0.211	0.57-11.82	1.69	0.512	0.35-8.24
Pregnancy	Preg/b-feeding	30	35.3	1 (ref)					
	Non Preg/b-feeding	72	55.4	2.28	0.004	1.29-4.00	1.78	0.111	0.88-3.63
Treatment Supporter	Yes	117	50.7	1 (ref)					
	No	17	77.3	4.34	0.012	1.38-13.64	9.22	0.001	2.38-35.79

IV. DISCUSSION

In this study, the retention was found to decline over time whereby more than two-thirds of HIV infected youth were not retained on HIV treatment by the end of 12 months after ART initiation. Being aged 20-24 years, being a male, being single or never married, having a baseline WHO clinical stage 2 or 3, and reporting having no treatment supporters were independent predictors of attrition among HIV infected youth initiated on ART in Rorya DC.

A higher decline in retention was noted in the first three months after enrolment. These findings were also observed from the other studies in Sub Saharan Africa. [14-15] The decline could be due to the fact that the first three months are critical in coping up with the diagnosis and new experience with the ARTs.[16] Drug side effects, stigma, and perceived well-being have been cited as contributing factors to lower retention rates of youth on care and treatment services. [17-18]

The proportion of clients retained at 12 months in this study are much lower compared to that reported by Mee, *et al.*, in his study conducted in Tanzania which reviewed the patterns of retention on HIV treatment between 2008 and 2016.[19] This difference may be explained by differences in study population. While the study by Mee, *et al.*, included extracted data for all the HIV infected youths in the country, we only studied youths in one council. However, the results from this study are comparable to those from other studies in Sub-Saharan Africa settings. [14][18] Complex and dynamic factors have been identified to specifically affect the optimal retention of PLHIV youth on care and treatment services. These include HIV associated-stigma, socioeconomic stressors, poor risk perception of consequences of non-adherence, and depression. [16,20-21] Engagement of peers in the delivery of HIV care and treatment services has been used to address the factors affecting retention of youths on care. [22] Adopting this strategy may promote retention in the current study area setting.

Moreover, active tracing of clients immediately after missing the appointment has been identified as one of the effective measures to improve the retention of clients prior to being categorized into attrition. [23,24] Further studies are needed to evaluate effective tracing mechanisms which will work better for the youth especially in the first three months of HIV treatment.

Attrition observed may have included a substantial proportion of clients self-transferred to another facility or died who could not be reached and reported. It is common for clients who find unfavourable service in one facility to silently transfer to another facility, retest for HIV, and get re-enrolled. The mobile clients may also be re-testing and get enrolled in multiple facilities due to unawareness of the procedures or when avoiding the procedures for collecting a transfer out letter each time they shift to another location. The current database can not capture clients already registered elsewhere unless the names are exactly the same. There is, therefore, a need to determine the true outcome of youth clients who are categorized as attrition. Innovations to deliver simplified and user-friendly ART services among mobile populations may reduce attrition and account for clients who will be refilled in the site other than the facility they were enrolled in.

In our study, we found that the males had higher attrition rates compared to the females. This is similar to other studies conducted in Subsaharan Africa. [25-26] The reasons could be due to their economic activity constraints and poor health-seeking behaviours. [9][28] A study in Kenya revealed that delivering care and treatment services near the youth males' residence or site of economic activity promotes the uptake of the services. [27] This may be attributed to the time and financial costs saved on increasing access to service. The analysis of the cost of accessing the ART services in 7 regions of Tanzania showed the males spent significantly higher travel costs than the females. [28] Furthermore, extending ART refill services to a community setting has been demonstrated to decrease the substantial risk for attrition among the males of 20 years and bove. [29-30] Further studies are needed to

evaluate the impact of community ART refill services on reducing the risk of attrition for the male youths.

In this study, being aged 20-24 years was an independent predictor for attrition. Koech, *et al.*, reported similar findings among youths enrolled on HIV care in Kenya. [31] At this age interval, youth may have already been economically independent which may be associated with increased mobility. The 2019 Guideline on the Differentiated Service Delivery of Care and Treatment Services in Tanzania suggests the provision of special consideration to the mobile population by offering longer ART refills adapted to the client's travel plans. Successful implementation of this approach may significantly improve the retention of youth in this age category. Contrary, the findings from other studies indicate that youth aged 15-19 have higher attrition rates compared to 20-24. [12,32] The differences may be due to varied socio-economic contexts from the current study settings.

In this study, being single or never married was found to be the independent predictor of attrition among the HIV infected youth on care and treatment services. The finding in this study is consistent with the finding by Mecha, *et al.*,[33]. This might be because of a lack of social support in adherence to treatment and dealing with the stigma. In contrast, other studies found no association between marital status and attrition among youth on HIV treatment. [31,35] Further exploration on the role of partner support among youth on HIV treatment is needed to derive streamlined strategies in retaining these clients on care and treatment.

