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Effects of Ivermectin therapy on the sperm functions of Nigerian onchocerciasis patients

Idonije O.B[a]., Asika E.C[b]., Okhiai, O[c] and Nweke I.N[d].

[a] *Department of Chemical Pathology, Ambrose Alli University Ekpoma, Edo State Nigeria*
[b] *Department of Pharmacology & Toxicology, faculty of Pharmacy, Madonna University Elele, Rivers State Nigeria*
[c] *Department of Nursing, Ambrose Alli University, Ekpoma, Edo State, Nigeria*
[d] *Department of pharmacology & Therapeutics, College of Medicine, Abia State University Uturu, Abia State Nigeria*

ABSTRACT

The effect of ivermectin, a broad spectrum antihelminthic on the sperm functions of animal models have been extensively studied, however data on humans are very scanty hence this present study. In this study we screened a total of 385 patients who were diagnosed of onchocerciasis. Out of which, 37 (9.6%) were eligible for further tests, as their sperm counts were normal while the remaining patients had very low sperm counts and were therefore not used for further tests or were too weak after the preliminary screening tests and were not considered eligible for further test/studies. We therefore investigated the effects of ivermectin therapy on the sperm functions of these eligible 37 diagnosed patients of onchocerciasis who were of ages between 28 and 57 years. The sperm functions were assessed via seminal fluid analysis using standard procedure and the following parameters were measured: sperm counts, sperm motility, sperm morphology, sperm volume, sperm viscosity and sperm liquefaction time. The above parameters were measured before and after the patients were treated with 150µg/kg body wt of ivermectin for eleven months and the results were compared and also with normal control reference range. We observed significant reduction in the sperm counts and sperm motility of the patients tested. On the morphology there was significant increase in the number of abnormal sperm cells. This took the forms of two heads, double tails, white (albino) sperms and extraordinarily large heads. It is suspected that the above alterations in the already determined parameters of the patients' sperm cells could only have occurred as a result of their treatment with ivermectin. However, we could not record any significant change or alteration in the sperm viscosity, sperm volume, and sperm liquefaction time of the patients. We therefore suggest that caution be seriously exercised in the treatment of male onchocerciasis patients with ivermectin to avoid the adverse effects it has on the patients' sperm functions.

Keywords: sperm function, ivermectin, onchocerciasis, morphology

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INTRODUCTION

For quite a number of years ivermectin has remained the best drug of choice for the treatment of onchocerciasis which is also known as river blindness. Within this period ivermectin has been

described by clinicians as a safe and effective antihelminthic drug for combating a variety of parasitic nematodes in animals. Its widespread use and therapeutic profile suggested that it might also be beneficial and useful for the treatment of onchocerciasis in humans. Phases one, two and three clinical trials of the drug were all successful and Relative to Diethylcarbamazine (DEC) and placebo, ivermectin resulted in significant and longer lasting decrease in microfilarial skin loads and most adverse effects were mild and non-ocular [1]. Subsequently the effects of ivermectin on microfilaridermia were evaluated with longitudinal skin surveys. The overall effect of ivermectin treatment in the various study areas was reduction of 96 – 99% in skin microfilaria within the first few months of treatment [2]. The multi-year follow up studies have shown a significant impact on decreasing microfilarial skin loads. After 5 years of treatment in some local communities in Enugu and Ebonyi States there was a 90% reduction in skin loads of microfilaria. In Gami-Central African Republic, a drastic reduction in both the prevalence and intensity of *Onchocerca volvulus* microfilaridermia was observed after 5 years of annual treatment in the community with ivermectin [3]. Repeated doses of ivermectin appear to have a cumulative effect and multiple annual doses of ivermectin have resulted in a slower repopulation of the skin by microfilariae [4].

Chijioke and Ononkwo [5] observed that ivermectin is a safe and well-tolerated drug and that the incidence, but not the severity of adverse effects attributed to ivermectin therapy is related to the pre-treatment intensity of microfilariae in the skin.

