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Fatty Acid Profile Analyses of Three Freshwater Fish Species from Igboho Reservoir, Oyo State, Nigeria

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Abstract: This study investigated the fatty acid profile of three freshwater fish species from igboho reservoir Oyo state Nigeria. This is as a result of a great degree of interest on fatty acids on human health. The fishes used in this study are snakehead (*Parachanna obscura*, Gunther 1844), African mud fish (*Clarias gariepinus*, Burchell, 1822) and African pike (*Hepsetus odoe*, Blotch, 1794). The fatty acid profile analysis showed the existence of Saturated (SFA), Monosaturated (MUFA) and Polysaturated (MUFA) fatty acids. Results obtained showed a wide range of Monounsaturated fatty acids (MUFA) (30.24-33.37%), Saturated fatty acids (SFA) (38.51-53.98%) and Polyunsaturated fatty acid (PUFA) (13.28-24.30%). Palmitic and Stearic acids were the major fatty acids in saturated fatty acid group, Oleic and Palmitoleic acids were the predominant fatty acids in Monosaturated fatty acid group while Docosahexaenoic acid (DHA) and Linoleic acid were the major Polyunsaturated fatty acids. The n-3/n-6 ratio values of the three fish species is below 1.0 which fall within the proposed dietary intake standard (0.25-1.0) and a good indication that the three fish species can supply the required essential acid needed by Man. Moreover, the PUFA/SFA coefficient ratio of P. obscura exceeded the minimum value by HMSO which represents an advantageous impart when consumed by Man.

Keywords: Fatty acid profile, *Clarias gariepinus*, *Parachanna obscura*, *Hepsetus odoe*, Igboho reservoir.

RESEARCH PAPER

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INTRODUCTION

Fish provides important nutrients to large number of people worldwide and this makes a very significant contribution to nutrition (Adewumi *et al*., 2014). The fish muscle which is the segment used for human consumption contains saturated fatty acids (SAF), Monosaturated fatty acids and long chain polyunsaturated acids (PUFA) that have significant role in human health.

Polyunsaturated fatty acids (PUFAs) are commonly categorised into two main groups; Omega-3 (n-3) and Omega 6 (n-6) depending on the position of the first double bond from the methyl-end group of the fatty acid (Abedi & Sahari, 2014).

The main W-3 PUFA playing important role in human health include alpha-linolenic acid (ALA), Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA) and Docosapentaenoic (DPA) and w-6 PUFAs which include Linoleic acid (LA) and Arachidonic acid (ARA) (Abedi & Sahari, 2014). These PUFAs are not synthesized in the human body and therefore inclusion in human diet is a necessity (Jabeen & Chaudhry, 2011; Oyase *et al*., 2016). The chief sources of PUFAs in human diet include fish (Gladyshev *et al*., 2012). Ultimately, it is important to take a proactive approach to ensure sustained access and uptake of PUFAs for proper maintenance of our health (Ozporlak, 2013).

The absorption of fat-soluble Vitamin K, E, A and D from diet and metabolism of cholesterol of the body is also regenerated by the PUFAs (Connor, 2003). N-3 PUFA also play a vital role in the development and function of the nervous system, photo-reception and reproductive system (Alsalvar *et al*., 2002; Sadihu, 2003). Bowman (1980) and Gibson (1983), also confirmed that polyunsaturated synthesis can regulate prostaglandin synthesis and hence induce wound healing. Polyunsaturated fatty acids (PUFAs) are particularly important due to their ability to prevent cardiovascular disease, psychiatric disorders and some other illnesses such as atherosclerosis, thrombogenesis, high blood pressure, cancer and skin diseases (Gladyshev *et al*., 2012). n-3 and n-6 ratio is a useful indicator for comparing the relative nutritional values of different fish oils (Peng *et al*., 2013). The ratio of n-3/n-6 PUFA in lipids of the freshwater fish varies between 0.5 and 3.8 whereas it varies between 4.7 and 14.4 in marine fish

(Guler *et al*., 2008). It was suggested that a ratio of 1.1 or 1.5 would constitute a healthy human diet (Osman *et al*., 2001).

