

## Knowledge, attitude, and practice of physicians towards familial hypercholesterolaemia in north regions of Saudi Arabia

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### Abstract

**Objective:** To assess knowledge, attitude and practice of physicians regarding familial hypercholesterolaemia.

**Method:** The cross-sectional study was conducted from October to December 2022 in Aljouf, Tabouk, Hail and northern border regions of Saudi Arabia after approval from the ethics review committee of Qurayyat Health Affairs. Those included were physicians specialising in familial hypercholesterolaemia and having experience in family medicine, endocrinology, paediatrics, cardiology, internal medicine and obstetrics and gynaecology. Their knowledge, attitude and practice were assessed using a predesigned questionnaire. Data were analysed using SPSS 20.

**Results:** Of the 430 subjects, 220(51.2%) were males, 89(20.7%) were aged 36-40 years, 169(39.3%) were residents, and 146(34%) had experience >15 years. Overall, 270(62.8%), 286(66.5%) and 287(66.7%) subjects did not know about the clinical diagnostic algorithms of Simon Broom, Dutch Lipid Clinic Network and United States MedPed programme investigated criteria, respectively. There were, 149(34.7%) subjects with high awareness level, and the level of awareness was significantly affected by gender, level of training, medical specialty, and familiarity with familial hypercholesteremia ( $p < 0.05$ ). There were 343(79.8%) subjects who said they had never diagnosed familial hypercholesterolaemia.

**Conclusion:** There were knowledge gaps, low awareness, and inappropriate practices related to familial hypercholesterolemia, indicating the need for targeted programmes.

**Key Words:** Familial hypercholesterolemia, Cholesterol, Genetic, Cardiovascular disease.

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### Introduction

Familial hypercholesterolaemia (FH) is one of the most frequent inherited disorders, classified as autosomal dominant, encountered in clinical practice. It is a complicated, genetic, multifactorial disorder characterised by increased serum levels of low-density lipoprotein (LDL) cholesterol, which leads to increased cholesterol precipitation<sup>1,2</sup>. Heterozygous FH (HeFH) prevalence is estimated to be 1 in 500 worldwide<sup>3</sup>, while the majority of global FH cases go undiagnosed<sup>4</sup>. Despite being rare, with a general prevalence of one in a million and being less common than HeFH, homozygous FH is a serious illness that first manifests in infancy<sup>5</sup>.

FH is mostly diagnosed and detected in patients either presenting with ischaemic heart disease (IHD) or having positive family history<sup>6</sup>.

The frequency of FH has currently been estimated to be 1

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in 250 in the United States which accounted for 0.47% of the total population, 1 in 113 in China, and 1 in 353-229 in Australia<sup>7</sup>. However, the severity of the issue is mainly unknown in the Middle East region, including Saudi Arabia<sup>8</sup>. In a meta-analysis comprising 11 million subjects, FH prevalence was estimated 0.32% in the general population<sup>9</sup>. From 17 countries in the Middle East and North Africa (MENA) region, only 57 mutations have been identified, compared to almost 500 reported from three Western countries. The inadequate reporting of FH by general practitioners (GPs) might be addressed, enhancing patient outcomes<sup>10</sup>. GPs requested over 92% of the lipid profiles in the community, indicating that they are crucial in identifying people with FH, with a multi-country study reporting varying rates of FH diagnoses, and studies pointing out that doctors' understanding and awareness of FH were lacking<sup>11,12</sup>. Also, the coronavirus disease-2019 (COVID-19) pandemic affected the detection and screening process for FH worldwide<sup>13</sup>.

