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Relevance of mutation in Myocilin gene as a genetic biomarker of adult-onset primary open-angle glaucoma in an African population

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Abstract
Background*: Glaucoma is a leading cause of irreversible blindness in the world. Primary open angle glaucoma (POAG) disproportionately affects individuals of African ancestry. Many gene linkage-based studies have identified several genes with varying contributions to glaucoma; one of which is mutation in myocilin gene. Identifying genetic biomarkerisdesirable for screening of high-risk individuals and early management to avoid vision loss.*
Objective: *The aim of this study was tofind out the relevance of mutation in myocilin gene as a genetic biomarker of adult-onset primary open-angle glaucoma among indigenes of Rivers State, Nigeria.*
Methodology: *This was a case-control study. Four hundred adult-onset POAG patients attending the Glaucoma Clinic at the University of Port Harcourt Teaching Hospital were compared with 400 age and sex matched phenotypically normal non-glaucoma indigenes of Rivers State, between June and November 2022. Venous blood samples were obtained for genomic analysis from the study population. DNA was extracted; amplified; with specific primers for myocilin using polymerase chain reaction. Single Nucleotide Polymorphism (SNPs) were detected after sequencing. Bioinformatic analyses were done with SMART software. SPSS Version 25 was employed for demographic and inferential statistics.* ***Results:*** *A total of 800 participants aged ≥40 years were recruited. Mean age of the study population was 56.7 ± 9.4years. The prevalence of mutation in the myocilin gene among POAG group was 9.5% and 2.5% among the control group. This observed difference was statistically significant (p=0.000). The sensitivity and specificity were 9.5% and 97.5% respectively. The Positive Predictive Value (PPV) was 79.1% and accuracy 50%.The presence of mutant myocilin gene was4.1x likely to be associated with adult-onset POAG.*
Conclusion: *Mutations in myocilin gene are associated with adult onset POAG and may be useful in as a biomarker for screening in POAGespecially in high-risk population of African descent.*
Keywords: *Myocilin gene mutation, Biomarker, Adult-onset Primary Open Angle Glaucoma, Rivers State.*

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I. Introduction

Glaucoma is a leading cause of irreversible blindness; accounting for approximately 0.3% of blindness worldwide (Bourne et al., 2017; WHO, 2020). It is also the leading cause of irreversible blindness in Nigeria – being responsible for 15-20% of blindness in Nigeria (Abdull et al., 2009). The Africa region has the highest incidence and prevalence of glaucoma (Quigley et al., 2006). Primary open angle glaucoma disproportionately affects individuals of African ancestry and is the most common cause of permanent blindness in Africa (Liu, 2011). Most patients in Africa have poor or inadequate knowledge of glaucoma and therefore present very late for clinical evaluation and treatment. In addition, there is often reluctance in the acceptance of medical or surgical interventions among African populations (Bowman et al., 2010.

Primary open-angle glaucoma is the most prevalent variant in Nigeria; and the Niger Delta Region has the highest number of glaucoma patients in Nigeria – being responsible for 20.8% of bilateral blindness (Pedro-Egbe et al., 2006). POAG is usually asymptomatic until it progresses to irreversible blindness. Blindness from POAG can be prevented if the pre-symptomatic stages are detected early and corresponding adequate treatment instituted.

The etiology of POAG is multifactorial and not well understood, however, several pathogenetic mechanisms have been advanced to explain the optic neuropathy that occurs in primary open -angle glaucoma. Genetic, mechanical, vascular and other interwoven factors are said to influence individual susceptibility to

optic nerve damage (Bowling, 2016). Genetical predisposition has been shown to play an important role in the pathogenesis of POAG (Fan et al., 2010; Fingert, 2011). Many gene linkage-based studies have identified several genes with varying contributions to glaucoma including mutation in myocilin gene. The extent to which mutation in myocilin gene could be used as a biomarker for the screening of high-risk individuals is still been investigated. This study investigates the relevance of mutation in Myocilin gene as a genetic biomarker of adult-onset primary open-angle glaucoma in Rivers State, Nigeria-an African population.