Fish fats also include large amounts of saturated fatty acids mostly associated with tricylglycerols and minor amount of phospholipids (Sahari & Wanasundara, 1998; Quari *et al*., 2014). The PUFA/SFA is used for the assessment of lipids on the basis of the proportions of different fatty acid groups.

The knowledge of fatty acid composition of important commonly consumed fish has received attention of researchers especially in respect of the recent dietary and medical emphasis on the fatty acid in human physiology. However, different species have variation in their fatty acid of composition and levels. The variation in fatty acid of fish is due to diet consumed, reproductive cycle, temperature, season and geographical location (Ozugol & Ozugol, 2007; Hartioglu, 2012; Ozpark, 2013).

Hence, this paper reports a quantitative composition of fatty acids in three freshwater fishes in Igboho reservoir, Igboho Oyo state Nigeria.

MATERIALS AND METHODS

(i) Sampling Site

Fresh fish samples of *P. obscura*; *C. gariepinus* and *H. odoe* were purchased from commercial fish landing at the bank of Igboho reservoir, Igboho Oyo state for an interval of 6 months (January, 2021- June, 2021). The fish samples were transported in an insulated iced container to Biology laboratory The Polytechnic Ibadan, Oyo state. The fish specimens were packed in a separate labelled polythene bags and stored in the freezer pending laboratory analysis.

(ii) Identification of Fish Samples

In the laboratory, the fish samples were identified using the taxonomy keys by Reed *et al*., (1967) Babatunde & Aminu (1998). The identified samples were then labelled in triplicates.

(iii) Storage of Samples

At the laboratory, all the fish were removed from ice boxes, cleaned, washed with fresh tap water to remove all the adhering sand and dust and freeze at -18^{o}C until analysis.

(iv) Fish Samples Preparation

The fish samples were gutted, filleted and muscle tissue were minced for analysis

(v) Chemical Analysis – Total Lipid

Samples were minced using a mincer. Total lipids were extracted from minced samples according to Folch method (Folch *et al*., 1957) using chloroform: methanol (2:1, v/v).

(vi) Methylation of fatty acids and Gas Chromatography (GC)

Fatty acids methyl esters (FAMEs) were performed according to the procedure of (Radwan, 1978). A sample of fish oil (50mg) was transferred into screw-cap vial. 2 ml benzene and 10ml sulfuric acid (1%) in absolute methanol were added. The vial was covered under a stream of nitrogen before heating in an oven 90c for 90 minutes. Ten ml of distilled water were added to the cooled vial and the methyl ester in each vial were extracted with 5 ml of petroleum ether for three times. The three petroleum ether extracts were combined and concentrated to its minimum volume by using a stream of nitrogen. Analysis of fatty acids methyl ester (FAMEs) was carried out by Gas chromatography using (Hewlett Packard, Palo Alto, CA, USA) (HP 6890) and (FID) detector was used at 250^{o}C. The fatty acid methyl siloxane capillary column HP-5 (30m x 0.32 mm I.D.x 0.25µm film thickness) was used. Nitrogen was used as the carrier gas (0.8m / mm gas flow). The injection temperature was 220^{o}c spiltless mode. The temperature program was 200^{o}C zero hold min (10^{o}C/min) until 250^{o}C (5^{o}C/ min) and held at this temperature for 9-minute total run time was 9 minutes. A standard mixture of methyl esters was analyzed under identical condition prior to running the samples. The retention times of the unknown samples of methyl esters were compared with those of standard.

(vii) Statistical Analysis

Data were analyzed by descriptive analysis and one way analysis of ANOVA. Differences were considered significant at an alpha level of 0.05. All means were given with ± standard deviation.

RESULTS AND DISCUSSION

Table 1: Fatty Acid Composition of *Parachanna obscura*, *Clarias gariepinus* and *Hepsetus odoe* in Igboho Reservoir, Oyo State, Nigeria

Fatty Acid	*P. obscura*	*C. gariepinus*	*H. odoe*
C.8: 0 Capric acid	ND	ND	ND
C10:0 Caprylic acid	ND	ND	ND
C12:0 Lauric acid	3.08± 0.02	0.07±0.01	0.13±0.02
C14:0 Myristic acid	4.18±0.02	5.23±0.02	5.81±0.03
C14:1 Myristoleic acid	0.19±0.02	ND	ND
C15:0 Pentadecanoic acid	0.29±0.02	1.40±0.02	1.52±0.03