However, a recent report showed that 85% of all male patients treated in a particular centre with ivermectin in the recent past who went to the laboratory for routine tests were discovered to have developed various forms, grades and degrees of sperm dysfunctions including, low sperm counts, poor sperm morphologies (two heads, Tiny heads Double tails absence of tail's, Albino sperm calls), azoospermia and poor sperm motility [6]. Several studies done on animals also showed similar findings [7, 8]. However, study on human on the effect of ivermectin therapy on male fertility is scanty. It is therefore the aim of this study to investigate the effect of ivermectin on the sperm functions of onchocerciasis patients.

MATERIALS AND METHODS

Subjects

In this study we screened a total of 385 patients who were diagnosed of onchocerciasis. Out of which, 37 (9.6%) were eligible for further tests, as their sperm counts were normal while the remaining patients had very low sperm counts and were therefore not used for further tests or were too weak after the preliminary screening tests and were not considered eligible for further test/studies. We therefore investigated the effects of ivermectin therapy on the sperm functions of these eligible 37 diagnosed patients of onchocerciasis who were of ages between 28 and 57 years. The sperm functions of these thirty-seven (37) onchocerciasis patients were evaluated/analyzed both before and after treatment with ivermectin after informed consent have been obtained from each subjects and the study was conducted in compliance with the Declaration on the Right of the Patient [9].

Collection of specimen and analysis

Collection of Sperm: A minimum of five days period of abstinence from sex was recommended before collecting semen from the subjects. Prolonged abstinence from sexual inter-course was discouraged. The method used in the collection of sperm throughout the work was masturbation. This helped to ensure that all sperms that were released by the subjects were measured. Precaution was taken to see that the semen specimens were collected not within extremes of

temperatures and the specimens were submitted to the laboratory not more than thirty minutes after collection.

Microscopic examination:

(a) **Sperm counts:** the diluting fluid consists of

- Sodium bicarbonate = 5gm
- Formalin Neutral = 1ml
- Distilled water = 100ml

The apparatus used consists of:

- WBC pipette
- Microscope
- Improved Neuber counting chamber
- Test tubes
- Slides and cover slips.
- Wash bottles

Procedures

A WBC pipette was used to draw the semen to the 0.5 mark. The prepared diluting fluid was used to dilute the contents and adequate mixture was achieved. The chamber was then charged and allowed to stay for 2 mins. After the 2 mins the spermatozoa in the chamber have settled and counting was done using the 4 square mm i.e. the 4 large squares. The total number of spermatozoa counted in the 4 large squares was multiplied by 50,000. This gave the number of sperm cells per millilitre of semen.

(b) **Sperm Motility:** A drop of semen was placed on a pre-warmed slide and cover-slipped. The cover slip was ringed with petrolatum. A count of 200 spermatozoa was done of the whole depth of the fluid.

Non-motile sperms that settled at the bottom were also included to assess motility. The percentages of sperms showing actual progressive motion were then recorded.

(c) **Sperm Morphology:** We assessed the sperm morphology by performing differential counts of morphologically normal and abnormal spermatozoa types and stained smears were made on slides as for blood smears.

- The smear was made on slide
- The smear was immediately placed in a fixative- 95% alcohol before drying occurred.
- The film was stained with Papanicolaou staining method.

We then examined 200 spermatozoa under oil emersion. The percentage of the abnormal forms was then noted.

(d) **Sperm viscosity:** Semen was poured from the pipette to a test -tube. The manner of drop was carefully observed. Semen falling by drops was considered of high viscosity- normal. Grading was by (+) (++) (+++) etc. in ascending order of normalcy.

(e) **Semen Liquefaction Time:** the time within which the semen liquefied was assessed and noted in minutes from the collection time.

(f) **Semen Volume:** The volume of the semen from each patient was measured with graduated containers e.g. measuring cylinder of various capacities depending on the size of the semen, the unit of expressing the semen volume was the cubic millimeters or cm^3.