II. Methodology

This was a case-control study. Four hundred adult-onset POAG patients attending the Glaucoma Clinic at the University of Port Harcourt Teaching Hospital were compared with 400 age and sex matched phenotypically normal non-glaucoma indigenes of Rivers State, Nigeria between June and November 2022. Venous blood samples from were obtained for genomic analysis from the study participants. DNA was extracted; amplified; with specific primers for myocilin using polymerase chain reaction [Table 1].

Table 1: Primer sequences for myocilin gene polymerase chain reaction

Primer Name	Primer Sequence	Amplified Sequence Length
Myo-1Fa	5'-CCTCACGTGGCCACCTCTGTC-3'	554 bp
Myo-1Ra	5'-GGTTTCCAGCTGGTCCCGCTC-3'	554 bp
Myo-2F	5'-GCCGGCAGCCTATTTAAATGTC-3'	404 bp
Myo-2R	5'-CCTGCTCTGACAAGGGAACAG-3'	404 bp
Myo-3Fa	5'-GCTGTCACATCTACTGGCTCTG–3'	736 bp
Myo-3Ra	5'-GTCATAAGCAAAGTTGACGGTAGC-3'	736 bp

Single Nucleotide Polymorphism (SNPs) were detected after sequencing [Table 2].. Bioinformatic analyses were done with SMART software for protein domain structure prediction and MEGAX for evolutionary genetic analyses. SPSS Version 25 was employed for demographic and inferential statistics.

Table 2: Mutation Screening by Allele Specific Restriction Digestion

Nucleotide change	Amino acid change	Location	Sequence of primer pairs (5' to 3') and PCR condition	Length of PCR product (bp)
144 G->T	Gln48His	Exon 1	CTTCTGTGCACGTTGCTGCA CTGGTCCAAGGTCAATTGGT 94 °C 30 s, 52 °C 30 s, 72 °C 60 s for 30 cycles using 1 mM $MgCl_2$	313
1109 C->T	Pro370Leu	Exon 3	ATACTGCCTAGGCCACTGGA CAATGTCCGTGTAGCCACC 94 °C 30 s, 58 °C 30 s, 72 °C 60 s for 35 cycles using 1 mM $MgCl_2$	198

III. Results

Sociodemographic characteristics of the study population

The male to female ratio was 1:1, with a mean age in both groups of 56.7 ± 9.4 years, and an age range of 40 to 93 years. The modal age was 55-64 years accounting for 14.4% of the study population in each of the two groups. The difference in the ages of the participants in the two groups was not statistically significant (p=1.000) [Table 3].

Table 3: Sociodemographic characteristics of the study subjects

Variables	Distribution in Adult onset POAG cases n=400		Distribution in Normal subjects n=400		Total	(%)	Chi-Square Value	p-Value
	(n)	(%)	(n)	(%)				
Gender								
Male	200	(25.0)	200	(25.0)	400	(50)		
Female	200	(25.0)	200	(25.0)	400	(50)		
Total	**400**	**(50)**	**400**	**(50)**	**800**	**(100)**		
Age Group (Years)								
40-54	91	(11.4)	91	(11.4)				
55-64	115	(14.4)	115	(14.4)				
65-74	110	(13.7)	110	(13.7)				
75-84	52	(6.5)	52	(6.5)				
85-Above	32	(4.0)	32	(4.0)				
Total	400	(50)	400	(50)	800	(100)		

Mean age = 56.7 ± 9.4 years	**Age Range 40 to 93 years**	**0.000**	**1.000**

Ethnicity of the study participants

The participants of this study were from the following ethnic groups in Rivers State: Andoni, Ekpeye, Engenni, Etche, Igbani, Ikwerre, Kalabari, Ogba, Okrika, and Ogoni with equal number of participants represented from each participating LGA[Figure 1].

