ISSN No:-2456-2165

Analysis of Frequency and Patterns of Cardiac Autonomic Neuropathy (CAN) in Haart Naive HIV Sero Positive Patients at Unth, Ituku-Ozalla, Enugu: An Application of Ewing's & Time and Frequency Domain Method

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Abstract:-

➢ ***Background:***

Human immunodeficiency virus (HIV) can affect sensory, motor, and autonomic nerves causing neuropathy. Autonomic dysfunction develops when nerves of the autonomic nervous system are damaged by HIV. Symptoms of CAN include abnormal exercise-induced cardiovascular performance, postural hypotension, cardiac denervation syndrome, arrhythmias, myocardial ischaemia and sudden cardiac death. Knowledge of the prevalence of CAN will promote early management of such patients and improve the general outcome.

➢ ***Methods:***

CAN was assessed using Ewing's battery of five cardiovascular autonomic reflex tests and short term time and frequency domain heart rate variability test on patients and control arm. Spectral analysis of the heart rate variability is a sensitive technique for measurement of cardiovascular differential assessment of parasympathetic and sympathetic components, as well as blood pressure response.

➢ ***Results:***

The frequency of CAN was 62.0% for the subjects and 42.0% for the control, p value 0.045 using Ewing's cardiovascular reflex test and the frequency of CAN was 28.0% for the subjects and only 6.0% for the control, p value 0.003 using short term time and frequency domain heart rate variability tests. However Ewing's method is a more reliable test for the presence of CAN (Fig 1). Gender is an essential factor in the stage of CAN, age and gender are positive predictive factors of CAN.

➢ ***Conclusion:***

Apart from opportunistic infections, cardiac autonomic dysfunction itself can contribute to morbidity and mortality in HIV infection.

Keywords: *Cardiac Autonomic Neuropathy in HIV is Largely Unrecognized and Not Considered in the Management of HIV Positive Patients. More Focus is on Distal Peripheral Neuropathy and its Presentation. This Study Seeks to Demonstrate and Emphasize the Frequency of Cardiac Autonomic Dysfunction in HIV Infection. This Research Brings Out the Need to Screen HIV Patients for CAN Especially before Undergoing Some Invasive Procedures. The Frequency of CAN is Significantly Higher in HIV Infected Subjects than in the Non HIV Infected General Population.*

I. INTRODUCTION

Human immuno-deficiency virus (HIV) is a retrovirus/lentivirus which infects immune cells. HIV infection and acquired immune deficiency syndrome (HIV/AIDS) is considered a pandemic.[1] Records show that in 2022, the HIV prevalence rate among adults ages 15-49 in Nigeria is 1.4%, which is lower than previous estimate of 2.8%.[2] Nigeria has the second largest HIV epidemic in the world.[3]

HIV can affect sensory, motor and autonomic nerves. Sometimes neuropathy is due to a group of anti HIV medications. Autonomic dysfunction is common among HIV infected individuals usually occurring as part of a more generalized neuropathic process including distal symmetrical polyneuropathy. Autonomic nerves are responsible for modulation of all internal organ systems.[4]

ISSN No:-2456-2165

Since the late 1980's and early 1990's researchers have also identified changes related to the autonomic system leading to alterations in heart rate, postural hypotension, valsalva maneuver, prolongation of the QT interval, deep breathing, response to isometric exercise, cold face test, urogenital problems and mental stress[5-7]. Most of these autonomic alterations were identified in advanced, untreated cases[5-7]. Additionally some studies found a correlation between HIV infection and specific autonomic alterations in cardiac innervation and consequently, cardiac function[8-10].

Heart rate variability (HRV) is a marker of cardiovascular autonomic tone and is also known to be reduced in association with cardiac dysfunction. Abnormal autonomic function tests are common in HIV infected individuals, but the contribution of heart disease to such findings is not known.[11] Spectral analysis of HRV is a sensitive technique for measurement of cardiovascular differential assessment of parasympathetic and sympathetic components. HIV infection may be associated with severe global autonomic dysfunction, which is not related to heart disease[12.] HIV positive patients should be screened for the presence of the complication, in view of the possible serious events associated with it, such as arrhythmia, myocardial ischaemia, orthostatic hypotension and sudden cardiac death.[13] Recently studies have evaluated heart rate variability in HIV infected patients[10, 14, 15] with most showing decreased heart rate variability similar to findings observed in patient with cardiomyopathy.[10, 14, 15] This paper seeks to assess the presence and frequency of CAN in HIV infection and the possible presentation and predictors.

II. METHODS

The study was a cross-sectional, descriptive and hospital based study. There are four HIV clinic days per week, approximately seventy patients are seen each clinic day. The study was carried out at the HIV clinics UNTH Enugu after due ethical clearance.

➢ *Inclusion Criteria:*

- *For Subjects with HIV Infection:*

✓ HIV sero positivity confirmed by western blot after pretest counseling.
✓ Patients with HIV infection that are HAART naïve.
✓ Patients who consent to the study
✓ Patients in WHO stage 1 or CDC Category A.
✓ Patients aged 18years and above.