Fatty Acid	*P. obscura*	*C. gariepinus*	*H. odoe*
C16:0 Palmitic acid	21.69±0.02	32.19±0.03	32.70±0.03
C16:1 Palmitoleic acid	3.61±0.02	13.24±0.02	13.59±0.03
C17:0 Heptadecanoic acid	0.67±0.02	3.07±0.02	3.34±0.03
C17:1 Heptadecenoic acid	0.19±0.03	0.19±0.03	0.31±0.03
C18:0 Stearic acid	7.93±0.02	9.51±0.03	10.31±0.02
C18:1 Oleic acid	25.90±0.02	16.20±0.04	17.18±04
C18:2 Linoleic acid	12.27±0.03	1.40±0.04	1.55±0.02
C18:3 Linolenic acid (Omega 3)	1.04±0.03	0.91±0.03	1.05±0.02
C18:3 Linolenic acid (Omega 6)	0.61±0.03	0.49±0.03	0.62±0.03
C18:4 Octadecatetraenoic acid	1.57±0.02	ND	ND
C20:0 Arachidic acid	0.22±0.01	ND	ND
C20:1 Gadoleic acid	2.47±0.2	0.60±0.03	0.75±0.02
C20:2 Eicosadienoic acid	0.60±.03	ND	ND
C20:3 Eicosatrienoic acid (Omega 3)	0.06±0.02	ND	ND
C20:3 Eicosatrienoic acid (Omega 6)	0.62±0.03	0.30±0.03	0.41±0.03
C20:4 Arachidonic acid (Omega 3)	0.33±0.02	ND	ND
C20:4 Arachidonic acid (Omega 6)	0.59±0.02	0.62±0.02	0.70±0.02
C20:5 Eicosapentaenoic acid (EPA)	1.03±0.02	0.73±0.02	0.81±0.02
C22:0 Behnic acid	0.11±0.03	ND	ND
C22:1 Cetoleic acid	1.29±0.03	0.18±0.03	0.31±0.02
C22:4 Docosatetraenoic acid	0.61±0.03	1.33±0.02	1.48±0.04
C22:5 Clupanodonoicacid	1.05±0.02	3.81±0.02	3.91±0.03
C22:6 Docosahexaenoic acid (DHA)	3.05±0.02	3.51±0.03	3.90±0.03
C24:0 Lignoceric acid	ND	0.07±0.02	0.14±0.02
Total SFA	38.51±0.06	51.54±0.08	53.98±0.10
Total MUFA	32.37±0.06	30.24±0.09	31.83±0.10
Total PUFA	24.39±0.16	13.28±0.13	14.74±0.06
PUFA/SFA	0.63	0.26	0.27
Total Omega 3 w-3	1.43±0.03	0.91±0.03	1.05±0.02
Total Omega 6 w-6	1.81±0.04	1.41±0.04	1.73±0.03
Omega 3/Omega 6 (w-3/w-6)	0.80	0.65	0.61
DHA	3.05±0.02	3.51±0.03	3.90±0.03
EPA	1.03±0.02	0.73±0.02	0.81±0.02
DHA+EPA	4.08 ± 0.04	4.24 ± 0.05	4.71 ± 0.05

Note: Values are percentage of eluted esters using means of triplicate and standard deviation

KEY

ND – Not detected
SFA – Saturated Fatty Acid
MUFA – Monosaturated Fatty Acid
PUFA – Polysaturated Fatty Acid
DHA – Docosahexaenoic Acid
EPA – Eicosapentaenoic Acid