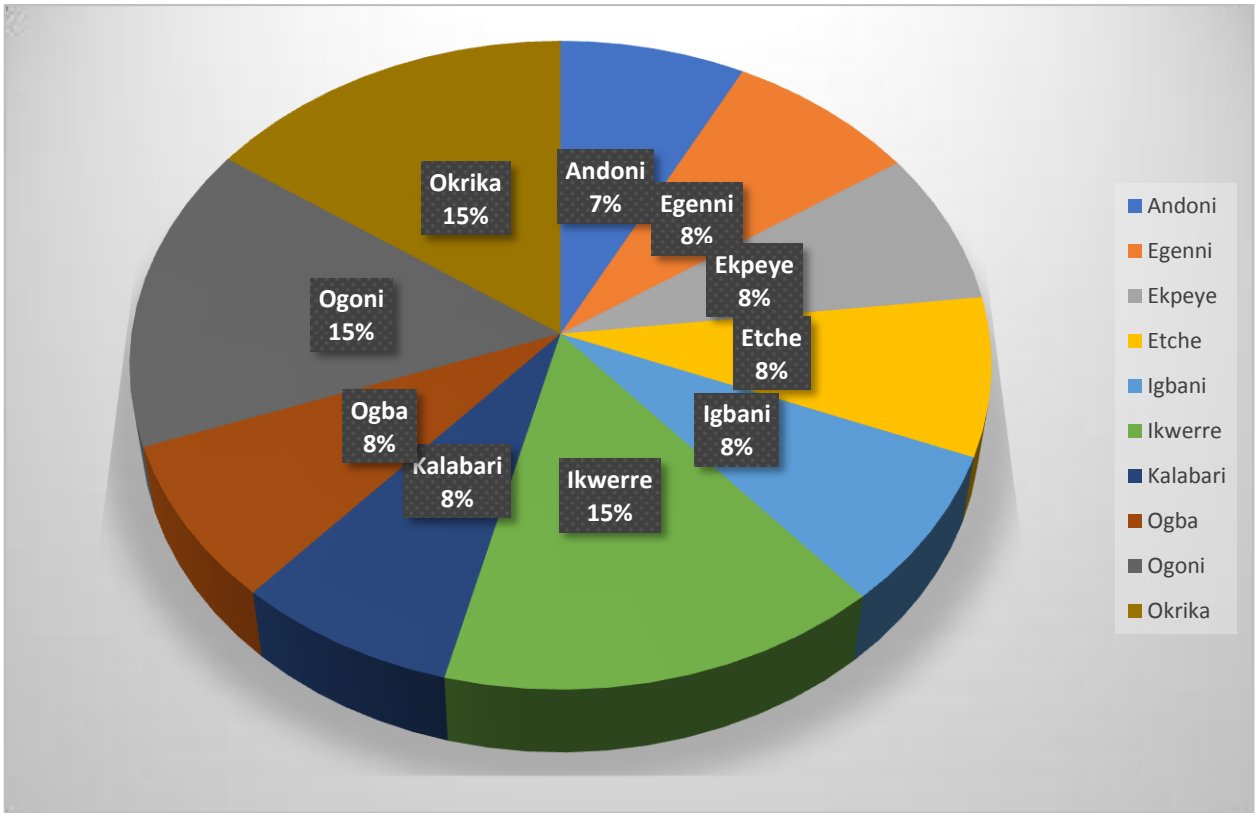

Figure 1: Ethnicity of the population studied

Table 4: Mutation Analysis of Single Nucleotide Polymorphisms (SNPs) in Myocilin Gene among the Study Population

S/N	Position in Genome	Mutation	POAG patients N (%)	Non-Glaucoma Subjects N (%)	Allelic Frequency Aden (%)	Thym (%	Consequences	Impact	Feature Type	Remark
1	Chrom 1: 171638779	A>T	15(3.8)	-	0.79	0.21	Missense Variant	Moderate	Transcript	Novel
2	Chrom 1: 171638703	A>T	8 (2.0)	-	0.74	0.26	Intron Variant	Moderate	Transcript	Novel
3	Chrom 1: 171638610	A>T	10 (2.5)	-	0.84	0.16	3 prime UTR variant	Moderate	Transcript	
4	Chrom 1: 171638608	G>A	5 (1.2)	10 (2.5)	0.88	0.12	Synonymous Variant	Low	Transcript	
	Total		**38(9.5)**	**10 (2.5)**			**p-value= 0.000**			