- *For Controls without HIV Infection:*

✓ Negative HIV screening test after due pre-test counseling.
✓ Patient who consent to study
✓ Patients aged 18years and above.

➢ *Exclusion Criteria:*

- History of pulmonary disorder that can limit the ability to perform Valsalva maneuver.
- History of diseases with affectation of the autonomic system such as diabetes mellitus, hypertension, leprosy, Parkinson's disease, malnutrition, chronic alcoholism.
- Physical examination showing signs of neurological dysfunction such as impaired cranial nerves, gait abnormality.
- Use of drugs that affect the ANS such as beta blockers, isoniazid, tricyclic antidepressants, oral nitrates.
- Pregnant women
- Patients on HAART
- Inability to perform any of the maneuvers.

➢ *Sample Size Estimation:*

Recently published results from the Nigeria HIV/AIDS Indicator and Impact Survey revealed an HIV prevalence of 1.4% among men and women in the age range of 15-49 years.[28] Using the WHO formula for sample size calculation in a finite population. The sample size (n) is thus calculated:

- $n=Z^2pq/d^2$
- Where n = minimum sample size
- Z = standard deviation at 95% confidence interval (1.96)
- p = prevalence of the disease (0.014)
- q = 1- p= 0.986
- d = sampling error tolerated (5%)
- Sample size is approximately 21.
- In order to increase the reliability of results and correct for possible attrition, the final number of patients to be recruited for this study N will be 50.
- Similar number of subjects (50) will be used as control.

Fifty subjects were recruited. Similar number of subjects (50) was used as control.

➢ *Patient and Public Involvement:*

Sub Saharan Africa has a huge HIV/AIDS burden, however much emphasis has been on distal polyneuropathy. This paper seeks to evaluate the ability of the virus to be directly implicated in cardiac autonomic neuropathy. Patients were not involved in the design and conduct of the study. However findings of this study will improve patient care and peri operative precautions in view of this existing cardiac abnormality.

➢ *Procedure:*

Socio demographic variables were obtained. Cardiac autonomic neuropathy was assessed using:

- Ewing's battery of five (5) cardiovascular autonomic Reflex tests.
- Short term (5 minutes epoch ECG recording) time and frequency domain Heart Rate Variability analysis.
- Subjects and control were advised to avoid alcohol, caffeine and rigorous activity.

48 hours before assessment. The assessment was carried out between 8am to 12 noon in a quiet room at ambient room temperature.

- Awake resting 5 minutes epoch electrocardiography (ECG) with patient lying supine and the head at about 45° using the Cardiax ECG machine with in-built

analogue to digital converter and connected to the laptop using USB cable. Beat to beat interval (R-R intervals) was calculated after correcting for artifacts. With the installed HRV analysis software, the following standard time and frequency domain measures of HRV was calculated. The time domain parameters are **SDNN** (the mean of the standard deviations for all R-R intervals) and **RMSSD** (root-mean square differences of successive R-R intervals). The frequency domain parameters are **LF** (low frequency power) representing sympathetic activity, **HF** (high frequency power) representing parasympathetic activity, and the ratio **LFIHF** (index of sympathovagal balance). A ratio of < 1.0 represents a greater parasympathetic outflow, whereas values >1.0 represents a shift toward increased sympathetic modulation.

- Thereafter, a continuous ECG monitoring was done while patient was sitting down and forcibly blowing into a mouth-piece connected to a sphygmomanometer maintaining a pressure of 40mmHg for 15 seconds. This was repeated for three times with one minute interval between them. Valsalva ratio, which is the ratio of the longest R-R interval after the maneuver and the shortest R-R interval during the maneuver, was calculated for each maneuver and the average used as the final value.
- The patient then breathes deeply and regularly at a rate of 6 breaths per minute while sitting up [5 seconds in and 5 seconds out for each cycle] for 1 minute. A continuous ECG monitoring was recorded throughout the period using marker to note onset of each inspiration and expiration. The difference between the maximum HR during inspiration and the minimum HR during expiration was calculated for each of three consecutive cycles and the average taken as the final value.
- The subject then stands from a supine position while the ECG was recorded. The point of starting to stand was marked on the ECG tracing. The ratio of the longest R-R interval around the 30th beat to that of the shortest R-R interval around the 15th beat after the point of starting to stand was recorded.
- The maximum voluntary contraction was estimated using a hand held sphygmomanometer cuff. Then the hand grip was maintained at 30% of the maximum voluntary contraction for 5 minutes. The blood pressure was measured every minute. A difference in diastolic BP just before release of hand grip and that after starting < 10mmHg is considered as abnormal.
- Finally, the supine BP of the subject was recorded after 10 minutes of rest. The subjects then stands up and the BP recorded after 2 minutes. A reduction of systolic BP of at least 20 mmHg or diastolic BP of at least 10 mmHg represents orthostatic hypotension.

Values obtained in the procedure above was interpreted in line with Ewing's proposal:

- *Normal:*

When all the tests are normal or a single borderline result.