Mutations bring on FH in LDL receptor apolipoprotein B, or the proprotein convertase subtilase/kexin type 9 (PCSK9) gene. The prevalence of angina pectoris and cardiovascular disease (CVD) increased by three-fold in patients with FH compared to unaffected persons<sup>14</sup>. Almost 20 million people have FH worldwide, yet the

majority goes undiagnosed due to a lack of national FH guidelines and medical professionals with specialised training in lipid disorders. It is also possible that primary care doctors (PCPs) working in suburban and rural areas lack access to specialised services. As a result, current therapy is inadequate.<sup>15,16</sup> The poor reporting of FH might be addressed by GPs through opportunistic screening, enhancing patient outcomes.<sup>17</sup>

GPs' understanding and awareness of FH can be better. Furthermore, the awareness and knowledge of FH among different specialisations (PCPs versus experts) have been reported to be similar.<sup>18</sup>

Low awareness of CVD genetic risk is shown by underreporting FH mutations in areas with high consanguinity rates.<sup>19</sup> In a study, even though most physicians believed their knowledge of FH ranged from being ordinary to above average, significant gaps were found. While two-thirds of the physicians identified FH properly, only a few were familiar with the prevalence, heredity and description of early CVD.<sup>20</sup>

A study in Saudi Arabia observed a significant knowledge gap regarding FH awareness, practice and detection in Saudi physicians. Only 7.1% of them had an adequate understanding of FH compared to 92.9% who needed better knowledge. Physicians who self-reported having medium or above-average acquaintance with FH had better mean knowledge scores than individuals with below-average familiarity (68.7%). Compared to trainees or registrars, consultant doctors were 4.2 times more familiar with FH. Comparing doctors with and without FH patients under their care, those treating patients with FH had elevated mean knowledge ratings. Additionally, age, degree of training, and number of years in practice significantly impacted physicians' mean knowledge scores.<sup>21</sup>

Another cross-sectional study discovered major deficiency in knowledge, understanding and familiarity with FH among Saudi physicians. Even though 72.4% of doctors said they were at least somewhat familiar with FH, 48.4% of the participants had poor FH knowledge, and only 51.6% had adequate FH knowledge. Also, 65.8% of the doctors said they frequently gathered comprehensive family histories, did physical exams, and closely screened relatives. The knowledge of several clinical algorithms for diagnosing FH patients was quite low (52%). Older participants had more training or years of practice and substantially higher mean FH knowledge and familiarity ratings.<sup>22</sup>

Pang et al. investigated physicians' knowledge,

awareness and attitude related to FH treatment in the Asia-Pacific region, and found significant gaps in physicians' knowledge and awareness of FH. Only 34% of the respondents said they were familiar with FH. Physicians from Japan and China were more likely than those from Vietnam and the Philippines to properly define FH and identify the usual lipid profile, scoring 72% accurate and 65% correct, respectively. About 35% of the doctors were aware of national or international management recommendations, which was much lower than the 61% of physicians from the United Kingdom. Knowledge of prevalence (24%) and inheritability (41%) of FH as well as that of the CVD risk (9%) were low. Most doctors thought statin medication was a suitable cholesterol-lowering treatment (89%) and that laboratory interpretative commentary (81%) helped manage FH.<sup>23</sup>

A study evaluated the awareness, knowledge and perception of FH among practicing doctors in Japan, South Korea and Taiwan. Of the 230 physicians, only 47% were aware of the heritability, 27% knew the prevalence, and 13% were aware of the CVD risk associated with FH. Majority of the doctors (70%) thought they had above-average familiarity with FH. PCPs (59%) and lipid specialists (41%) were considered the best healthcare providers for FH patients, including services for cascade screening. Cardiologists and endocrinologists were thought to have a lower role, while nursing staff had no significant influence. Only 35% of physicians were aware of local specialised clinical care for lipid diseases.<sup>24</sup>

The current study was planned to assess knowledge, attitude and practice of physicians regarding FH in Saudi Arabia.

## Subjects and Methods

The cross-sectional study was conducted from October to December 2022 in Aljouf, Tabouk, Hail and northern border regions of Saudi Arabia. After approval from the ethics review committee of Qurayyat Health Affairs, the sample size was calculated based on literature<sup>21</sup>, with expected FH familiarity being average to above-average at 68%. Margin of error was kept at 5% and confidence interval (CI) 95%. To account for non-response, the sample size was inflated by about 30%.

Those included were physicians working in Saudi Arabia's northern region specialising in FH and having experience in family medicine, endocrinology, paediatrics, cardiology, internal medicine and obstetrics and gynaecology (OB/GYN). Those not meeting the inclusion criteria were excluded.