A number of studies have shown less attrition among PLHIV clients with treatment supporters compared to their counterparts. [27,36,37] Nakamanya, *et al.,* described the role of treatment supporters in providing psychological coping and reminding clients to take their medication and, therefore, encouraging adherence to ART services. [36] The current study affirms the significance of the role of the treatment supporter in the retention of HIV-infected youths on care and treatment services.

4.1 Strength and limitation of the study

The strength of this study relies on the use of the national standardized data sources that facilitated capturing of consistent data. However, this study has some limitations that may have influenced the results. The study relied on the retrospective data collected from the routine care and service delivery which could be subjected to errors and data incompleteness. These were minimized by using multiple data sources to complement and validate the information. The study focused on only data that are routinely captured in a day to day care and treatment services. Other important information such as Key and Vulnerable Population (KVP) category, occupation and the level of education which were found predictors of attrition among PLHIV in literature could not be assessed as the records reviewed did not capture these data. Attrition may have included clients with unknown outcomes such as unascertained death, self transfers, and clients who were later re-engaged to care. However, the findings still provide an insight on the rate and its association with baseline characteristics.

V. CONCLUSION

Retention of HIV-infected youth who are initiated on ART declines over time with a significant drop in the first 3 months of enrolment. Predictors of attrition include sex, age, marital status, WHO stage, and having no identified treatment supporter.

VI. RECOMMENDATIONS

This study revealed rates of retention decline over time among HIV-infected youth enrolled on care and treatment and highlighted significant predictors of attrition. Urgent implementation of the following recommendations by Rorya DC health management may improve the retention of youth on HIV care and treatment services: 1) Focusing on youth's retention specifically during the first three months on ART should be intensified. The adoption of youth PLHIV peers in navigating through care and treatment services and providing peer counselling may be needed to optimally address youth's specific barriers to retain on care. Youth with no identified treatment

supporters will also benefit from peer support; 2) The male youth and those who are in the 20-24 years age category have been found to be at risk of attrition and is attributed to their economic activity constraints and high mobility. Consideration of extending care and treatment services through community ART refills and offering longer hours for ART services will promote their retention.

In addition, further research to determine the outcomes of attrition among youth is recommended to account for clients who may have silently transferred to another facility or died. Moreover, there is an urgent need for qualitative analysis of the predictors for attrition to develop a deeper understanding and streamline relevant intervention measures.

Abbreviations

AIDS: Acquired Immune Deficiency Syndrome; ART: Anti-retroviral therapy; CBHS: Community Based HIV Services; CTC: Care and Treatment Clinic; HIV: Human Immuno-deficiency Virus; KVP: Key and Vulnerable Population; PITC: Provider Initiated Testing and Counselling; PLHIV: People Living with HIV; VCT: Voluntary Counselling and Testing; WHO: World Health Organization.

ACKNOWLEDGMENTS

The authors appreciate the technical expertise of Dr. Amon Sabasaba whose contribution in data analysis and review of the report is greatly appreciated. Special thanks also go to Mara Region Administrative Secretary and Rorya District Medical Officer for granting permission to conduct the study. We also thank the Rorya DC healthcare workers who gave us cooperation during the data collection process in the midst of their tight working schedules.

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Final considerations: It demonstrates the importance of exploring further discussions on the subject with students, even if religious aspects are not the main influencer, but to deconstruct the abortion-related taboo in order to obtain professionals with a holistic and humanized view.

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Classification: NLM Code: WG 440

Language: English

Great Britain Journals Press

LJP Copyright ID: 392815

London Journal of Medical and Health Research

Volume 1 | Issue 1 | Compilation 1.0

Perception of Nursing Academics at a University Center in Brasília on Induced Abortion

Percepção Dos Acadêmicos De Enfermagem De Um Centro Universitário Em Brasília Sobre Aborto Induzido

Bizerra, N. C[α], Santana, L. G[σ], Santos, T. O. S[ρ] & Silva, S. H. R[Ω]

RESUMO

Objetivo: Conhecer e descrever a percepção de acadêmicos do curso de enfermagem acerca do aborto induzido e identificar fatores que influenciam em seu posicionamento sobre o tema.

Método: Foi realizado um estudo descritivo com abordagem qualitativa. Eleitos 19 estudantes do curso de Bacharelado em Enfermagem matriculados no Centro Universitário Euro-Americano (UNIEURO). A coleta de dados ocorreu por meio de entrevistas com roteiros semiestruturados.

Resultados e Discussão: Emergiram três categorias temáticas: 1) Conhecimento sobre a legislação brasileira a respeito do aborto; 2) Posicionamento pessoal com relação à prática do aborto no Brasil; 3) A influência da religião, da moral e da ética.