Fatty and composition of *C. gariepinus*, *P. obscura* and *H. odoe* is shown in Table (1). The total fatty acid in the three freshwater fish species were further categorized into saturated fatty acid (SFA), Polyunsaturated and Saturated fatty acid (SFA). The total saturated fatty acid (SFA) ranges from 38.51% in *P. obscura*, 51.54% in *C. gariepinius* and 53.98% in *H. odoe*. However, the most abundant fatty acids were palmitic (C16:0) 21.69 – 32.70% and stearic acid (18.0) 7.93 – 10.31%. The total Polyunsaturated fatty acid (PUFA) ranges 24.39% in *P. obscura*, 13.28% in *C. gariepinus* and 14.74% in *H. odoe*. The most abundant PUFA were Linoleic acid (C18:2) 1.40 – 12.27% and Docosahexaenoic acid (DHA) (22.6) 3.05 – 3.90% and Clupanodonic acid (C22.5) 1.04 – 3.91%. The total Monounsaturated fatty acid (MUFA) ranges from 30.24% in *C. gariepinus*, 31.83% in *H. odoe*, and 32.37% in *P. obscura* while the highest proportion of MUFA were Oleic acid (18.1) 15.20 – 17.18%, Palmitoleic acid (16.1) 3.61 – 13.59% and Gadoleic acid (C20.1) 0.75 – 2.47%. Among the detected SFAs, Palmitic acid (16.0), Stearic (C18.0) and Myristic (C14.0) acids were the major SFAs. These findings are in accordance with the findings of Guil-Guerrero *et al*., 2011 and Alhsan Hammed, 2017. High prevailing quantity of SFAs may be due to less efficiency of fish species in utilizing the SFAs as core energy source which resulted in the rise of SFAs (Nath and Banerjee, 2012). Dominancy of SFAs was found over MUFAs and PUFAs which is similar to the findings of Alhsan Hammed, 2017.

The results showed that SFAs from C8.0 to C24.0 existed among the fish species. Regarding the SFA values, Palmitic acid (C16:0) was the most abundant in the three fish species similar to previous work of Osibona *et al*., 2009, Osibona, 2011; Kumaran *et al*., 2012).

Mystric acid (C14.0) has been implicated in hypercholesterolemia in humans (Fernandes *et al*., 2014), although has low amounts are beneficial to human health.

This study revealed comparable abundance of Stearic acid. Mary, (2008), stated that stearic acid could promote iron utilization in human body.

Mystric acid is a very important fatty acid that stabilizes many different proteins including proteins used in the immune system and to fight tumors – myristoylation, while lauric acid has antimicrobial function and stabilizes certain protein having similar function as Mystric acid and palmitic acid (Erasmus *et al*., 2008; Mary, 2008).

The fish species studied contain appreciable amounts of Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are the document w-3 PUFAs found in the three freshwater fish species. This finding is similar to Zenebe *et al*., 1998 and Gorgun & Akpinar, (2012) who reported that the most abundant FAs in freshwater species are EPA and DHA. The domination of these w-3 PUFAs might be attributed to the feeding habit of the three fish species (Robert *et al*., 2014).

The fish species feed lower in the food chain mainly on microalgae (diatoms and dinoflagellates) which are excellent sources of EPA and DHA. Meziane *et al*., (2007), reported that diatoms and dinoflagellates contain high concentrations of EPA and DHA respectively and have been used as markers of diatoms and dinoflagellates in aquatic food web.

The percentages of Eicosapentaenoic acid (EPA) ranges between 0.73 – 1.03% and docosahexaenoic acid (DHA) ranged between 3.05%-3.90% respectively. Similar to findings reported by Osibona *et al*., 2009; Ozogul & Alagoz, (2007); Memon *et al*., (2010), Moreover, the observed values of Eicosapentaenoic acid (EPA) were found to be lower than the value of docosahexaenoic acid (DHA) in the three fish species. This supports an earlier finding by Taiwo *et al*., (2014).

The proportions of EPA and DHA are responsible for the variations of n-3/n-6 ratio. Both EPA and DHA cannot be synthesized in the human body and thus need to be supplemented through dietary intake (Gunstone, 1996; Alsalvar *et al*., 2002).

Dietary intake of DHA and EPA plays an important role in human health, as preventing several diseases mainly of cardiovascular system (Simpoulos, 2009; Nachi *et al*., 1998). Thus, high values of these PUFAs in human diets are desirable.

EPA is likely to be retained in membranes than DHA (Subhadra *et al*., 2006). This could be the reason for the lower levels of EPA as recorded in this study than DHA in the muscles of the fishes. Thus, the levels of DHA in this current research work suggest that the three fish species can have a healing effect for muscle pain and inflammation (Moshin & Ambak, 1983).