After taking written informed consent from all the

subjects, data was collected using a 27-item survey questionnaire in the English language, which was distributed both in paper and electronic forms, as preferred by each participant. The questionnaire was adapted from a similar study conducted previously in Saudi Arabia<sup>21</sup>, and was evaluated for appropriateness, accuracy and relevance by a panel of GPs who were also asked to comment on its content.

There were two main components to the questionnaire. The first part related to the physician's demographic information, including gender, level of education and training, years of experience, and medical specialty. The second component explored FH knowledge, awareness and practice. The participants had to choose the accurate response from a list of possible answers to each question. There were no open-ended questions.

Data was analysed using SPSS 20. Data was expressed as frequencies and percentages. The T-test was used to further analyses. P<0.05 was considered significant.

## Results

Of the 430 subjects, 220(51.2%) were males, 89(20.7%)

**Table-1:** Demographic data.

Variables	Description (n=430)
<b>Age</b>	
30 or less	86 (20)
31-35	83 (19.3)
36-40	89 (20.7)
41-45	58 (13.5)
46-50	42 (9.8)
> 50	72 (16.7)
<b>Gender</b>	
Male	220 (51.2)
Female	210 (48.8)
<b>Level of training</b>	
Resident	169 (39.3)
Registrar\ specialist	120 (27.9)
Senior registrar \ specialist	75 (17.4)
Consultant	66 (15.3)
<b>4- Your medical specialty</b>	
Family medicine	143 (33.2)
Internal medicine	104 (24.2)
Paediatrics	57 (13.3)
OB/GYN	48 (11.2)
Endocrinology	41 (9.5)
Cardiology	37 (8.6)
<b>5-Number of years in practice for participants</b>	
1-5	99 (23)
6-10	99 (23)
11-15	86 (20)
> 15	146 (34)

OB/GYN: Obstetrics and gynaecology.

were aged 36-40 years, 169(39.3%) were residents, and 146(34%) had experience >15 years (Table 1).

**Table-2:** Knowledge level of the physicians.

Questions and answers	Description (n=430)
<b>6-How familiar are you with familial hypercholesterolaemia? The following a 10-point scale where 1 means "Not at all familiar" and 10 means "Extremely familiar."</b>	
1-3	153 (35.6)
4-7	210 (48.8)
8-10	67 (15.6)
<b>7- Best description for familial hypercholesterolaemia (FH)</b>	
One of the family members has been diagnosed with a high level of blood cholesterol.	58 (13.5)
A genetic disorder marked by very high levels of blood cholesterol associated with a family history of premature heart disease	197 (45.8)
The presence of multiple lipid abnormalities that caused by genetic disorder	56 (13)
A very rare, fatal genetic condition caused by a very high level of blood cholesterol which may be up to six-fold of the normal level.	65 (15.1)
Don does not know	54 (12.6)
<b>8- What is the prevalence of heterozygous FH among the general population?</b>	
1 in 100 persons	60 (14)
1 in 500 persons	86 (20)
1 in 1000 persons	85 (19.8)
1 in 2000 persons	40 (9.3)
1 in 5000 persons	23 (5.3)
Don't know	136 (31.6)
<b>9- What is the percentage of likelihood that first-degree relatives of an individual who has FH would have FH (i.e., siblings, parents and children)</b>	
0	24 (5.6)
0.25	109 (25.3)
0.5	147 (34.2)
0.75	46 (10.7)
1	24 (5.6)
Don't know	80 (18.6)
<b>10- Across all age groups, how much greater is the risk of untreated FH patients to premature coronary heart disease compared to the normal individuals?</b>	
2 times greater	51 (11.9)
5 times greater	68 (15.8)
10 times greater	104 (24.2)
20 times greater	86 (20)
Don't know	121 (28.1)

There were 210(48.8%) physicians who reported moderate familiarity with FH. The largest proportion of the physicians 197(45.8%) described FH as a genetic disorder marked by very high levels of blood cholesterol associated with a family history of premature heart disease. There were 136(31.6%) who didn't know the prevalence of heterozygous FH among the general population, 80(18.6%) did not know the percentage of likelihood that first-degree relatives of an individual who has FH would have FH, and 121 (28.1%) had no knowledge

of the risk of untreated FH patients to premature CHD compared to the normal individuals (Table 2).