Table 5: Prevalence of mutation in myocilin gene in the study population of both groups

	Mutation in Myocilin gene PRESENT	Mutation in Myocilin gene ABSENT	TOTAL	Prevalence
POAG Group	38	362	400	9.5%
Non-Glaucoma Group	10	390	400	2.5%
TOTAL	48	744	786	

Chi-Square Goodness of Fit Test =21.772 df = 2 p-value = 0.000

Measure of Association of Myocilin Gene Mutation with adult-onset POAG-Odds Ratio (Prevalence Odds Ratio)

An odds ratio is a statistic that quantifies the strength of association between two events: the presence of mutation in myocilin gene and the POAG. It indicates how much higher the odds of the mutant myocilin gene are among the POAG cases than in the normal phenotypically non-glaucoma cases. In this study, The Prevalence Odds Ratio or the likelihood of individuals with mutations in myocilin gene; having adult-onset POAG was 4.1; implying that mutant myocilin gene is 4.1 x likely to be associated with adult-onset POAG. This was statistically significant (p=0.000) [Table 5].

Table 5: Prevalence Odds Ratio of Mutant Myocilin Gene

	Adult-onset POAG	No POAG	Total
Mutant Myocilin Gene Present	38	10	48
Mutant Myocilin Gene Absent	362	390	752
Total	400	400	800
Pearson Chi-Square 405.674; p=0.000			

Prevalence Odds Ratio (POR) = $\frac{38\ x\ 390}{10\ x\ 362} = \frac{14{,}820}{3{,}620}$ = 4.1. The presence of mutant myocilin gene is 4.1x likely to be associated with adult-onset POAG.

Possibility of Using Myocilin Gene Mutation as a Screening Test for Adult-Onset POAG

The sensitivity and specificity of using mutation in myocilin gene for detecting cases of adult-onset POAG in the study population was 9.5% and 97.5% respectively. The Positive Predictive Value (PPV) in this study was 79.1% and accuracy 50% [Table 6].

Table 6: Screening Test for adult-onset POAG using Myocilin Gene Mutation as a Biomarker

Screening Test (Mutation in myocilin Gene)	**Gold Standard (Clinical Assessment) Diagnosis of POAG**		**TOTAL**
	Disease (+)	**Disease (-ve)**	
Positive	38 [True Positives (TP)]	10 [False Positive (FP)]	48 (TP + FP)
Negative	362 [False Negative (FN)]	390 [True Negative (TN)]	752 (FN + TN)
TOTAL	**400 (TP + FN)**	**400 (FP + TN)**	**800 (TP +FP +FN +TN)**

Sensitivity $= \frac{TP}{TP+FN}$ X 100 $= \frac{38}{400}$ X 100 = 9.5%

Specificity $= \frac{TN}{TN+FP}$ X 100 $= \frac{390}{400}$ X 100 = 97.5%

Positive Predictive Value $= \frac{TP}{TP+FP}$ X 100 $= \frac{38}{48}$ X 100 = 79.1%

Negative Predictive Value $= \frac{TN}{TN+FN}$ X 100 $= \frac{390}{752}$ X 100 = 51.8%

Likelihood Ratio Positive $= \frac{Sensitivity}{1-Specificity} = \frac{0.095}{1-0.97} = \frac{0.095}{0.03} = 3.2$

Likelihood Ratio Negative$= 1 - \frac{Sensitivity}{Specificity} = 1 - \frac{0.095}{0.975} = 1 - 0.097 = 0.9$

Odd Ratio $= \frac{LR+}{LR-} = \frac{3.2}{0.9} = 3.6$

Youden's Index = (Sensitivity + Specificity) – 1

= (0.095 + 0.97) - 1

= 1.065 – 1

= 0.065

Accuracy $= \frac{TP+FN}{Total}$ X 100 $= \frac{400}{800}$ X 100 = 50%

IV. Discussion

This work is a case-control study to show the relevance or otherwise of myocilin gene mutation in the diagnosis of adult-onset primary open angle glaucoma. Four hundred (n=400; 50%) established cases of adult-onset primary open angle glaucoma undergoing various treatments in the Ophthalmology Clinic of the University of Port Harcourt Teaching Hospital, were compared with 400 (n=400; 50%) age and sex matched phenotypically normal non-glaucoma subjects who are indigenes of Rivers State. The two groups were identical in age, sex and racial characteristics; thereby minimizing the influence of some inherent confounding factors.