Monosaturated fatty acids (MUFAs) are quite helpful in maintenance of good cholesterol level and prevention of occurrence of prostate cancer, diabetes and obesity with natural effects on the level of cholesterol (Alhsan Hameed *et al*., 2017).

The MUFAs identified during analysis of the three fish species ranged from 30.24% to 32.37%. The most abundant MUFA in all the fish species was Oleic acid (C 18:1) which is related to the findings of Rodrigues *et al*., (2019).

P. obscura contained higher amount of oleic acid than other fish species analysed. Oleic acid in fish is directly related to dietary fatty acid content (Ackman, 1989) and depends on the metabolism of each species which may lead to variations in the final fatty acid amount in fish fillets.

Moreover, Oleic acid is used by the body to fight inflammation to reduce arthrosclerosis, to maintain blood sugar balance and to boost the immune system (Wendy, 2006). Under the action of ACAT (acylCoA-Cholesterol acyltransferase, oleic acid binds to cholesterol (Legrand, 2007). The results of the fatty acid profile also revealed that Myristoleic acid (14:1), a promising antiprostatic cancer therapeutic fatty acid (Iguchi *et al*., 2001) was detected in *P. obscura* (0.19%) whereas Palmitoleic acid (16:1) was detected in all fish species examined. Epidemiological reports disclosed that Palmitoleic acid involves in hemostasis, cholesterol metabolism and insulin sensitivity, lower risk of diabetes, lower levels of inflammation with net mixed effects on serum lipid (Bernstein *et al*., 2014).

Previous studies conducted on freshwater fish species such as *L. rohita*, *C. mrigala*, *C. catla* (Memon *et al*., 2010), *O. mykiss* (Rebole *et al*., 2015) as well as *Cichla* sp (Inhamus *et al*., 2009); *Prochlodus* spp (Luzia, 2003) and *P. corroscans* Andrade *et al*., 1995 revealed high Palmitic acid (16:0) and Oleic acid (18:1) contents which corroborate the findings of this present work in respect of *P. obscura*, *C. gariepinus* and *H. odoe* from Igboho Reservoir, Oyo State.

All fish species demonstrated high PUFA content which is corroborated with previous studies conducted on freshwater fish species from River Niger, Edo State, Nigeria (Oyase *et al*., 2016). However, the relative distribution of fatty acids in all the three fish species studied showed that P. obscura was the richest in Polyunsaturated fatty acid (PUFA) with a value of

24.39%; *H. odoe* (14.74%) and *C. gariepinus* with a value of 13.28%.

Linoleic acid was the dominant Polyunsaturated fatty acid with a value of (12.27%) in *P. obscura*. Similar to the previous work of Effiong & Fakunle, (2015) that reported 13.52% Linoleic acid in *C. gariepinus* visceral oil and also a similar report by Pourshamisin *et al*., (2012).

Wendy, (2006) stated that Linoleic acid get converted in the body to a substance which help to regulate inflammation and blood pressure as well as heart, gastrointestinal and kidney functions. Moreover, excessive intake of Linoleic acid in humans leads to synthesis of alpha-linoleic (ALA) that is beneficial as eisosapentaenoic acid (EPA) and decosahexaenoic acid DHA (Oyase *et al*., 2016). Das, (2008) earlier reported that direct intake of various PUFAs alter the cell membrane fatty acid composition, which in turn modulates cell tissue response to infection, injury and inflammatory actions.

This present research work further revealed that P. obscura contained Arachidonic acid (C 20:4) a precursor for prostaglandin and thromboxane biosynthesis (Pompela *et al*., 2002) although the level of this acid in P. obscura was very low (0.33%). Arachidonic acid can interfere with the blood clotting process and usually attach to endothelial cells during wound healing (Abd-Rahman *et al*., 1995; Bownman *et al*., 1980). Also, this acid plays a role in growth.

The n-3: n-6 fatty acid ratio has been suggested to be a useful indicator for comparing relative nutritional value of fish oils (Osibona, 2009) Zhang *et al*., (2012), affirmed that nutrional composition indices are important in determining the n-3/n-6 PUFA ratio.

The ratio of n-3: n-6 fatty acids in freshwater fish ranges between 0.5 and 3.8 whereas it ranges between 4.7 and 14.4 in marine fish (Henderson & Tocher, 1987). However, it was suggested that a ratio of 1.1 or 1.5 would constitute a healthy human diet (Kleimenov, 1971; Osman *et al*., 2001).