**Table-3:** Practice level of the physicians.

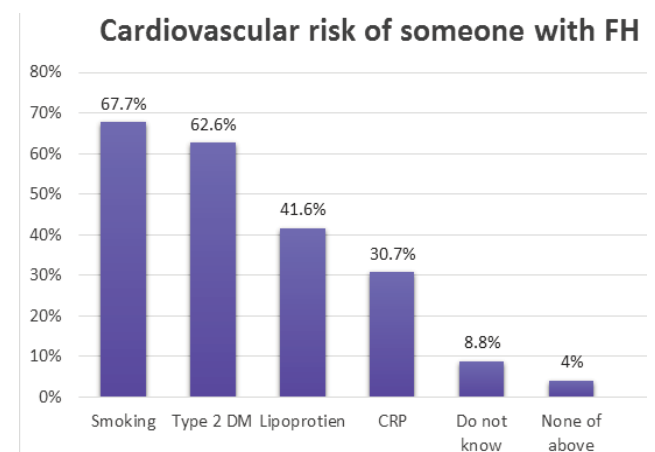
Questions and answers	Description (n=430)
<b>11-Which of the following performs routinely in patients with documented premature coronary artery disease?</b>	
Ask for a detailed family history of coronary artery disease	65 (15.1)
close relatives screening for hypercholesterolaemia	52 (12)
Examine for tendon xanthomata	48 (11.2)
Examine for arcus cornealis	40 (9.3)
All of the above	211 (49.1)
None of the above	14 (3.3)
<b>12-A genetic testing is the only accurate way to diagnose familial hypercholesterolaemia (FH). Is this statement true or false?</b>	
True	137 (31.9)
False	185 (43)
Don't know	108 (25.1)
<b>13- Which, if any, of the following have been recognized to further increase the cardiovascular risk of someone with familial hypercholesterolaemia? Please select all that apply.</b>	
Elevated Lipoprotein	179 (41.6)
Smoking	291 (67.7)
Elevated C-reactive protein	132 (30.7)
Type 2 Diabetes	269 (62.6)
Don't know	38 (8.8)
None of the above	17 (4)
<b>14- Which of the healthcare providers in the following would be most effective at early diagnosis of familial hypercholesterolaemia and screening first-degree relatives? You can select up to two.</b>	
Family medicine \ primary care physicians	297 (69.1)
Lipid specialists Paediatricians	102 (23.7)
Cardiologists	129 (30)
Lipid specialists	104 (24.2)
Endocrinologists	87 (20.2)
OB/GYNs	25 (5.8)
<b>15- Have you ever diagnosed a patient with FH?</b>	
Yes	87 (20.2)
No	343 (79.8)
<b>16- Have you ever followed a patient with FH?</b>	
Yes	162 (37.7)
No	268 (62.3)
<b>17- Based on the following choices, which one could help you in the diagnosis of FH in your field practice?</b>	
Laboratory comment on lipid profile result which alerting a possible FH warning by the clinical healthcare system during the practice	72 (16.7)
A direct contact with the laboratory	53 (12.3)
All of the above	48 (11.2)
None of the Above	182 (42.3)
Do not know	33 (7.7)
<b>18- do you routinely screening the close relatives of any patients with FH by lipid profile?</b>	
Yes, the patient's children and other close relatives	204 (47.4)
Yes, the patient's children only	89 (20.7)
No	61 (14.2)