Considerações finais: Mesmo que os aspectos religiosos não sejam os principais influenciadores, conclui-se a importância de se explorar ainda mais discussões sobre o tema com alunos para desconstruir o tabu relacionado ao aborto a fim de obter profissionais com uma visão holística e humanizada.

Palavras-chaves: percepcao; estudantes de enfermagem; aborto induzido.

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I. INTRODUÇÃO

De acordo com a Organização Mundial da Saúde (OMS, 2000), o aborto é conceituado como remoção de um feto pesando menos de 500g, o que equivale há mais ou menos 20 semanas de gestação. Tal evento é classificado quanto à idade gestacional – precoce ou tardio; quanto ao grau de eliminação – completo ou incompleto; situação clínica – evitável e inevitável e quanto ao tipo – espontâneo ou provocado (SOUZA et al., 2008; BRASIL, 2012).

O aborto induzido pelas próprias mulheres ou em clínicas clandestinas é uma das maiores causas de

mortalidade materna, o que o torna um problema de saúde pública. Segundo a OMS (2013), estima-se que a cada ano são feitos 22 milhões de abortamentos em condições inseguras, acarretando a morte de cerca de 47.000 mulheres além de disfunções físicas e mentais em outras 5 milhões de mulheres. A maior ocorrência de morte decorrente de aborto induzido acontece em comunidades carentes e estão associadas a múltiplas dimensões da pobreza – como a falta de recursos financeiros, a dificuldade de acesso à informação e direitos humanos, a insalubridade, dentre outros (BRASIL, 2011; MARTINS et al., 2017).

Santos et al., (2017) demonstraram que o método abortivo mais utilizado entre as mulheres é o medicamentoso e que os sintomas apresentados após o uso foram sangramento, vômitos e cólica, a pesquisa foi realizada com 855 puérperas, onde se observou a variação de idade entre 13 a 44 anos.

Quanto à incidência de abortamento constatou-se que o menor número de abortos foi observado em mulheres com maior grau de escolaridade, 51,3% das mulheres que praticaram o ato não concluíram o ensino fundamental e 3,0% eram analfabetas. Dentre as entrevistadas 42,6% referiam ser solteiras. Quanto ao aborto induzido 33,2% confirmaram a indução sendo as principais justificativas a falta de condições financeiras e estrutura familiar. Observou-se também uma grande parte dessa prática 57,2% foi realizada em casa com ajuda de amigos ou familiares.

A realidade notada é que as mulheres iniciam o processo abortivo fazendo uso de remédios, sendo o mais comum o Cytotec® o qual tem como princípio ativo o misoprostol originalmente é utilizado no tratamento de ulceras gástricas, e finalizam o processo com internação hospitalar para curetagem (DINIZ; MADEIRO 2012).

Nos casos mais graves, quando não há ocorrência de morte materna, algumas complicações consideráveis podem ocorrer isso vai depender do método utilizado para realização da prática, tais como: hemorragias, infecções de leves a gravíssimas, histerectomias, transfusão sanguínea, lesões de vulva ou de vagina, desgarro de colo uterino, perfuração do útero e até mesmo lesão de órgãos vizinhos (FORNEL et. al., 2010).

Muitas mulheres que procuram os serviços de saúde nessa situação sofrem discriminação e julgamento por parte dos profissionais, esperando horas por atendimento, ameaça de denúncia à polícia, desrespeito verbal e físico durante atendimento, internação coletiva com puérperas e recém-nascidos (DINIZ; MADEIRO, 2012).

De acordo com Código Penal Brasileiro de 1940 art. 128, são permitidos os casos de aborto quando há risco de morte da mãe e em casos de estupro. O Supremo Tribunal Federal em 2012 autorizou a interrupção legal da gestação nos casos de anencefalia. O código de ética de Enfermagem prevê em seu art.73, é proibido aos enfermeiros provocar aborto, ou cooperar em prática destinada a interromper a gestação, exceto nos casos permitidos pela legislação vigente.

A crescente prática abortiva é evidente. As mulheres ficam mais propensas a buscarem serviços ilegais e em ambientes inadequados ou insalubres, o que pode ser prejudicial e até mesmo fatal. Esse tema é complexo e abrange diversas opiniões, passando pela esfera religiosa, ideológica, ética, politica e aspectos jurídicos (BRASIL, 2008; BRASIL, 2011; BRASIL, 2017).

No ambiente hospitalar, a mulher entrará em contato com profissionais que serão responsáveis pela sua assistência, que deverá ocorrer de forma holística e humanizada destacando-se a equipe de enfermagem que se faz presente de forma integral, esse contato necessita de uma preparação maior por parte do profissional para que a atenção à mulher seja livre de preconceitos religiosos ou morais. Isso se dá por meio de debates e discussões acerca do tema durante a formação acadêmica, analisando seus diversos aspectos e promovendo treinamentos para propiciar atendimento satisfatório por meio da humanização e impessoalidade (BRITO, et al., 2015).