Socio-demographic characteristics of the study population

Age and Sex Characteristics

Adult-onset primary open-angle glaucoma occurs from the age of 40 years (Allen et al., 2015; Fan et al., 2010; Kyari et al., 2015; Awoyesuku et al., 2012). Working independently and in different periods of time, Murdoch et al., in a study among 1563 people of Hausa/Fulani ethnic extraction of Nigeria; reported that POAG was more prevalent in individuals aged 45 years and older (Murdoch et al., 2001) while Adeoye in South Western region of Nigeria observed that POAG was more prevalent in individuals aged 50 years and older; and that POAG accounted for 11.1% of blindness in Nigeria (Adeoye, 2001).

Corroborating with the findings of this work are the works of Leske et al., (1994) in the Barbados Eye Study which observed that adult-onset POAG was predominately in populations 45 years and older and that POAG significantly increases with age in all populations.

In this research, we recruited equal participants aged 40 years and older of both sexes and age-matched populations of the same ethnic and socio-cultural background. This was deliberate as the study-design from the onset was intended to eliminate influences from differences in age, sex and racial identities in the two groups. Moreso, it was intended to make the comparative groups as similar in characteristic features as possible, thereby achieving some level of homogeneity.

The participants of this study were from the following ethnic groups in Rivers State with similar socio-cultural background: Andoni, Ekpeye, Engeni, Etche, Igbani, Ikwerre, Kalabari, Ogba, Okrika, and Ogoni. We recruited equal number of participants in the various local government areas giving a good and wide-spread of the study population - both riverine and upland communities.

Mutation in the Tertiary Structure of Myocilin Protein in the Study Population

This study noted a prevalence of mutations in the myocilin gene of 9.5%% in the glaucoma group and 2.5% in the control group. The observed differences in the mutation points in POAG patients and non-glaucoma subjects was statistically different (p=0.000). The mutations were observed in exon-2, anserine (ASN) was replaced by valine (VAL) and as a consequence, the amino acid adenine was replaced by thymine in the genomic sequence. This alteration in the amino acid sequence of myocilin protein could be responsible for its altered physiological function. This assertion needs further investigations as gene variants (mutations) are known to prevent one or more proteins from working properly.

Although the role of myocilin in the pathogenesis of glaucoma is currently debated, its mutation is known to be associated with adult-onset POAG. The secretion of wild-type myocilin is inhibited in the presence of co-expressed mutant myocilin and its aggregation in the endothelial reticulum (ER) is induced by the presence of mutant myocilin protein (Caballero et al., 2000; Jacobson et al., 2001). The aggregation of wild-type/mutant myocilin in the anterior chamber is harmful to trabecular meshwork (TM) cells and leads to over proliferation of MYOC thereby hastening the process of apoptosis (Liu et al., 2004; Joe et al., 2003).

There is growing evidence in the body of knowledge that paucity of normal myocilin (Wiggs et al., 2001; Kim et al., 2001; Lam et al., 2000; Gould et al., 2004) or its overexpression are associated with the expression of mutant/misfolded myocilin (Joe et al., 2015) which play vital roles in the morphological changes in the TM and the process of cell apoptosis (Hamanaka et al., 2017).