RESULTS

(A) Sperm Counts:

There was a significant drop in the sperm counts of the patients after their treatment with ivermectin. For example in a particular patient the sperm count dropped from 125 million cells per ml of sperm before therapy with ivermectin to 105 million per ml after the treatment *[Normal Control Range = 60 – 120 x 10^6 per ml, see table 1]. There was also a significant reduction in the motility of the cells after the treatment with ivermectin. These reductions were not concurrent, nor where they in proportion to one another. As for the morphology, there was a significant increase in the number of abnormal cells after the treatment with ivermectin *[Normal control Range of normal motility = above 50%, see table 1].

Normal control values for the various semen variables are as shown below in table 1 as this was also used in comparism with the variables analyzed in the test patients.

TABLE 1: Normal values of semen variables (WHO 1992) [10]

Standard tests	
Volume	2.0 ml or more
pH	7.2-8.0
sperm concentration	$20x10^6$ spermatozoa/ml or more
total sperm count	$40x10^6$spermatozoa per ejaculate or more
Motility	50% or more with forward progression(categories a and b)or 25% or more with rapid progression(category a)within 60 minutes of ejaculation
morphology	30% or more with normal forms
Vitality	75% or more live,i.e.,excluding dye
white blood cells	fewer than $1x10^6$/ml
immunobead test	fewer than 20% spermatozoa with adherent particles
MAR test	fewer than 10% spermatozoa with adherent particles
Optional tests	
α -Glucosidase(neutral)	20 mU or more per ejaculate
zinc(total)	2.4 μ -mol or more per ejaculate
citric acid(total)	52 μ -mol or more per ejaculate
acid phosphatase(total)	200 U or more per ejaculate
fructose(total)	13 μ -mol or more per ejaculate

TABLES 2: Summary of sperm counts

S/N	Pre ivermectin therapy Sperm Count (million/ml)	Post ivermectin therapy Sperm Count million/ml
1	125	100
2	60	40
3	79	51
4	80	53
5	113	102
6	94	91
7	55	40
8	82	51
9	48	30
10	67	55
11	83	45
12	94	51
13	130	106
14	150	148
15	160	117
16	168	158
17	93	68

18	101	100
19	125	113
20	128	104
21	120	100
22	138	129
23	77	54
24	150	125
25	67	45
26	79	55
27	128	100
28	123	100
29	153	132
30	110	100
31	123	115
32	119	109
33	134	120
34	140	115
35	148	115
36	98	55
37	120	104

In Table 2 shown above, there is a reduction in the sperm count of all the patients examined ranging from little reduction to much reduction in the sperm count when pre ivermectin and post ivermectin sperm counts are compared.

Also sperm motility was found to reduce drastically (see table 3) when pre ivermectin and post ivermectin motility tests are compared. Sperm with abnormal morphology were also found to have increased after ivermectin therapy as shown in table 4

TABLES 3: Results of sperm motility test

S/N	Pre ivermectin therapy motility (%)	Post ivermectin therapy motility (%)
1	65	25
2	73	61
3	85	40
4	90	53
5	98	17
6	80	39
7	67	50
8	77	43
9	85	60
10	86	68
11	81	15
12.	90	40
13	65	40
14	67	51
15	50	60
16	49	20
17	38	30
18	20	11
19	98	50
20	69	51
21	79	31
22	87	40
23	55	36
24	25	23

25	99	50
26	59	16
27	78	40
28	76	55
29	74	62
30	71	18
31	80	30
32	81	39
33	70	41
34	55	50
35	88	58
36	79	50
37	87	48