- *Early Autonomic Neuropathy*:

One of the three (HR) test is abnormal or there is two borderline results for the HR tests.

- *Definite Autonomic Neuropathy*:

Two or more of the HR tests are abnormal.

- *Severe Autonomic Neuropathy*:

Two or more abnormal HR test results plus one or both abnormal blood pressure result.

The reference values for the different cardiovascular reflex tests as proposed by Ewing was used as follows:

- *Valsalva Maneuver*:

The index of measurement is the valsalva ratio. Normal valsalva ratio is (> 1.21), borderline is (1.11-1.20) and abnormal valsalva ratio is <1.10).

- *HR Response to Standing*:

The index of measurement is the 30:15 ratio. Normal ratio is (>1.04), borderline is (1.01-1.03) and abnormal ratio is (<1.00).

- *HR Response to Deep Breathing*:

The index of measurement is the Maximum - Minimum HR in (beats/minutes). Normal is (> 15 beats/min), borderline response is (11-14 beats/min) and abnormal result is <10 beats/min).

- *BP Response to Postural Change*:

The index of measurement is the fall in systolic BP in (mmHg). Normal response is <10mmHg), borderline response is (11-30mmHg) and abnormal response is (>30mmHg).

- *BP Response to Sustained Handgrip*:

The index of measurement is a rise in diastolic BP in (mmHg). Normal response is (> 16mmHg), borderline response is (11-15mmHg) and abnormal response is (<10mmHg).

The values for the HRV analysis of the control served as normal and was used to compare as no normative values for HRV exists.

A thorough neurological examination will be carried out in a quiet room assessing functions of the higher centers, cranial nerves, motor and sensory systems.

➢ *Analytical Techniques:*

The 5th percentile of time and frequency domain parameters of the control was computed to determine a cut-off point between abnormal value and normal value; which hence was used as a reference point for the subjects. **Abnormal values were values below the 5th percentile as adopted from a similar study[16].**

Statistical tools employed in the data analysis include descriptive statistics (mean, standard deviation, simple percentages, and bar charts), chi-square test of association,

Mann-Whitney U test, Kruskal-Wallis test non-parametric equivalent for one-way ANOVA and multivariate logistic regression mechanisms. The descriptive statistics such as mean, standard deviations, and proportional estimates (percentages) were used to describe the data characteristics while the inferential chi-square test, Mann-Whitney test and Kruskal-Wallis were used to establish comparative differences across the subjects and control groups. However, the multivariate logistic regression analysis was used to ascertain the predictors of CAN among the sample groups. All inferential statistics were judged at 0.05 level of significance, and the data analysis was aided by Statistical Package for Social Science (SPSS) version 25 and Microsoft Excel 2016.

III. RESULTS

➢ *Analysis of Socio-Demographic Characteristics of the Research Participants:*

Table 1 Socio-Demographic Characteristics of the Participants

Characteristics	Measures	Subjects (n = 50)	Control (n = 50)	Statistical difference		
				Stat.	df	p-value
Sex	Male	19(38.0)	25(50.0)	1.932	1	0.164
	Female	31(62.0)	25(50.0)			
Age brackets	20-29 yrs	18(36.0)	29(58.0)	5.728	3	0.126
	30-39 yrs	8(16.0)	5(10.0)			
	40-49 yrs	17(34.0)	9(18.0)			
	50+ yrs	7(14.0)	7(14.0)			
Mean±Std		38.02±12.11yrs	33.34±12.42yrs			
		Overall: 35.68±12.27 years				
BMI						
- Mean±Std		23.78±4.27	25.86±5.34	2.165	-	0.331+
- Range		19.72-32.60	19.38-40.79			
Inferential Statistics: Chi-Square Test (*); Mann-Whitney Test (+); BMI (normality test): K-S stat.=0.182, p=0.004						

The socio-demographic result of the participants presents statistics of the gender, age brackets (and mean age) as well as the Body Mass Index (BMI). The gender (Sex) statistics shows that out of the 50 sampled subjects, 19(38.0%) are males while 31(62.0%) are females. Meanwhile, in the control group, we have 50:50 percentage of males and females. The statistical comparative result indicates that there is no significant variation in the gender distribution (*Chi-Sq.*=1.932, p=0.164>0.05).

The age statistics shows that the study participants are of ages 20 years and above. The mean age for the subjects is 38.02±12.11 years, while for the control group, the ages are mostly clustered around 33.34±12.42 years. With the above statistics, the overall average age of the study participants is 35.68±12.27 years. The comparative result shows no significant difference in age distribution across the subjects and control groups (*Chi-Sq.* = 5.728, p=0.126>0.05).

The BMI series of the subjects averaged 23.78±4.27 and ranges from 19.72-32.60, while for the control group, the BMI series averaged 25.86±5.34 and ranges from 19.38-40.79. The result shows that BMI readings of the control samples are a little bit higher in comparison with the BMI series from the subject samples. However, the comparative result (Mann-Whitney U test) on the ground that the data series were confirmed not to be normally distributed as provided by one-sample Kolmogorov-Smirnov (K-S) test (K-S = 0.182, p=0.004<0.05) shows no significant difference in the BMI readings across the subjects and control groups (Mann-Whitney U stat. = 2.165, p=0.331>0.05). In other words, the result supports independence of BMI series from the subject and control samples.