In this study, the chromosomal location of the mutant myocilin gene associated with adult onset POAG was in chromosome 1-GLC1A. This agrees with the work of Stone et al. Stone et al., in 1997 first identified and reported the association of mutations in myocilin with POAG mapped to the GLC1A locus at 1q24.3-q25.2 - OMIM: 601652 (Hewitt et al., 2008). However, this study found 4 single nucleotide polymorphisms associated with mutations in the myocilin gene in the adult-onset primary open angle glaucoma subjects (chromosome 1: 171638779; chromosome 1: 171638703; chromosome 1: 171638610 chromosome 1: 171638608), thus corroborating polygenetic etiology of adult-onset primary open-angle glaucoma (Wang et al., 2018). This finding also compares well with the results in Pakistan who reported a novel SNP rs879255525 in a mutant myocilin gene associated with glaucoma that varied significantly between POAG patients and controls ($p<0.01$) (Nazir et al., 2018).

In the study of Nazir et al., the change in the nucleotide sequence of rs74315341 resulted in the substitution of serine for arginine and the change in rs879255525 resulted in the substitution of asparagine for lysine.The study of Nazir et al. showed the replacement of guanine with thymidine. Nazir et al utilized a case-control epidemiological method with 100 patients and 100 controls subjects (40 males and 55 females had positive family histories of glaucoma, whereas none of the control subjects had a positive family history).

Our work compares well with the works of Challa et al. in Accra, Ghana and Fingert et al., in Iowa, United States of America. Challa et al. in the Ghanaian study observed that 4 individuals with severe adult-onset POAG had novel missense mutations in exon 3.

Myocilin has three exons (the sequence of DNA present in messenger RNA, some of which encodes the amino acids of a protein) and contains two major homology regions, the N- and C-terminus (Aroca-Aguilar et al., 2005; Yang et al., 2015; Wang et al., 2018). Notably, majority of myocilin mutations are localized in exon

3, which encodes a 504-amino acid glycoprotein (Yang et al., 2015). This position supports the findings in this research. Over 278 different myocilin mutations have been reported, among which pathogenic mutations account for 37.77% (Aroca-Aguilar et al., 2005; Hewitt et al., 2008).

Results from this work corroborates the findings of Challa et al. in Ghana which reported mutation whereby aspartate was replaced by lysine in the adult onset POAG group and this was not detected in 152 ethnically matched control subjects. Also in the Ghanaian study, 14 adult-onset POAG individuals and 8 non-glaucoma subjects exhibited a translationally silent polymorphism in codon 325 (Thr325Thr).

Possibility of Using Myocilin Gene Mutation as a Screening Tool for Adult-Onset POAG

Screening in epidemiology is an active search for disease among apparently healthy people (Dobrow et al., 2018). Screening test is done to detect potential health disorders or diseases in people who do not have any symptoms of disease. The goal is early detection and lifestyle changes or surveillance, to reduce the risk of disease, or to detect it early enough and commence most effective treatment (Dobrow et al., 2018).

While screening for glaucoma in populations at risk and specifically adult-onset glaucoma sounds a reasonable idea and continues to draw the attention of ophthalmologists and epidemiologists, objective considerations are hereby laid for better understanding and deductive reasoning.

First and foremost, screening involves the specific examination of a population at risk to identify an existing illness at a presymptomatic stage or to identify susceptibility to a particular disease.Applying the consolidated 10 screening principles/criteria of Wilson and Jungner's of screening of diseases, although frosted with limitations are often regarded as the authority for screening decisions (Dobrow et al., 2018); adult-onset POAG may fail to be considered a disease for screening because of the low sensitivity and specificity of the proposed screening tests.

However, the impact of glaucoma as a public health problem is well established. Vision 2020-The Right to Sight (a global program that was established in 1999 by International Agency for prevention of Blindness in partnership with the World Health Organization) identified glaucoma as one of the major leading causes of blindness and therefore a public health issue (https://www.iapb.org). Glaucoma is the second commonest cause of blindness after cataract and a leading cause of irreversible blindness and the World Health Organization (WHO) estimates that over 80 million people are incapacitated by glaucoma (Bourne et al., 2017; WHO, 2020). Because the future prevalence of POAG is likely to increase in developed countries, open-angle glaucoma will become an even greater public health concern, and screening may become crucial to decrease morbidity (Quigley, 2003). Effective screening can be achieved by targeting high-risk populations, such as older people or first-degree relatives of glaucoma patients.