Baseado nisso, um estudo foi realizado em uma universidade no Rio Grande do Norte com objetivo de conhecer a opinião de estudantes de enfermagem sobre aborto. O estudo foi realizado

com 111 estudantes, destes 103 eram mulheres escolhidas de forma intencional com requisitos antes estabelecidos. Dos entrevistados, 60,4% afirmaram ser contra, 20,7% informaram ser favoráveis e 18,9% preferiram não declarar opinião acerca do assunto. Além disso, 32,4% afirmaram a oposição ao aborto mesmo em casos previstos em lei e que não devia ser realizado sob nenhuma circunstância, 31,5% referiram que a escolha cabia à mulher e 24,4 manifestaram que tal decisão cabia ao profissional de saúde (BRITO, et al., 2015).

Os dados obtidos nesse estudo mostram o quão importante são as discussões acerca do tema, visto que a maioria das participantes são jovens, envolvidas com as tecnologias e assuntos mundiais, estas precisam reformular e repensar a maneira como lidam com o assunto, pois se trata de uma questão atual (MACHADO, et al., 2012; DONATI, et al., 2010; BRITO, et al., 2015).

No estudo feito com estudantes de graduação em enfermagem e medicina do Rio de Janeiro, por meio de uma análise documental demonstrou que dos temas encontrados nos programas de disciplinas do curso de graduação em Enfermagem a palavra aborto apareceu apenas 2 vezes e dentre os achados temáticos da graduação em medicina foi encontrado apenas 1 vez, logo, temática foi pouco abordada nos dois cursos, os programas das disciplinas não estão apropriados para uma formação onde inclua o aprendizado satisfatório sobre o aborto (MARCONSIN, et al., 2013).

Na pesquisa realizada por Goés e Lemos (2010) com acadêmicos de enfermagem sobre o que estes pensam sobre o aborto provocado, 71,34% se mostraram contrárias à prática, mas apontaram algumas situações aceitáveis como estupro, risco de vida da mãe e mal formações congênitas, além disso, alguns comentários sobre a mulher que realiza a prática como: desamparada, carente, desesperada, covarde, fraca, criminosa, dentre outros percebemos então a discriminação e que a culpa recaí sobre a mulher tirando a responsabilidade dos outros participantes da situação.

Importante também ressaltar a opinião dos profissionais de enfermagem acerca do tema, na análise feita no Rio Grande do Sul em que dos participantes 7 eram enfermeiros e 12 técnicos, totalizando 19 participantes, o que pôde-se abstrair que os discursos tiveram um comportamento implícito talvez discriminatório, menor interação com a paciente e maior focalização nos aspectos clínicos, mas tiveram outros que ressaltaram a importância do apoio psicológico e do cuidado humanizado. É preciso que os profissionais ampliem seu olhar sobre o aborto se desvinculando de estereótipos considerando a totalidade do ser humano tanto no decorrer da formação profissional e durante o exercício da profissão (STREFLING, et al., 2015).

Acredita-se que a extensão de discussões acerca desse tema no período da graduação é de suma importância, pois o modo como os estudantes veem a prática abortiva determinará a qualidade do atendimento prestado as mulheres que procurarem o serviço de saúde (BRITO, et al., 2015).

Tendo dito isso, surgem os seguintes objetivos deste estudo: Conhecer e descrever a percepção de acadêmicos do curso de graduação em enfermagem acerca do aborto induzido e identificar fatores que influenciam em seu posicionamento acerca do tema.

II. MÉTODO

Foi realizado um estudo descritivo com abordagem qualitativa. A pesquisa qualitativa prioriza explicar o porquê dos fenômenos, suas especificidades e se preocupa com a análise da dinâmica das relações sociais. Foram considerados a subjetividade, experiências, crenças, valores atitudes e inspirações dos entrevistados. O pesquisador deve ser totalmente imparcial de modo que seus pensamentos não influenciem no andamento da entrevista, pois, o desenvolvimento da pesquisa é imprevisível (GOLDENBERG, 2002; MINAYO, 2004).

A amostra pesquisada neste estudo se deu por conveniência, foram eleitos estudantes do curso de Bacharelado em Enfermagem, com idade

maior ou igual a 18 anos, ambos os sexos e de diferentes períodos da graduação, regularmente matriculados no Centro Universitário Euro-Americano (UNIEURO) que é uma instituição de ensino superior particular brasileira fundada no ano de 1998, com sede em Brasília, no Distrito Federal e com unidades nas regiões do Plano Piloto e Águas Claras. Esta pesquisa foi executada nas unidades: I, Asa Sul; II, Águas Claras e III, Asa Norte, nos turnos matutino e noturno, dos diferentes períodos da graduação.