TABLE 4: RESULTS OF SPERM MORPHORLOGY TEST

N/S	**Pre ivermectin therapy Morphology (%) Abnormal**	**Post ivermectin therapy Morphology (%) Abnormal**
1.	10	25
2.	15	45
3.	11	38
4.	25	47
5.	40	60
6.	28	75
7.	39	58
8.	43	62
9.	10	75
10.	18	69
11.	15	70
12.	16	55
13.	11	68
14.	21	52
15.	20	44
16.	26	80
17.	28	13
18.	49	57
19.	35	66
20.	11	70
21.	10	59
22.	25	72
23.	26	67
24.	18	54
25.	49	77
26.	49	63
27.	27	48
28.	15	35
29.	18	38
30.	12	15
31.	22	61
32.	36	73
33.	27	49
34.	33	61
35.	20	62
36.	18	35
37.	40	67

TABLE 5: Table showing drop in sperm count following ivermectin therapy

RANGE OF FALL SPERM COUNTS (MILLIONS/ML)	TOTAL % OF THE ENTIRE POPULATION.
20 – 29	2.9 %
30 – 39	20.2 %
40 – 49	17 .2%
50 – 59	17. 2%
60 – 69	20.1 %
70 – 79	2 .2%
80 – 89	17 .3 %
90 – 99	2.9 %
TOTAL	**100 %**