➢ *Assessment of CAN among HAART naïve HIV Sero Positive Neurologically Asymptomatic Subjects in UNTH Enugu:*

Table 2 Assessment of CAN using Ewing's method

Parameters	Measures	Subject (%)	Control (%)	Statistical difference	
				stat.	p-value
Parasympathetic parameters					
Valsalva ratio	No dysfunction	44(88.0)	47(94.0)	1.099	0.295+
	Dysfunction	6(12.0)	3(6.0)		
Heart rate response to deep	No dysfunction	20(40.0)	30(60.0)	4.000	0.046*

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breathing	Dysfunction	30(60.0)	20(40.0)		
Heart rate response to standing	No dysfunction	50(100.0)	50(100.0)	-	-
	Dysfunction	0(0.0)	0(0.0)		
Overall Parasympathetic activity					
	No dysfunction	19(38.0)	27(54.0)	6.575	0.037+
	1 Dysfunction	26(52.0)	23(46.0)		
	2 Dysfunctions	5(10.0)	0(0.0)		
	3 Dysfunctions	-	-		
Sympathetic parameters					
BP response to standing	No dysfunction	50(100.0)	49(98.0)	-	1.000+
	Dysfunction	0(0.0)	1(2.0)		
BP response to Handgrip	No dysfunction	2(4.0)	5(10.0)	1.382	0.240+
	Dysfunction	48(96.0)	45(90.0)		
Overall Sympathetic activity					
	No dysfunction	2(4.0)	5(10.0)	2.460	0.269+
	1 Dysfunction	48(96.0)	44(88.0)		
	2 Dysfunctions	0(0.0)	1(2.0)		
Presence of CAN					
	Yes	31(62.0)	21(42.0)	4.006	0.045*
	No	19(38.0)	29(58.0)		
Inferential Statistics Used: Chi-Square Test (*); Fishers Exact Test (+)					

Result of the CAN parameters using the Ewing's method (Table 2) highlights the parasympathetic and sympathetic activities of the participants, showing those who has dysfunction and those who do not have.

Under the parasympathetic activity, Valsalva ratio of the participants was determined. The result shows that those whose Valsalva ratio has no dysfunction (88.0%) were slightly lower in comparison with those whose Valsalva ratio has dysfunction (94.0%) across the subject and control groups. However, the Valsava ratio among the subjects who have dysfunction (12.0%) is higher in comparison to those who have similar case in control group. Although, the distribution across subjects and control did not vary substantially (Chi-Sq. = 1.099, p=0.295>0.05).

Another parasympathetic parameter, the heart rate response to deep breathing was seen to swing oppositely across the subjects and the control samples; and the swing was ascertained to vary significantly across the subjects and control group (Chi-Sq. = 4.000, p=0.046<0.05). Meanwhile, considering the heart rate response to standing, there was no difference across the subjects and control groups. The overall result indicates a significant difference across the subjects and control group (Chi-Sq. = 6.575, p=0.037<0.05).

Moreover, the sympathetic parameters captured were BP response to standing and BP response to handgrip. Results of these two parameters across the subject and control group shows no statistically significant difference (p>0.05), and confirmed by the overall estimate (Chi-Sq.=2.460, p=0.269>0.05). A combined result of the parasympathetic and sympathetic activities submits that identification of CAN with the use of Ewing's method vary significantly across the subjects and control samples (Chi-Sq. = 4.006, p=0.045<0.05); an indication that Ewing's method is a reliable method for assessing/identifying CAN among HAART naïve HIV Sero Positive Neurologically Asymptomatic Subjects in UNTH Enugu.

Table 3 Assessment of CAN using Time and Frequency (TF) Domain Method

		Subject (%)	**Control (%)**	**Statistical difference**	
				Stat.	**p-value**
Both sympathetic and parasympathetic					
SDNN	No dysfunction	38(76.0)	49(98.0)	**10.698**	**0.001***
	Dysfunction	12(24.0)	1(2.0)		
Parasympathetic parameters					
RMSSD	No dysfunction	43(86.0)	48(96.0)	3.053	0.081
	Dysfunction	7(14.0)	2(4.0)		
PNN50	No dysfunction	41(82.0)	48(96.0)	**5.005**	**0.025***
	Dysfunction	9(18.0)	2(4.0)		
HF ms^2	No dysfunction	42(84.0)	48(96.0)	-	-
	Dysfunction	8(16.0)	2(4.0)		