Os alunos foram abordados em sala de aula com a proposta de participação da pesquisa, após aceite estes dirigiram-se individualmente para um ambiente reservado, foi feita a leitura do Termo de Consentimento Livre e Esclarecido e assinatura em duas vias para aqueles que aceitaram, logo foi iniciada entrevista. Realizada a gravação e posterior transcrição dos dados. Como instrumento para coleta de dados foi utilizado um roteiro semiestruturado composto primeiramente por questões sócio demográficas (identificação, data de nascimento, sexo, semestre em curso, turno, ocupação remunerada, estado civil, cidade onde mora, número de filhos e crença religiosa, espiritual ou filosofia de vida) e em seguida por 5 questões abertas sobre o conhecimento sobre a legislação, seu posicionamento sobre a prática, se tiveram algum tipo de contato com aborto, como seria seus cuidados como enfermeiros frente a uma situação de aborto induzido por um paciente e como as práticas religiosas, espirituais ou filosofia de vida impacta na opinião sobre o tema. O número necessário de estudantes para a pesquisa respeitou o técnica de saturação de dados de Glaser e Strauss (1967), quando houve repetição do conteúdo.

Para analisar as entrevistas optou-se pela Análise de Conteúdo de Bardin. A Análise de Conteúdo utilizada neste estudo foi operacionalizada em três etapas: pré-análise, exploração do material e tratamento dos resultados obtidos e interpretação (BARDIN, 2011).

A etapa da pré-análise é a fase da organização propriamente dita. Realizou-se leituras sucessivas do material coletado, com objetivo de obter a saturação do conteúdo pesquisado. Logo após, procedeu-se a constituição do “corpus”, visando responder as regras: da exaustividade, representatividade, homogeneidade, e pertinência, tendo sempre em foco os objetivos propostos. Nesta fase também foi selecionado alguns trechos ou fragmentos das entrevistas e para garantir o anonimato dos participantes estes foram identificados pela letra E, seguido do número ordinal á ordem que foi realizada as entrevistas (E-1, E-2...).

Na etapa de exploração do material, ocorreu a separação, classificação e agrupamento dos dados, que forneceram as informações exatas para responder aos objetivos do presente estudo.

E por fim na etapa de tratamento dos resultados obtidos e interpretação foi realizada a seleção das informações para serem interpretadas de maneira que respondam as questões levantadas no objetivo do estudo e analisadas á luz da literatura concernente ao tema (BARDIN, 2011).

Este trabalho está de acordo com as normas estabelecidas para pesquisas com seres humanos de acordo com a Resolução 466/2012 e complementares, do Conselho Nacional de Saúde. Este trabalho foi aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos do UNIEURO sob o protocolo nº 2.591.145. Todos os participantes deste estudo assinaram o Termo de Consentimento Livre e Esclarecido TCLE em duas vias.

III. RESULTADOS E DISCUSSÃO

Foram entrevistados 19 acadêmicos de enfermagem com duração máxima de 10 minutos em cada entrevista. Pôde-se notar uma diversidade em relação à faixa etária, a qual variou de idade mínima 18 a máxima 36 anos em que a média foi de 22 anos (D.P. 3,9). É importante destacar os dados sociodemográficos, pois, segundo Brasil (2011) o aborto atravessa aspectos sociais, culturais, econômicos, jurídicos, religiosos e ideológicos, nos dados analisados nesta pesquisa observa-se na Tabela1, que 17 participantes eram do sexo feminino e 2 do sexo masculino, em relação ao estado civil 18 afirmaram ser solteiros, 1 casado e apenas 1 dos participantes alegou ter 1 filho.

Tabela 1: Dados Sócio Demográficos dos Participantes, Brasília – DF, 2018 Continuação

Variáveis	n	(%)
Filhos		
0 (zero)	17	89,4
1 (um)	1	5,2
sem resposta	1	5,2
Trabalho Remunerado		
Sim	9	47,3
Não	10	52,6
Crença religiosa, espiritual ou filosofia de vida:		
Católicos	6	31,5
Evangélicos	1	5,2
Espírita	2	10,5
Nenhum	5	26,3
Outros	5	26,3

Fonte: Elaboração Própria

A correlação entre os dados sociodemográficos e o aborto, pôde-se abstrair que por uma pequena diferença os estudantes do turno noturno são mais favoráveis do que os estudantes do turno matutino, quanto ao sexo como a grande maioria eram mulheres estas também lideraram no quesito totalmente favorável a prática, dos 2 participantes do sexo masculino 1 foi favorável e o outro não sendo que este desfavorável afirmou estado civil casado e alegou ter filho.

