IJBPAS, January, 2021, 10(1): 310-325

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

NON-COMMUNICABLE DISEASES IN SAUDI WOMEN AND THEIR ASSOCIATION WITH AB AND RH ANTIGENS

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Received 4th Nov. 2020; Revised 25th Nov. 2020; Accepted 12thDec. 2020; Available online 1stJan.2021

https://doi.org/10.31032/IJBPAS/2021/10.1.5654

ABSTRACT

Background: Earlier studies have suggested that every year stroke kills more women than men. As per American stroke association and CDC'S national center for health statistics, stroke causes almost double the number of fatalities than fatalities caused by breast cancer. According to the American heart association, in 2017, there were 84,738 women fatalities due to the stroke which constituted 57.9% of all fatalities caused by stroke in USA. **Methods**: We collected relevant information from one thousand two hundred fourteen female control subjects (selected randomly) and 772 female ischemic stroke patients (IS). We also collected blood test results from 213 patients who were hospitalized for brain hemorrhage (HS). Additionally, we also collected total lipid profile and the blood test results from 307 female hypercholesterolemia (HC) patients who were admitted to King Suleiman hospital in Hail SA. Patient data was collected only from patient files by fifth year male and female medical students and the medical interns. **Results**: Since our sample size was large, we used Z test to calculate statistical significance of blood antigen distribution frequencies between controls and HS and HC female patients. Results of statistical analysis show a significantly reduced expression of "A" and "AB" antigens in HS female patients in comparison to the control female subjects. In contrast we saw significant rise in "B" antigen distribution in HS female patients as compared to the controls. Likewise, there was a high prevalence of hypercholesterolemia in B^+ patients. **Conclusions**: Our results suggest a correlation between blood group

distribution, hypercholesterolemia and HS diseases. B blood type shows a significantly higher distribution in HS and hypercholesterolemia female patients as compared to controls.

Keywords: Cholesterol, Diabetes, Obesity, Stroke

Abbreviations: AHA: American Heart Association; CHD: Coronary heart disease; HDL: High density lipoproteins; HS: Hemorrhagic stroke; HC: Hypercholesterolemia; IS: Ischemic stroke; LDL-C: LDL Cholesterol; TGs: Triglycerides; TS: Transient stroke.

INTRODUCTION

Every year we lose many lives due to the three major types of strokes (IS, HS, and TS). These incidences of strokes are also responsible for not only causing the large number of untimely deaths they also cause a large number of disabilities all across the globe **[1]**. Although it is a well-known fact that IS causes most of stroke related deaths and the disability however the "hemorrhagic stroke (HS)", is also responsible for causing high number of fatalities. An uncontrolled high blood pressure (hypertension) is believed to be the main reason for HS. According to the Saudi Arabian stroke society, the ischemic stroke is responsible for majority of the stroke related loss of life (80-85%) and only 15% fatalities are caused by the intracerebral hemorrhage.

According to the "American Stroke Association" (ASA) the third and the less common type of stroke is also known as the "transient ischemic stroke" or the "mini stroke". According to the American Stroke Association, people should take an incidence of "mini stroke" as the warning sign and get serious about their everyday life style by exercising and making the drastic changes to their diet.

Several major risk factors have now been identified for causing almost 90% of all the incidences of strokes **[2]**. In 2017, globally 6.2 million people died due to the cerebrovascular disease (stroke). Out of these 2.7 million (43.5%) died due to the occurrence of ischemic stroke and 3.0 million (48.4%) due to the intracerebral hemorrhage and about 0.4 million (6.5%) due to subarachnoid hemorrhage **[3]**. Studies published earlier have even suggested that, the middle-aged subjects who have reached the age of 55 years are twice more likely to have an episode of stroke, every 10 years **[4-5]**. Moreover, it was recently reported that 'stroke was among the top three reasons of causing women fatalities' **[6]**. Yet another study reported that each year, stroke is known to kill more women when compared to the males, and that it was more likely that a woman between the ages of 20-39 will have a stroke as compared to the men in

similar age bracket **[7]**. According to CDC and ASA, in women stroke causes almost double the number of fatalities than the fatalities caused by the breast cancer. In 2017, there were 84,738 women fatalities due to stroke which constituted about 57.9% of all fatalities caused by stroke in USA **[8]**.