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Overall Parasympathetic activity					
	No dysfunction	38(76.0)	45(90.0)	6.390	0.094+
	1 Dysfunction	4(8.0)	4(8.0)		
	2 Dysfunctions	4(8.0)	1(2.0)		
	3 Dysfunctions	4(8.0)	0(0.0)		
Sympathetic parameters					
LF ms^2	No dysfunction	40(80.0)	48(96.0)	**6.061**	**0.014***
	Dysfunction	10(20.0)	2(4.0)		
VLF ms^2	No dysfunction	44(88.0)	48(96.0)	2.174	0.140+
	Dysfunction	6(12.0)	2(4.0)		
Overall Sympathetic activity					
	No dysfunction	39(78.0)	46(92.0)	5.976	0.053+
	1 Dysfunction	6(12.0)	4(8.0)		
	2 Dysfunctions	5(10.0)	0(0.0)		
Presence of CAN	Yes	14(28.0)	3(6.0)	**8.575**	**0.003***
	No	36(72.0)	47(94.0)		
	Inferential Statistics Used: Chi-Square Test (*); Fishers Exact Test (+)				

The assessment of CAN using Time and Frequency Domain method (Table 3) presents that the method is inconsistent (significant difference) in determining the SDNN which is both a sympathetic and parasympathetic parameter for assessing CAN. However, considering only the parasympathetic parameters, the TF Domain method was seen to be consistent (no significant difference) in measuring the RMSSD and HF ms^2 ($p>0.05$) while inconsistent in estimating the PNN50 ($p=0.025<0.05$). In overall, it was ascertained that the TF Domain method is consistent in measuring the parasympathetic activity among the HAART naïve HIV Sero Positive Neurologically Asymptomatic patients.

Also, estimating the sympathetic activity, the TF Domain method was seen to be consistent in measuring the VLF ms^2 ($p=0.140>0.05$) while inconsistent in determining the LF ms^2 ($p=0.014<0.05$) across the subjects and control groups. Meanwhile, an overall result depicts consistency of the TF Domain method in measuring the sympathetic activity of the patients.

Conclusively, the final result irrespective of the activity (parasympathetic or sympathetic or both) shows that the Time and Frequency (TF) Domain method is equally a reliable method of ascertaining the level of CAN among HAART naïve HIV Sero Positive Neurologically Asymptomatic patients (Chi-Sq. = 8.575, $p=0.003<0.05$). Hence, the need for comparing the method with the Ewing's method.

The comparative statistics of both the Ewing's and TF Domain methods in assessing CAN among the HAART naïve HIV Sero Positive Neurologically Asymptomatic patients is as presented in figure 1 below.

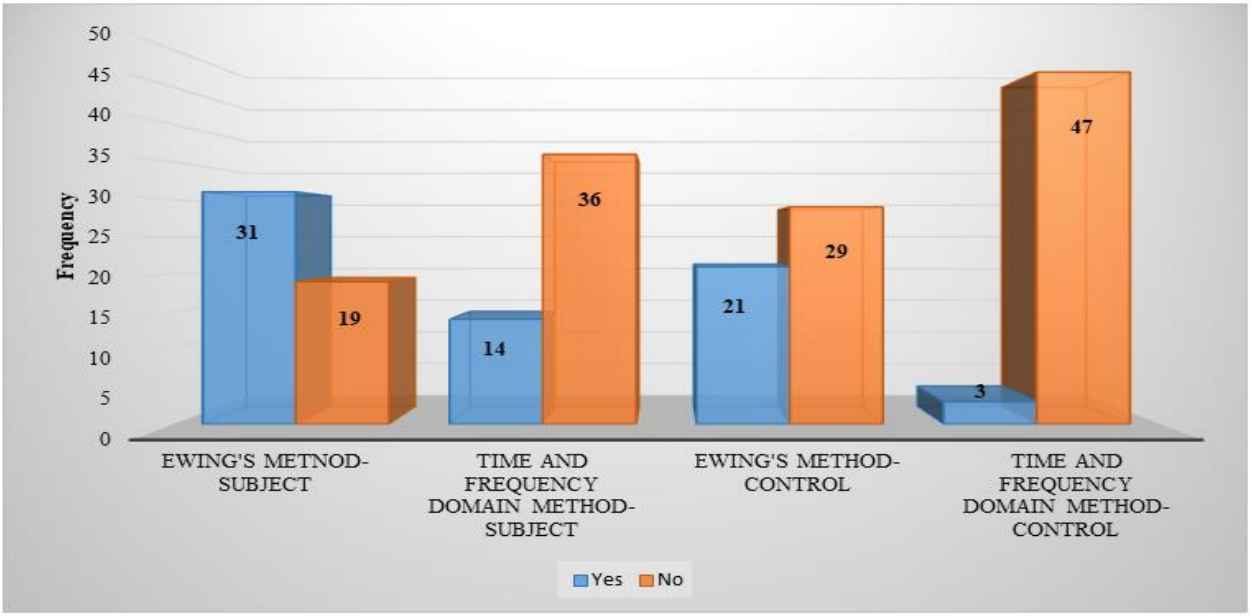

Fig 1 Assessment of CAN using both Ewing's and Time and Frequency Domain Methods

ISSN No:-2456-2165

Comparative assessment of the both methods: the Ewing's and Time and Frequency Domain method as presented (fig. 1) above confirmed that the Ewing's method of determining CAN among HAART naïve HIV Sero Positive Neurologically Asymptomatic patients is better in comparison with the Time and Frequency Domain method. Evidence is as provided by closeness of the variations in 'Yes' and 'No' bars of the Ewing's method across the subjects and control groups with respect to similar socio-demographic characteristics.