Para facilitar a compreensão das falas, seguindo o rigor metodológico escolhido para análise dos dados, emergiram três categorias temáticas: 1) Conhecimento sobre a legislação brasileira a respeito do aborto; 2) Posicionamento pessoal com relação à prática do aborto no Brasil ; 3) A influência da religião, da moral e da ética;

3.1 Conhecimento Sobre a Legislação Brasileira a Respeito Do Aborto

Durante a entrevista os acadêmicos foram interrogados sobre o conhecimento a respeito da legislação do aborto no Brasil, pois, sabe-se que as situações permitidas em lei são: Estupro, risco de morte da mãe e casos de anencefalia (BRASIL, 1940; BRASIL, 2012). O número de estudantes que conhecem cada situação, permitida em lei, para o aborto pode ser observado na Tabela 2.

Tabela 2: Conhecimento Dos Estudantes Sobre as Situações Permitas Em Lei Para O Aborto Induzido. Brasília – DF, 2018

Variáveis	n	(%)
Estupro	12	63,1
Anencefalia	4	21
Risco de morte da mãe	5	26,3
Nenhum	7	36,8

Fonte: Elaboração Própria

Observa-se que a maioria dos estudantes relatam os casos de estupro, um total 4 alunos na categoria de anencefalia e de risco de morte da mãe 5 tinham conhecimento e um dado muito importante é que 7 alunos afirmaram não saber nenhum dos casos previstos pela lei, como podemos vislumbrar nas falas a seguir:

"É eu sei que o aborto ele é um crime quando você induz é, mas ele é respaldada pela lei quando você sofre estupro ou quando traz risco à Vida da mãe né e no mais é isso." (E-1)

"A legislação do aborto? É sei dos artigos da constituição e dos penais também que tá na lei é isso que eu sei que tipo o aborto ele, ele é liberado em casos de estupro, tem lei também que libera quando a mãe tem risco iminente de vida também é liberado e tem jurisprudência pra casos de anencefalia." (E-12)

"Ah eu não tipo, não to muito antenada nesse negócio, como lá na sala toda vez que a gente entra nessa discussão eu prefiro ficar calada porque é algo que acaba criando muita inimizade [...] A gente quase não conversa sobre isso, então eu to meio que por fora sobre isso." (E-4)

Pode ser que os alunos ingressantes não tivessem domínio sobre o conteúdo questionado, devido a grade curricular a partir do 4° semestre os alunos têm contato com a matéria de Ética/Bioética/Legislação e a partir do 6° semestre a matéria de Saúde da mulher. Chamou atenção que alunos do 1°, 2° e 3° semestres afirmaram não ter conhecimento nenhum sobre o assunto, já 1 acadêmico do 9° semestre sabia relatar apenas dos casos de estupro e quanto a legislação completa apenas 2 alunos do 3° período demonstraram aprazamento do conteúdo.

Considera-se que o universo acadêmico, em especial o da formação em enfermagem, tem o propósito de instruir os graduandos, a fim de adquirirem não só competências técnicas, mas também temáticas e éticas capazes de servir como base para o exercício profissional (BRITO, et al., 2015).

3.2 Posicionamento Pessoal Com Relação À Prática Do Aborto No Brasil

Quando questionados sobre seu posicionamento sobre a prática como resultado obteve-se 3 subcategorias em que 9 foram totalmente favoráveis a prática, 6 parcialmente favoráveis (apoiam apenas os casos regidos por lei) e 4 totalmente desfavoráveis. Fato curioso, pois, o que se pensa é que a maioria seria contra e que a depender do semestre cursado pelo estudante a opinião mudaria, mas, na verdade, neste estudo mostra o contrário a maior parte foi completamente favorável e nas 3 subcategorias citadas obteve-se estudantes de vários semestres, de forma homogênea nos grupos apresentados.

Já no estudo qualitativo, sobre o que pensam os acadêmicos de enfermagem sobre aborto induzido, realizado no Rio de Janeiro por Goés e Lemos (2010) demonstrou que 71,34% dos estudantes se mostraram contrários a prática e usaram argumentos para se referir como: Ato proveniente de uma irresponsabilidade, ato brutal, violento, agressivo e aborto é um homicídio.