In our study, Myocilin gene mutation test had a low sensitivity of 9.5% and high specificity of 97.5%. Both sensitivity and specificity tests are needed to fully understand the test's strengths as well as its shortcomings. Sensitivity measures how often a test correctly generates a positive result for people who have the condition that's being tested for. The more sensitive a test is, the less likely an individual with a negative test will have the disease and thus the greater the negative predictive value. The more specific the test is, the less likely an individual with a positive test will be free from disease and the greater the positive predictive value.

Sensitivity and specificity are inversely proportional, meaning that as the sensitivity increases, the specificity decreases and vice versa. In this study, we observed a high specificity of 97.5%. This implies that testing for mutation in myocilin gene could identify over 97% of patients who do not have the disease.

The Positive Predictive Value (PPV) in this study was 79.1%. PPV is the percentage of patients with a positive test who actually have the disease. This tells us how many of the test positives are true positives; and if this number is higher (as close to 100 as possible), then it suggests that this new test is doing as good as ′gold standard. ′ Positive and negative predictive values are directly related to the prevalence of the disease in the population. Assuming all other factors remain constant, the PPV will increase with increasing prevalence; and NPV decreases with increase in prevalence (Parikh, et al., 2008).

Diagnostic test accuracy provides evidence on how well a test correctly identifies or rules out disease and informs subsequent decisions about treatment for clinicians. Screening the population for adult onset POAG, using myocilin gene mutation in our study, had an accuracy of 50%. A good diagnostic test should have 70% and above accuracy while 100% is excellent.

Youden's index integrates sensitivity and specificity information under circumstances that emphasize both sensitivity and specificity, with a value that ranges from 0 to 1. It is used to estimate and compare diagnostic accuracies of tests. Perfect tests YI = 1), tests with poor diagnostic accuracy (YI = 0). Youden's index is not sensitive to differences in sensitivity and specificity.It is not affected by disease prevalence. The cut-off points for having an acceptable Youden index is 50%. Any value below 50% denote an overall lack of the diagnostic test to detect either disease or health. Our result yielded 6.5%.

However, our study showed that the Prevalence Odds Ratio or the likelihood of individuals with mutations in myocilin gene; having adult-onset POAG was 4.1; implying that mutant myocilin gene is 4.1 x likely to be associated with adult-onset POAG. This was statistically significant (p=0.000)

From the foregoing and the evidence provided in this study, mutation in myocilin gene does not have sufficient power of accuracy and specificity to be a screening tool for adult-onset POAG. However,mutationin myocilin gene could beused as a screening tool (biomarker) for adult-onset POAG among high-risk subjects(relatives of POAGpatients). This position is shared by the works of Fingert, et al. (2007) and Stone et al. (2012).

By identifying high-risk subjects who carry the mutant myocilin gene, the physicians would know when to recommend closer monitoring and earlier treatment to prevent or minimize vision loss. If mutant myocilin gene is identified in an individual, genetic testing will warrant for the screening of relatives to determine if they also would benefit from close surveillance.

In cases where myocilin gene mutation is negative, the family members can be reassured that their risk of developing POAG is likely no higher than the general population. Also, genetic testing may help in locations with poor economic resources like ours as scarce clinical resources could be directed to those areas that mostly need them (Stone, et al., 2012).

Genetic testing is widely available for myocilin gene mutations for POAG in the United States of America, (Fingert, et al., 2011). Testing unselected patients with POAG for mutations in the gene would result in low yield as only 9.5% of POAG patients had a positive result from this study. Conversely, testing high-risk populations may have a much higher yield and utility. Specifically, patients that are relatives of those known to have mutations in myocilin genecould have as much as 4.1 times risk of having POAG.

V. **Conclusion**:

The presence of mutant myocilin gene is 4.1x likely to be associated with adult-onset POAGand could be useful in as a biomarker for screening in POAG especially among high-risk population of African descent.Therefore,screening forof mutation in Myocilin gene as a genetic biomarker of adult-onset primary open-angle glaucoma is still relevantin contemporary ophthalmic and public heath practice.

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