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NA	76 (17.7)
<b>19- Are you familiar with this Clinical diagnosis approach to diagnose FH patients? [The Simon Broome criteria]</b>	
Yes	160 (37.2)
No	270 (62.8)
<b>19- Are you familiar with this Clinical diagnosis approach to diagnose FH patients? [The Dutch Lipid Clinic Network DLCN criteria]</b>	
Yes	144 (33.5)
No	286 (66.5)
<b>19- Are you familiar with this Clinical diagnosis approach to diagnose FH patients? [The United States MedPed Programme]</b>	
Yes	143 (33.3)
No	287 (66.7)
<b>20-At which age would you screen a young individual for hypercholesterolaemia in a patient with a family history of premature coronary heart disease?</b>	
0-6	60 (14)
7-12	87 (20.2)
13-18	101 (23.5)
None of the Above	105 (24.4)
Don't know	77 (17.9)

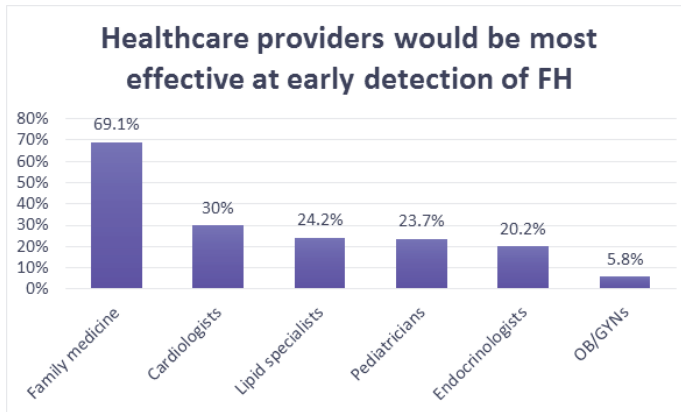
There were 12 questions investigating the practices of the participants (Table 3). Smoking was the major risk factor reported to increase CVD risk of someone with FH (Figure 1). Family medicine and primary care physicians were



**Figure-1:** Factors increasing the cardiovascular risk. FH: Familial hypercholesterolaemia, DM: Diabetes mellitus, CRP: C-reactive protein.

considered to be most effective in terms of early FH detection (Figure 2). Regarding factors that could help in the detection of FH in clinical practice, all choices were selected by 182(42.3%) (Figure 3).

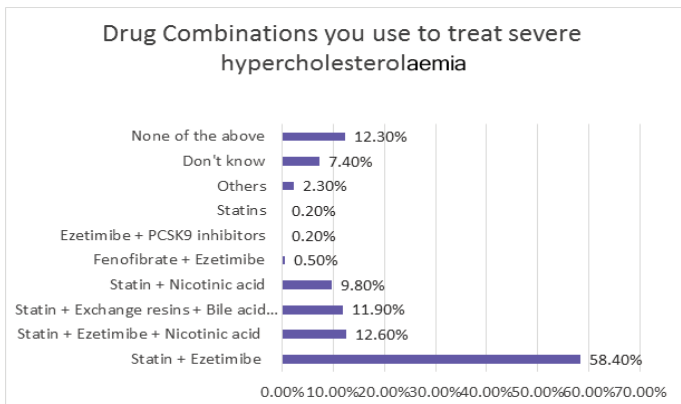
There were 10 questions investigating the participants' level of awareness (Table 4). The most used drug combination to treat severe hypercholesterolaemia was statin and ezetimibe, whereas the least used combination was ezetimibe and PCSK9 inhibitors (Figure 4).



**Figure-2:** Opinion regarding the most effective healthcare providers to detect familial hypercholesterolaemia (FH). OB-GYN: Obstetrics and gynaecology.



**Figure-3:** Choices that could help in the detection of familial hypercholesterolaemia (FH).



**Figure-4:** The most used combination to treat severe hypercholesterolaemia.. PCSK9: Proprotein convertase subtilisin/kexin type 9.