Alguns argumentos utilizados neste estudo para esclarecer seus posicionamentos foram:

"Eu acho que o aborto deveria ser legalizado [...] Na cabeça da sociedade eles acham que a mulher vai engravidar e abortar todos os dias, todo os dias, gente não é assim é um processo difícil, é um processo doloroso e ninguém é obrigado a ser mãe tem gente que não nasceu com esse dom e eu acho que a sociedade, eu acho que isso deveria ser mais conversado, conversado dentro de casa, conversado nas escolas, conversado nas universidades, deveria ser um assunto que não traz medo." (E-14)

"Tá vou falar do ilegal, primeiro eu sou totalmente contra o aborto ilegal por que ele traz vários tipos de complicações pra paciente [...] E sobre aborto legal eu acho a prática sim válida nos casos que são, são normatizados por aborto, os legais eu sou a favor." (E-5)

"Bom, pra mim é independente da situação, por exemplo, tem algumas situações que a pessoa a foi estuprada, ou alguma coisa do tipo é independente da forma que a pessoa ficou grávida eu não sou a favor do aborto, eu sei que assim dependendo de como isso aconteceu é algo traumático pra pessoa e a pessoa não quer o filho, mas eu vejo assim que tem muitos, muitas famílias que querem, tem muitos casais que não conseguem ter filhos então eu acho que poderia entregar essa criança pra um lar que fosse cuidar." (E-18)

Também interrogados sobre o contato que já tiveram com algum tipo de aborto 8 dos participantes relataram contato com aborto induzido por conhecidos, amigos ou familiares, 9 deles disseram que nunca tiveram contato nenhum com aborto, 2 apenas com aborto espontâneo e 1 relatou além do contato por terceiros ter cometido aborto induzido, foi separado algumas falas para demonstrar isso:

> *"Uma colega minha que ela usou e eu não sei, acho que ela tava de uma semana, ou algumas semanas, ou 12 semanas não sei, que ela utilizou a pílula do dia seguinte e sofreu o aborto e quando ela sentiu dores fortes no abdômen e quando ela foi fazer xixi saiu o feto no vaso, então aquilo ficou, eu fiquei um pouco chocada. "* (E-10)

> *"Os abortos que eu tenho como experiência são de vivência do próprio ambiente hospitalar de pacientes que já chegaram no hospital com o feto já sem vida [...] Uma paciente que inclusive veio a óbito quando a mesma tentou fazer o aborto de maneira ilegal e apresentou hemorragia, apresentou infecção, febre e ai quando chegou no hospital até por vergonha porque sabia que chegando lá ia ser identificada a causa e essa paciente chocou, então a gente infelizmente não teve como reverter o quadro da paciente então eu acredito que se fosse o meio legal, talvez seria mais uma morte evitada."*(E-16)

> *"Eu fiz o aborto, eu acho que vai fazer dois anos em novembro, minha mãe é ciente, minha mãe inclusive participou comigo, não deixei nada fechado, minha mãe é minha amiga, então fiz o ilegal, da forma consegui, não me aconteceu nada, foi praticado e foi concluído com sucesso [...] em Salvador inclusive tem lugares que você consegue fazer com a de você conseguir não com a medicação e sim pra você abrir, retirar e tudo então é fácil entendeu? O ilegal acaba se tornando, eles dá maneiras pra outras pessoas exercerem e ganharem por isso. "*(E-19)

No entanto, quando questionados sobre como seria sua conduta como enfermeiros frente a uma situação de aborto induzido por um paciente 12 afirmaram prestar atendimento humanizado independente de sua opinião pessoal, 5 demonstraram tensão em responder sobre como seria sua atuação frente a esta situação (sendo que 4 destes afirmaram ser totalmente favoráveis a prática) e além disso 2 declaram que não participariam do atendimento. Foi separado trechos que mostram isso:

> *"Aí fica difícil. (risos) Deixa eu pensar aqui assim no legal eu tentaria, porque no ilegal não participaria."* (E-5)

> *"Então a gente fica assim meio receosas né porque como é proibido, a gente fica acabando com medo[...]É difícil, a gente tá ali pra ver a pessoa como um todo então. A gente vai ter que, sei lá né? Não vou pode abandonar a paciente numa situação dessa também ir lá e criticar, e punir né? Não a gente vai ter que saber lidar."* (E-6)

> *"Eu não discriminaria a mulher em momento algum é uma prática que eu particularmente não adotaria né e nem aconselharia a fazer, mas no caso que ela já tivesse feito [...] Poder ajudar né porque os danos consequentes vai surgir de qualquer forma. "* (E-13)

> *"Então primeiramente eu não iria julgar, mas orientar conforme o que preconiza o ministério da saúde porque é a minha profissão fazer isso e depois deixar minhas opiniões por fora."* (E-7)

No estudo realizado por Madeiro e Rufino (2017) sobre assistência a mulheres que realizaram aborto provocado, mostrou que uma em cada três mulheres participantes se sentiram desrespeitadas e maltratadas durante a internação. Isso tem um impacto muito grande para as pacientes que estão vivenciando aquele momento, violam seu direito ao atendimento digno e respeitoso. Demonstrando a importância que se tem na humanização no cuidado e equidade para com os clientes um dos princípios fundamentais da assistência à saúde no Brasil.