Table 4 Stages of CAN in relation to Gender, Age and BMI [using Ewing's method]

		Stages of CAN						Stat. difference	p-value
		Early		Definite		Severe			
Variable	**Parameters**	**Sub.**	**Ctrl**	**Sub.**	**Ctrl**	**Sub.**	**Ctrl**		
Gender	Male	10 (20.0%)	14 (28.0%)	8 (16.0%)	7 (14.0%)	1 (2.0%)	1 (2.0%)	6.146	0.0213
	Female	6 (12.0%)	10 (20.0%)	21 (42.0%)	12 (24.0%)	4 (8.0%)	1 (2.0%)		
	Overall %	**24.0%**	**48.0%**	**58.0%**	**38.0%**	**10.0%**	**4.0%**		
Age	20-29 yrs	4 (8.0%)	7 (14.0%)	6 (12.0%)	5 (10.0%)	2 (4.0%)	0 (0.0%)	1.096	0.3047
	30-39 yrs	9 (18.0%)	5 (10.0%)	13 (26.0%)	10 (20.0%)	0 (0.0%)	1 (2.0%)		
	40-49 yrs	2 (4.0%)	9 (18.0%)	6 (12.0%)	7 (14.0%)	1 (2.0%)	1 (2.0%)		
	50+ yrs	1 (2.0%)	3 (6.0%)	4 (8.0%)	7 (14.0%)	2 (4.0%)	0 (0.0%)		
	Overall %	**32.0%**	**48.0%**	**58.0%**	**58.0%**	**10.0%**	**4.0%**		
BMI		26.13±4.65		23.28±3.09		24.54±2.83		0.674	0.5621

††Sub. Stands for Subject group, Ctrl stands Control Group; Inferential Statistics Used: Chi-Sq. for count data and Kruskal-Wallis test for continuous data

Result of stages of CAN in relation to Gender, Age and BMI using Ewing's method is as presented in table 4 above. Looking at the result based on gender of the patients, a total of 72.0% of the confirmed CAN are in their early stage (subjects: 20% from males and 12% from females; control: 28% from males and 20% from females); a total of 96.0% of the confirmed CAN are in their definite stage (subjects: 16% from males and 42% from females; control: 14% from males and 24% from females); while 14.0% of the confirmed CAN are in their severe stage (subjects: 2% from males and 8% from females; control: 2% from males and 2% from females). However, the statistical differences across the subjects and control groups were confirmed to be significant, an indication that gender is an essential factor in the stage of CAN among HAART naïve HIV Sero Positive Neurologically Asymptomatic patients in the area (Chi-Sq. = 6.146, p=0.0213<0.05).

Based on the age brackets of the patients, a totality of 80% of the identified CAN are in the early stage (subjects: 32%; control: 48%); 116% of 58% each from subjects and control group are at the definite stage, while 14% of 10% from subjects and 4% from control group are in the severe stage. The comparative test shows no significant difference across the ages (Chi-Sq. = 1.096, p=0.3047>0.05).

Finally, estimates based on Body Mass Index (BMI) of the patients shows that CAN at early stage was seen in patients with average BMI of 26.13±4.65; Among people of BMI 23.28±3.09, CAN is still at definite stage, while among patients of BMI 24.54±2.83, CAN was confirmed to be at severe stage. The Kruskal-Wallis test result shows no statistically significant variation with respect to BMI across the stages. The overall comparative result is as presented in figure 2 below:

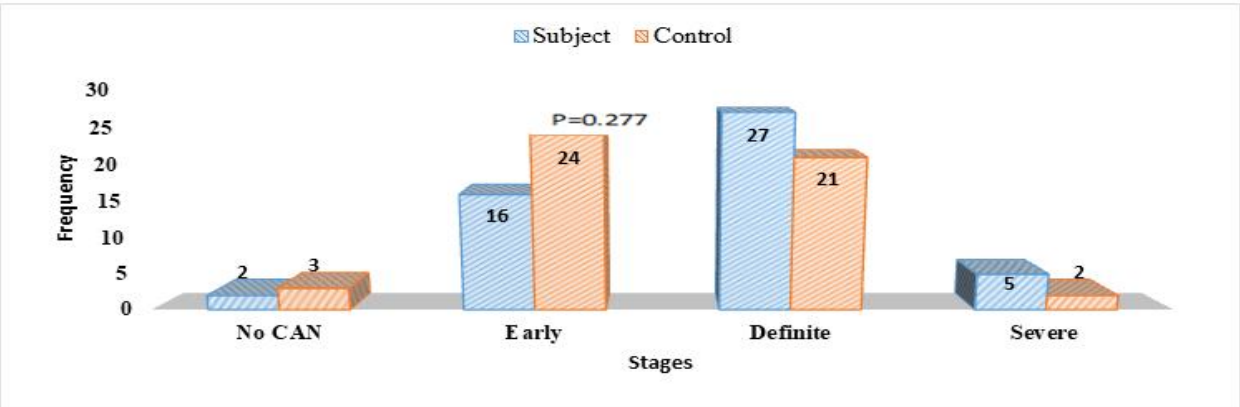

Fig 2 Stages of CAN based on Ewing's Method

ISSN No:-2456-2165

The result in fig. 2 above shows that based on Ewing's proposal, CAN were seen mostly at definite stage for the subjects, followed by early autonomic neuropathy (early stage), then severe stage and finally, the disappearance (No CAN). Meanwhile, for control group, CAN were seen majorly at early stage, followed by definite stage, then severe stage, and lastly No CAN. The result shows no significant variation with respect to the groups (p=0.277>0.05).

➢ *Determinant factors of CAN among HAART naïve HIV Sero Positive Neurologically Asymptomatic Subjects in UNTH Enugu:*

Table 5 Multivariate (Logistic Regression) result of predictive factors of CAN in HAART naïve HIV sero positive neurologically asymptomatic subjects: Using both the Ewing's and Time and Frequency Domain methods

Ewing's test							95% C.I. for EXP (B)	
Parameters	B	S.E.	Wald	df	p-value	Exp. (B)	Lower	Upper
Age<30 years	-0.027	0.028	0.983	1	0.322	0.973	0.922	1.027
Gender(1)	0.947	0.720	1.731	1	0.188	2.577	0.629	10.560
Constant	0.924	1.098	0.708	1	0.400	2.519		
Summary of Model Estimates & Results of Adequacy Tests *Hosmer and Lemeshow (H-L) Test: Chi-Sq. = 39.112, p=0.0026<0.05* *Nagelkerke R-Square = 0.273* *Overall specificity and sensitivity estimate = 39.2%*								
Time & Frequency (TF) Domain test								
Age<30 years	0.020	0.029	0.463	1	0.496	1.020	0.964	1.079
Gender(1)	-0.251	0.728	0.119	1	0.730	0.778	0.187	3.241
Constant	-1.227	1.168	1.104	1	0.293	0.293		
Summary of Model Estimates & Results of Adequacy Tests *Hosmer and Lemeshow (H-L) Test: Chi-Sq. = 21.897, p=0.0164<0.05* *Nagelkerke R-Square = 0.219* *Overall specificity and sensitivity estimate = 22.5%*								

Our result in table 5 above, confirmed that the multivariate logistic model using either of the methods (Ewing's method or TF Domain method) is fit for the data series. In other words, there is no significant variation between the observed and predicted model. The evidence is provided by Hosmer and Lemeshow (H-L) test [Chi-Square (H-L) test stat. = 39.112 & 21.897; p=0.0026<0.05 & p=0.0164<0.05]. However, the explanatory power of the model (Pseudo R-square) as estimated by the Nagelkerke R-Square for the two methods are 0.273 and 0.219 respectively; an indication that the logistic regression models containing age and Gender can only explain about 27.3% and 21.9% respectively of the total variations in CAN among HAART naïve HIV sero positive neurologically asymptomatic patients at UNTH Enugu. Also, the overall percentage estimate from the specificity and sensitivity analysis are 39.2% and 22.5% which are both below the 50.0% cut-off point. We can therefore infer that the models captured only about 39.2% and 22.5% respectively of the predictive factors of CAN among the patients.

Based on the factors in the model, Age (Ewing's: p = 0.322; TF domain: p = 0.496), and gender (Ewing's: p =0.188; TF domain: p =0.730), we can deduce that Age and Gender are positive and insignificant predictors of CAN among HAART naïve HIV sero positive neurologically asymptomatic patients at UNTH Enugu. Meanwhile, the odd ratio (O.R) estimates indicates that the odds of developing CAN does not increase with increase in age and that the odds of developing CAN is same across the gender.

A comparative assessment using the model summary statistics of the both methods confirmed that the Ewing's results are better than the TF Domain method.

IV. DISCUSSION

CAN have previously been described to be associated with HIV infection. Craddock et al[17] first described this problem in 1987, and reported four cases of cardiorespiratory arrest (one ending with the death of the patient) in five AIDS patients who were undergoing fine needle aspiration biopsy of the lung. One of these patients and other four were subsequently identified as affected by cardiovascular autonomic neuropathy (CAN), on the basis of abnormal responses to at least two of three tests of heart rate variability.

➢ *Socio Demographic Characteristics:*

Both groups (Table 1) were properly matched and inferences can be made from the results. The influence of age and gender on cardiac autonomic innervations,[16] was shown to cause differential autonomic tone in both gender, with females having more parasympathetic tone. The study by Abhishekh[16] also noted decreasing vagal tone and sympathetic predominance with increasing age.