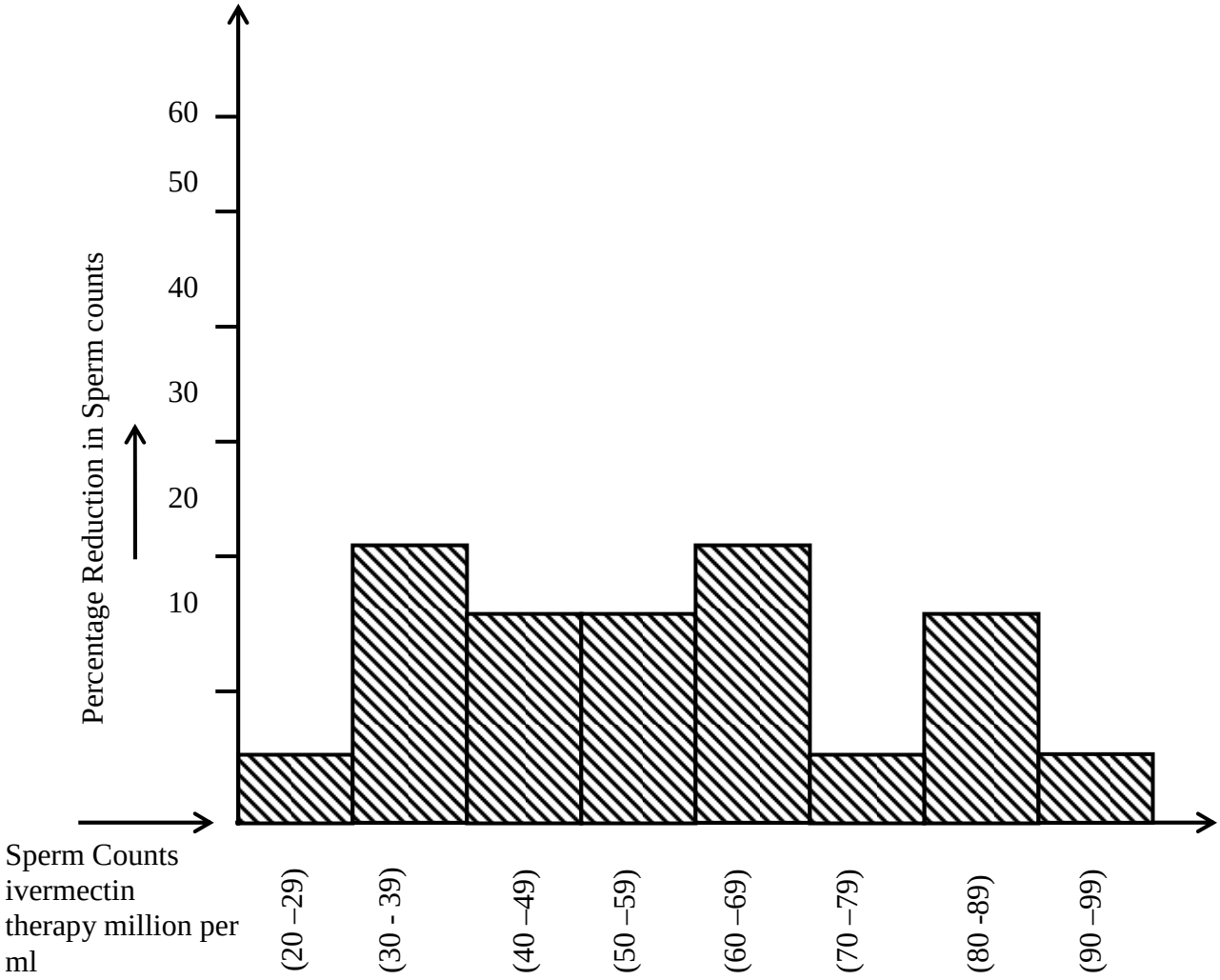

Fig. 1: Drop in sperm count following ivermectin therapy

TABLE 6: Table showing the drop in sperm motility following ivermetin therapy

RANGE OF FALL IN SPERM MOTILITY	TOTAL % OF THE ENTIRE POPULATION
1 – 9	5.7%
10 -1.9	5.7%
20 – 29	22. 9%
30 – 39	8. 6%
40 – 49	20%
50 – 59	17. 1%
60 – 69	8 .6%

70 – 79	5.7%
80 – 89	5.7%
TOTAL	**100%**

TABLE 7: Table showing the increase in the abnormal forms of sperm morphology

RANGE OF INCREASE IN ABNORMALITY	TOTAL % OF THE ENTIRE POPULATION
20 – 29	2.9%
30 – 39	2.9%
40 - 49	5.7%
50 – 59	20%
60 – 69	22.9%
70 – 79	14.3%
80 – 89	22.9%
90 – 99	11.4%
TOTAL	**100%**