3.3 A Influência Da Religião, Da Moral E Da Ética

Quando questionados sobre a influência que a religião tem sob sua opinião acerca do aborto induzido 12 participantes afirmaram que não

intervém e 7 declaram que a religião influi diretamente em sua opinião pessoal, como se pôde verificar a maioria declarou que a religião em si não afeta no seu posicionamento pessoal, logo, o que se pensa é que o posicionamento dos participantes desta pesquisa está mais correlacionado com sua moral, ética e valores pessoais. Para confirmar este dado foram separadas as seguintes falas:

> *"Eu não tenho religião, mas eu acredito assim em Deus, mas eu acho que o aborto deveria ser legal sim, pois realmente, realmente nem todo tem a coisa de ser mãe e nem todo mundo quer nem tá preparado naquele momento de vida, tem muita adolescente que corre risco fazendo aborto ilegal com isso e não deveria, é isso."* (E-3)

> *"Questão religião eu nem fui muito influenciada com isso eu já nasci com essa de que é um ser humano, não justifica, não justifica matar."* (E-2)

> *"Segundo minha religião eu não deveria ser a favor, mas é como já falei eu não acredito que a religião deva influenciar na vida da pessoa, cada um tem que decidir se quer ou não na questão do aborto."* (E-9)

> *"Bom primeiro porque tipo eu sou cristã e segundo a minha religião é proibido né porque tipo Deus deu a vida e nós não temos o domínio de tipo simplesmente acabar com a vida porque mesmo sendo nosso corpo, mas desde quando ele é um feto já é uma vida então não sou a favor pela minha religião."* (E-11)

> *"Eu sou favorável apesar de, da minha religião, da minha opção religiosa ser contra, mas eu como pessoa sou a favor porque eu acho que é uma opção de cada um."* (E-16)

Ética e moral aparecem com diferentes conceitos e definições, a depender da situação entendido como sinônimos ou opostos. A moral vem do latim (mos-mores), significa costumes, as regras do comportamento, remete ao agir humano, normas, leis e hábitos. E a ética, do grego (ethos), também significa costumes, regras de comportamento aparentemente ambas têm o mesmo significado, mas a diferença é que a ética de ordem mais reflexiva procura entender o agir do humano, a natureza entre o bem mal e à moral como de ordem mais prática, normativa e legislativa (DURAND, 2003).

No estudo realizado com estudantes de enfermagem acerca do posicionamento ético sobre situações dilemáticas em saúde, realizado em Minas Gerais, no ano de 2010, em que um dos temas era sobre aborto a maioria dos acadêmicos se posicionou contrária em relação à prática (69,29%), defendendo neste caso a sacralidade da vida que era um dos argumentos escolhidos para explanar sua opinião. Essa posição espelha diretamente os dogmas centrais das religiões demonstradas pela maioria dos entrevistados, já que 74,28% declararam-se católicos e 10,73%, protestantes/evangélicos. Isso demonstra nitidamente que neste estudo a religião dos acadêmicos entrevistados afeta diretamente em sua opinião, fazendo oposição a este estudo (RATES; PESSALACIA, 2010).

IV. CONCLUSÃO

Ao concluir o presente estudo pôde-se perceber que a opinião dos estudantes sobre o aborto pode não estar baseada apenas em aspectos religiosos, mas em sua maioria por questões éticas, morais, culturais e ideológicas. Observa-se também que muitos estudantes se dizem favoráveis a prática e outra grande parte apoiam os casos regidos por lei, outro ponto positivo é que muitos dos participantes afirmaram realizar um bom atendimento a essas pacientes quanto enfermeiros, porém, pôde-se perceber pela fala de alguns que é um tema polêmico, traz certa tensão e não é muito desbravado em sala de aula.

Sabe-se que o aborto ainda é problema de saúde pública e enquanto o governo não cria programas de saúde específicos para buscar uma melhora neste quadro não cabe apenas a educação em saúde para as pacientes ou termos legais, mas também aos profissionais da saúde e estudantes de enfermagem a prestar atendimento humanizado, livre de julgamentos, preconceitos e com maior embasamento teórico para obtenção de resultados satisfatórios na assistência a estas pacientes.

Este estudo não teve o objetivo de defesa ou oposição ao aborto, mas sim conhecer e demonstrar a opinião dos estudantes para com estes dados explicitar a importância de salientar a temática com os alunos, moldando os mesmos para que ao final da faculdade sejam profissionais com uma visão holística e humanizada independente de valores e crenças.

A importância de se falar da temática em sala de aula é ampla, não com objetivo de mudar os valores e aspectos éticos ou morais dos acadêmicos, mas sim com o propósito de incentivar discussões ideológicas sobre o aborto e também salientar a importância do conhecimento legal, biológico, direitos reprodutivos das mulheres e educação em planejamento familiar.

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