**Table-4:** Awareness level of the physicians

Questions and answers	Description (n=430)
<b>21- Are you familiar with the cascade screening for a patient with familial hypercholesterolaemia?</b>	
Yes	145 (33.7)

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No	285 (66.3)
<b>22- Do you know of any clinical specialist who provides services for lipid disorders to whom you can refer a patient with familial hypercholesterolaemia (FH)?</b>	
Yes	241 (56)
No	189 (44)
<b>23- A low-density lipoprotein (LDL) target for adults with FH is:</b>	
<1.8	60 (14)
<2.5	164 (38.1)
<3.3	72 (16.7)
Don't know	134 (31.2)
<b>24-An LDL target for patients with FH and known case of congenital heart disease (CHD) or diabetes is:</b>	
<1.8	166 (38.6)
<2.5	96 (22.3)
<3.3	40 (9.3)
Don't know	128 (29.8)
<b>25-Patients with FH should receive which one of the following medications as first-line treatment? Please select one option only.</b>	
Statins	220 (51.2)
Ezetimibe	45 (10.5)
Fibrates	38 (8.8)
Exchange resins/bile acid sequestrates	31 (7.2)
Nicotinic acid	22 (5.1)
Don't know	74 (17.2)
<b>26-Which of combinations medications can be used to treat severe hypercholesterolaemia? Please select all that apply.</b>	
Statin + Ezetimibe	251 (58.4)
Statin + Exchange resins/bile acid sequestrates	51 (11.9)
Statin + Ezetimibe+ Nicotinic acid	54 (12.6)
Statin + Nicotinic acid	42 (9.8)
Fenofibrate + Ezetimibe	2 (0.5)
Ezetimibe and adenosine monophosphate (AMP); Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (like evolocumab)	1 (0.2)
Statins	1 (0.2)
Other	10 (2.3)
don't know	32 (7.4)
None of the above	53 (12.3)
<b>27- Are you aware of these medications for FH patients use with statins? [PCSK9 inhibitors.]</b>	
Yes	188 (43.7)
No	242 (56.3)
<b>27- Are you aware of these medications for FH patients use with statins? [Lomitapide microsomal triglyceride transfer protein (MTP) inhibitor.]</b>	
Yes	76 (17.7)
No	354 (82.3)
<b>27- Are you aware of these medications for FH patients use with statins? [Mipomersen (an antisense oligonucleotide inhibitor)]</b>	
Yes	72 (16.7)
No	358 (83.3)
<b>27- Are you aware of these medications for FH patients use with statins? [Evinacumab monoclonal antibodies Angiopoietin Like 3 (ANGPTL3)]</b>	
Yes	91 (21.2)
No	339 (78.8)



**Table-5:** Correlations with awareness level.

Variables	Awareness level		P value*
	High	Low	
<b>Age (year)</b>			
30 or less	30 (20.1)	56 (19.9)	0.054
31-35	28 (18.8)	55 (19.6)	
36-40	35 (23.5)	54 (19.2)	
41-45	13 (8.7)	45 (16)	
46-50	10 (6.7)	32 (11.4)	
> 50	33 (22.1)	39 (13.9)	
<b>Gender</b>			
Male	93 (62.4)	127 (45.2)	0.001
Female	56 (37.6)	154 (54.8)	
<b>Level of training</b>			
Resident	41 (27.5)	128 (45.6)	0.000
Registrar\ specialist	33 (22.1)	87 (31)	
Senior registrar \ specialist	34 (22.8)	41 (14.6)	
Consultant	41 (27.5)	25 (8.9)	
<b>4- Your medical specialty</b>			
Family medicine	72 (48.3)	71 (25.3)	0.000
Internal medicine	37 (24.8)	67 (23.8)	
Paediatrics	7 (4.7)	50 (17.8)	
OB/GYN	4 (2.7)	44 (15.7)	
Endocrinology	16 (10.7)	25 (8.9)	
Cardiology	13 (8.7)	24 (8.5)	
<b>5-How many years have you been in practice since completing your medical school?</b>			
1-5	37 (24.8)	62 (22.1)	0.871
6-10	32 (21.5)	67 (23.8)	
11-15	31 (20.8)	55 (19.6)	
> 15	49 (32.9)	97 (34.5)	
<b>6- How familiar are you with familial hypercholesterolaemia? The following a 10-point scale where 1 means "Not at all familiar" and 10 means "Extremely familiar."</b>			
1-3	20 (13.4)	133 (47.3)	0.000
4-7	88 (59.1)	122 (43.4)	
8-10	41 (27.5)	26 (9.3)	

OB-GYN: Obstetrics and gynaecology.