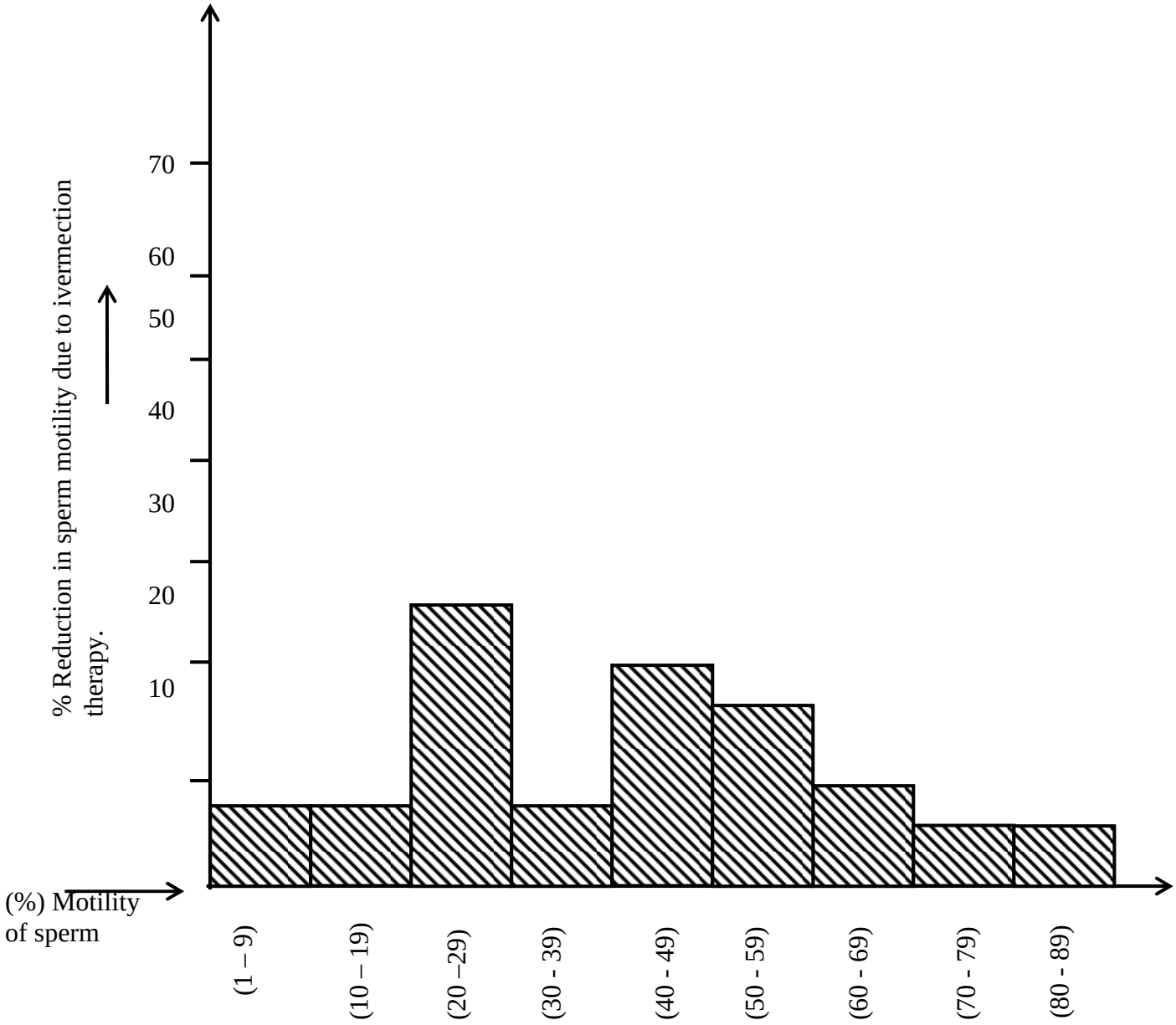

Fig.2: Drop in sperm motility following ivermectin therapy

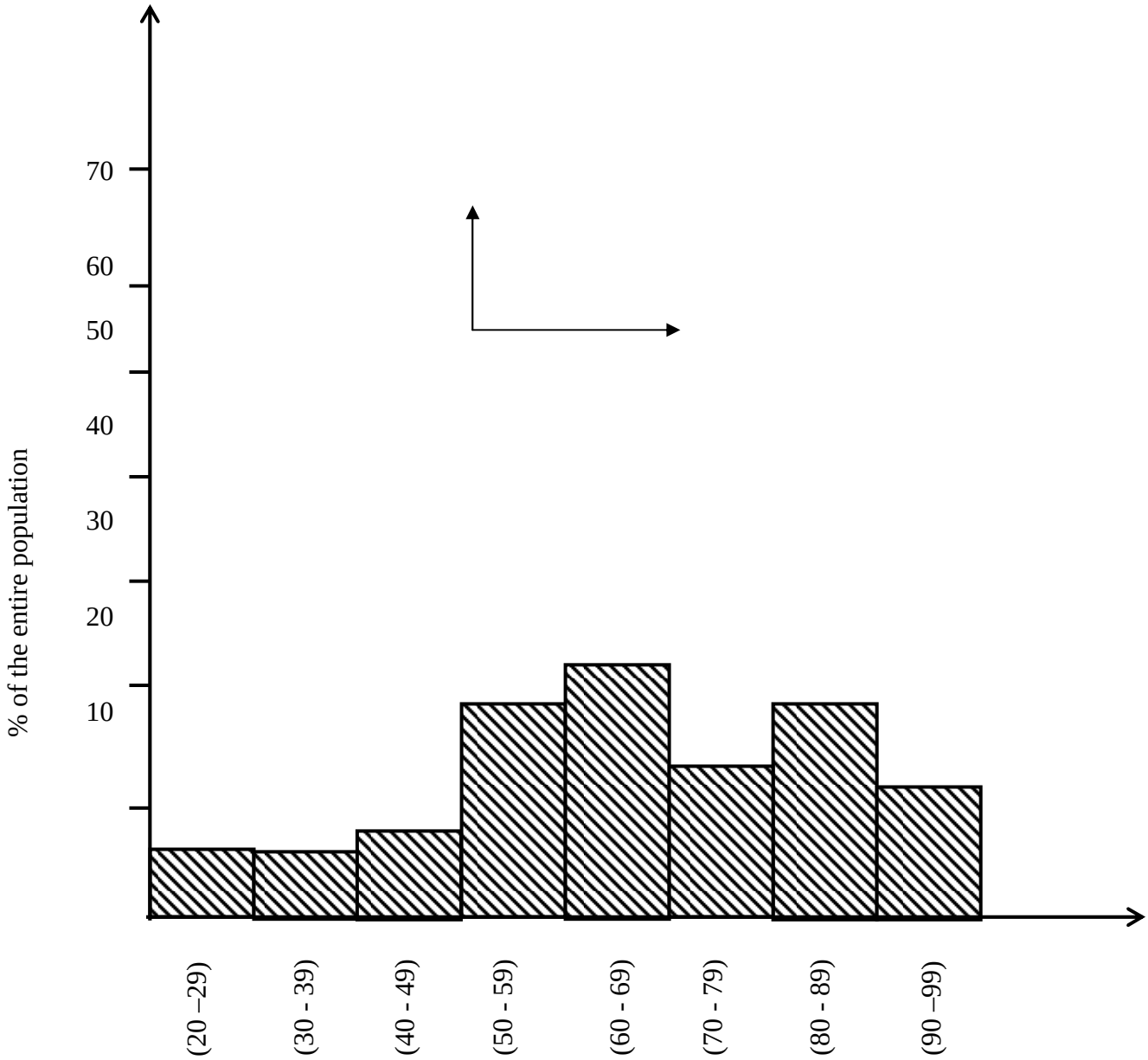

Fig 3: Range of increase in sperm abnormal morphology

Tables 5, 6 and 7, with figures 1, 2 and 3 showed the range of fall in sperm count, motility and increased abnormality in morphology respectively after statistical computation

DISCISSION

For normal fertilization to occur the sperm functions must meet the minimum required sperm functional capacity as shown in table 1[10] which serve as normal control in this study. From the

results obtained, it is evident that ivermectin therapy has significant adverse effects on the sperm functions of male onchocerciasis patients so treated. There was a significant reduction or drop in the sperm counts of the patients after their treatment with ivermectin. Furthermore, the study showed a significant and remarkable drop in the sperm motility of the patients after their treatment with ivermectin. As for the morphology of the sperm, there was a rise in the abnormal sperms after treatment compared with the morphology before the commencement of treatment. These changes no doubt are as results of the effects of the drug on the sperm function of the patients.

Although, there were no noticeable changes in the sperm volumes, sperm viscosity and the sperm liquefaction time the results of this study is enough to cause infertility in these patients.

This is similar to the findings of Tanyıldızı and Bozkurt, [7, 8] in animals, thus, they recommended caution in the use of ivermectin in animals met for breeding.

We therefore recommend caution in the use of ivermectin in male onchocerciasis patient and that further researches be conducted to establish:
a. the mechanism of this adverse effect of ivermectin on the sperm functions of the onchocerciasis patients.
b. whether ivermectin therapy also has adverse effects on other organs of the body such as – the liver, kidney, heart, lungs and red blood cells.
c. At what dosage does ivermectin cause such adverse effects?
And also, pharmaceutical companies and other stakeholders in the Drug Producing Industries may have the need to reformulate the drug – ivermectin- with a view to averting the above side effects.

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