There are different reports with some differences in the optimal ratio recommendations for healthy human diets. Sergent, (1977), recommended an optimal ratio of 0.2 while Simopoulos, (2008), indicated that optimal ratio may vary in consideration of complexities and differences of diseases, hence n-3/n-6 ratio range of 0.25 to 1.0 was proposed as a dietary intake standard. However, considering the nutritional benefits, the Food and Agriculture Organization and the World Health Organization suggested a high ratio of > 0.2 (FAO, WHO, 1994).

The n-3 and n-6 PUFAs have been discovered to have positive effects on cardiovascular diseases and certain types of cancer (Iwasaki *et al*., 2011). An increase in n-3 / n-6 ratio is essential to help the body to use fatty acids since n-6 FAs could have an antagonist effect with n-3 FAs (Polak - Juszczak and Komar - Symczak, 2009).

Generally, fish lipids have much higher n-3/n-6 ratio then recommended (1.5) and from a physiological stand point, this is highly beneficial and desirable for daily human diet. A balanced n-3/n-6 ratio in the diet is essential for normal growth and development and may play an important role in the prevention of coronary artery disease, diabetes, hypertension and cancer, rheumatoid arthritis (Kinsella *et al*., 1990). Moreover, they also affect neuron development in infants (Montano *et al*., 2001). Hitherto, n-3/n-6 polyunstaurated fatty acids are considered essential but since they cannot be synthesized in human body, they must be obtained through diet (Mahan and Escott Stump, 2005). Thus, the increase of fish consumption can improve the quality of diets, decreasing the risk of several disorders (Memon *et al*., 2011; Fernandes *et al*., 2014).

The n-3/n-6 ratio obtained was 0.80 for *P. obscura*, 0.65 for *C. gariepinus* and 0.61 for *H. odoe* which were within the recommended ratio for fresh water fish. The results of n-3/n-6 FA ratio were similar to the findings of other studies (Diraman & Dibeklioglu, 2009; Usydus *et al*., 2011. Nevertheless, it has been established that a well-balanced diet of n-3/n-6 FAs (1/1) to (1/2) is recommended (Zhu *et al*., 2010).

This result showed variation in the three fish species as reported by Hearn *et al*., (1987), and is comparable to those detected in the wild fish as reported by Zhang *et al*., (2012), with values that varied from 1.0 to 2.5. This discrepancy can be attributed to the dependence on the available food based on the local where fish were caught with other environmental factors (Zhang *et al*., 2014).

PUFA/SFA has been suggested as useful in evaluations regarding the nutritional quality of fish lipids (Ozogul *et al*., 2007; Mert *et al*., 2015). Values of the PUFA/SFA above 0.45 and below 4.0 respectively have been recommended by the UK department of Health and Social security and the UK Department of Health (HSMO, 1994).

The values obtained in this research work were *P. obscura* (0.63), 0.26 for *C. gariepinus* and 0.27 for *H. odoe*. This finding is similar to report of Osibona *et al*., (2009), that reported PUFA/SFA of 0.60 for *C. gariepinus* and 0.22 for *T. zilli* respectively. However, Salma *et al*., 2016 reported PUFA/SFA range of 1.26 - 1.65 for *Scomber scombus* fillets from South East of Tunisia while Rodrigues *et al*., (2017), recorded PUFA/SFA values of 2.11 - 3.47 for five farmed Brazilian fresh water fish species.

Similar PUFA/SFA results were recorded for *Esox licius* (2.46) (Mert *et al*., 2015) and *Lates niloticius* (3.15) (Ugoala *et al*., 2009).

However, the maintenance of these rates is caused by permanent and balanced fish lipid ingestion and leads prevention of cardiovascular disorders.

CONCLUSION

This study revealed that the three freshwater fish species (*P. obscura*, *C. gariepinus*, *H. odoe*) from Igboho reservoir contain important fatty acids but the extent of their presence differ greatly from one to another. Moreover, these fish species are sources of essential fatty acids particularly Eicosapentaeinoic acid (EPA) and Docosapentaenoic acid needed for good health, prevention and healing of diseases in Man.

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