➢ *Assessment of CAN Using Ewing's Battery of Five, Cardiovascular Reflex Tests:*

The study (Table 2) showed that the HIV subjects had a significantly higher prevalence of CAN 62%, compared to the controls 42%. This is statistically significant, with p value of 0.045. The results are also similar to the prevalence reported by Craddock[17], to be between 5 and 77%.

The presence of autonomic neuropathy in HIV-positive subjects was first observed almost 30 years ago.[17] Some 19 studies on adult patients have been published on this subject,

which recruited a total of 733 patients: amazingly, prevalence rates ranged between 0 and 100%. In the majority of the studies, patients already on ARV treatment were included, often together with treatment-naïve patients; ARV treatment can alter the response of the autonomic system to different stimuli, and can introduce itself cardiovascular pathology, thus making it important to study patients not on ARV treatment.[13]

In the following three publications, a group of treatment-naïve patients were studied (54 patients in Nzuobontane's study, 53 in Brownley's and 40 in Correia's)[18,19]. The Authors reached opposite conclusions though: Nzuobontane reported autonomic dysfunction to be present in 27.6% of AIDS presenters and 4.2% of HIV positive non-AIDS patients observed in Yaounde (Cameroon); Brownley reported no alteration in autonomic function both in symptomatic and asymptomatic HIV positive subjects observed in Miami (FL-USA), while Correia stated that sympathetic and parasympathetic cardiac dysfunction was present in the whole group of HIV-positive treatment-naïve Brazilian patients.

Different prevalence rates of autonomic cardiac neuropathy were noted in the two African studies done: 84% of the patients studied by Rogstad[15] had at least one abnormal autonomic test, while CAN was present in almost 17% of the group of AIDS and non-AIDS patients studied by Nzuobontane[20] (even though up to 80% of them had some abnormality in the autonomic tests performed).

Both studies report worse results of autonomic test in AIDS patients compared to non-AIDS HIV-positive patients.

Compostella et al[13] recorded a lower prevalence of HIV – related CAN which was present in 30% of the African HIV positive patients observed, with no direct correlation to their immunological status in a study done in African treatment naïve HIV positive patients[13]. However the prevalence of clinical autonomic neuropathy in HIV has been variable depending on HIV disease status and treatment, ranging from 0 to 84%.[19, 21, and 22]

Recently studies have evaluated heart rate variability in HIV infected patients[10, 14, 15] with most showing decreased heart rate variability similar to findings observed in patient with cardiomyopathy[10, 14,15]and also similar to findings observed in this study.

Some studies also found a correlation between HIV infection and specific autonomic alterations in cardiac innervations and consequently, cardiac function[23-25].

It is also noted that the HIV sero positive group significantly differs in the HR response to deep breathing and also in the overall parasympathetic activity using Ewing method, with the least response in heart rate response to standing. Kumar[26] found a significant difference in the heart rate response to deep breathing and least response in heart rate response to standing, but Rogstad et al[15] and Nzuobontane[20] reported no significant difference in heart rate response to deep breathing.

- *Assessment of CAN using the Time and Frequency Domain Measures:*

The frequency of CAN in this study (Table 3) was also noted to be significantly higher in the subjects 28% compared to the control 6%, with a p value of 0.003. The above finding was in agreement with the results from the Ewing score although the percentage of individuals affected is less. It can therefore be inferred from the above that HIV infection was significantly associated with CAN, with lower HRV values. The subjects had significant dysfunction in SDNN, PNN50, $HFms^2$ and $LFms^2$, hence more parasympathetic dysfunction was noted as in the Ewing battery of five. This was similar to the study done by Compostella et al[13] and Chow DC[27]. Both noted a decrease in HF value confirming that in these patients sympathovagal balance was altered, with sympathetic predominance. However comparing both techniques employed in assessing CAN, Ewing's test technically presents to be a more reliable method of assessing CAN (Fig 1).

- *Staging and Predictors of CAN:*

Gender is seen to be an essential factor in the stage of CAN with statistical significance, however overall comparisons show no significant difference in both groups with respect to age, gender and BMI. (Table 4). Differential autonomic tone in both gender, with females having more parasympathetic tone and decreasing vagal tone with sympathetic predominance are seen in increasing age as observed in a similar study.[16] Age and are gender are also shown to be positive but insignificant predictors of CAN in HIV patients.

V. CONCLUSION

Apart from opportunistic infections, cardiac autonomic dysfunction itself can contribute to morbidity and mortality in HIV infection. Clinicians should have an increased awareness of this health challenge to improve outcome of managing HIV patients.

- *Funding Statement:*

This research received no specific grant from any funding agency in the public, commercial or not-for profit sectors.

- *Disclosure:*

There are no competing interests in this research article.

- *Contributor Ship Statement:*

Professor Onwuekwe IO was instrumental to the research topic, planning and supervision of the research as my dissertation for Fellowship in West African College of Physicians. Prof Anisiuba BC and Prof Ulasi I.I aided my access to the cardiax machine and analysis software. Professor Chukwuka C J approved the research at the HIV clinics of the University of Nigeria Teaching Hospital. Dr Nathaniel Ndionuka Anokwute proofread this manuscript.

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