FH awareness level has significant correlations with gender, level of training, medical specialty, and the degree of familiarity with FH (Table 5).

## Discussion

The awareness and knowledge of physicians regarding FH are generally suboptimal<sup>18,24</sup>. Therefore, the current study was planned to assess the knowledge, awareness and practice of physicians in northern regions of Saudi Arabia regarding FH. Less than half the physicians were moderately familiar with FH. The same proportions correctly defined FH, whereas the knowledge of physicians regarding the prevalence of FH was not good. Also, there were gaps in the knowledge of the physicians regarding the probability of having FH due to the presence of FH among first-degree relatives, and

inadequate knowledge regarding the risk of CHD among untreated FH patients.

In a previous Saudi study on family physicians in a government hospital, it was found that most physicians (72.4%) placed their familiarity with FH on average or above, but 48.4% of all the participants showed poor FH knowledge, whereas 51.6% had acceptable knowledge<sup>22</sup>. This reflects the presence of gaps in the knowledge of the family physicians in the previous study, similar to the current findings. Inadequate knowledge among physicians in Saudi Arabia seems to be a major problem, as in another Saudi study conducted on physicians, it was found that 92.9% of the physicians had poor overall knowledge and 68.7% placed their familiarity with FH as average or above<sup>21</sup>. A study conducted on medical interns in Jeddah showed that 76.5% knew the definition of FH, whereas the knowledge regarding the prevalence was low at 43.5%<sup>25</sup>. In the current study, a lower proportion defined FH correctly, and there was a high proportion of those who did not know the prevalence of FH, or reported the wrong rate. The situation outside Saudi Arabia is not better, as a study in India showed that only 31% of GPs correctly described FH, 51% rated themselves as having above-average of familiarity with FH, and only 28% knew about its prevalence<sup>26</sup>. In another Indian study, 27.9% physicians put themselves as having above-moderate familiarity with FH, whereas 71.4% correctly described FH<sup>27</sup>. In the current study, the total awareness was high in 34.7% cases, whereas 65.3% showed low awareness. High awareness of FH was associated significantly with male gender, family physicians' specialty, and a moderate degree of familiarity with FH, whereas low awareness was associated with residents. In a previous Saudi study conducted on family physicians, the mean score of knowledge of physicians was affected by age, level of training, years of experience, and familiarity with FH<sup>22</sup>. In the current study, residents were more likely to have a lower level of awareness, but the same proportion of consultants and residents reported high awareness. Additionally, the mean score of knowledge was associated with the age of physicians, level of training and years of experience. Only 43% reported that an accurate diagnosis of FH cannot be made by genetic testing alone, and that smoking was the major reported factor increasing the risk of CVDs for a person with FH. More than half the participants reported that family medicine or primary care physicians were the personnel who can most effectively detect FH early. Similar to the current findings, very low awareness was found regarding the clinical algorithm for the diagnosis of FH (52%) in a previous Saudi study<sup>22</sup>. A study done in India's Tamil Nadu state revealed that 41.4% of the physicians were not

aware and were uncertain whether they had a patient with FH under their care<sup>27</sup>.

The current study had some limitations. One of the main issues was that physicians working in primary and secondary healthcare settings often have limited facilities to diagnose and follow up with patients having FH. Additionally, limited data was available regarding the number of physicians in each department, which made it difficult to distribute the questionnaire equally. Furthermore, challenges were faced in obtaining an adequate sample from some specialties due to an insufficient number of clinicians working in the study region.

## Conclusion

The participating physicians showed gaps in knowledge, low awareness, and inappropriate practice related to FH. A high level of awareness was associated with the male gender, family medicine, and those with a moderate degree of familiarity with FH. Residents and specialist physicians appeared less aware of FH than senior staff. This could be due to differences in training or exposure to the condition. Educational and training programmes are necessary to increase the knowledge and awareness levels of physicians toward FH, and to improve their practice for the correct diagnosis of FH.

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**Conflict of Interest:** None.

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### Author Contributions

**AAA:** Sole responsibility for study conception, design, data collection, analysis, interpretation of results and manuscript preparation.