Many earlier studies reported some relationship between the inherited blood groups and many non communicable diseases **[9-26]**. For example, we and others recently reported some association between ABO blood groups and non-insulin dependent diabetes **[9-10]**, hypertension **[11-16]**, ischemic heart conditions **[17-21]** including the incidences of heart attacks or myocardial infarctions in male and female adults **[22-26]**. Like many other non-communicable diseases, several recently published reports have also shown a strong relationship between the incidences of IS and HS stroke and the A and B blood group antigens **[27-30]**,however, the results are not consistent.

METHODS

We selected 1240 female subjects (average age 33.7 years and their age ranged between 14 to 68 years) as controls, and asked them a few questions that were specifically prepared for this study. We collected information only after they had agreed to disclose their information voluntarily. Questions were asked related to their health such as if they or any of their immediate family members had any preexisting conditions, their height and weight was also recorded for calculating their body mass index (BMI), their smoking habits and their blood type was also recorded. Similar information was also gathered from the 213 HS female patient-files by the fifth-year medical students and medical interns. The data was collected only from the available HS patient's files from cardio center of one of the major hospitals in Hail, the King Khaled hospital (KKH). These patients had registered at KKH between1/1/2008-1/6/2018.

For the hypercholesterolemia patients we also collected some specific information from the patient files only after receiving the permission from manager incharge of patient records office. Information was gathered about their body weight, blood pressure, tobacco product use if any and the existence of any preexisting conditions either in the patient or in their immediate family members. The test results of the patient's lipid profiles were also recorded, including the results of their last two or three blood tests showing their LDL-C, total-C and their triglyceride levels. Just like the control subjects and the HS patients, we also recorded the individual blood type from each

of the 307 female hypercholesterolemia patient's file who had registered at KS hospital between November 2018 thru April 2020. Due to the Covid-19 lockdown regulations there was never any direct contact with any of the female patient at any point. The data was only collected from the available patients files in King Suleiman hospital by the fifth-year medical students and the medical interns, only after receiving an official authorization from the manager in charge of the record's office.

RESULTS

In this study, we collected data from the 1214 female controls and 213 female patients who had registered at a local hospital for the incidence of hemorrhagic stroke. In addition, the medical students also collected clinical data from 307 female hypercholesterolemia patients (with poorly controlled LDL, total Cholesterol and or triglyceride levels). Relevant data was also collected from HS patient files who had registered at the local hospital between 1/1/2008 to 1/6/2018. At no point during the whole duration of this study medical students and or the interns had any direct communication with any registered stroke or hypercholesterolemia female patients at the hospital. Among the control subjects, we found only 7.2% to be the regular tobacco users (smokers), 7.6% were hypertensive and 17.5% had the type 2 diabetes (T2DM). On the contrary, among the female hypercholesterolemia patients we found 43.6% to be hypertensive (a 5.7-fold increase over the control group) and only 7.2% patients were diabetics.

Comparative distribution of subjects with O versus non-O blood type in controls and HS female patients:

Several studies that were published earlier have shown a correlationship between the A, B, and the AB blood types (non-O blood groups) and the certain non-communicable diseases. However, in the current study, a thorough comparative data analysis did not reveal any significant changes in distribution of O blood types (48.4% versus 50.7% in HS females) or the non-O blood types (51.6% versus 49.3% in HS females) between the control group and the patient group **(Figure 1)**. Results were not significant at $p < .05$. Therefore, our results did not reveal any such correlations with O versus non-O blood types in the control or the patient group.

Percent distribution of A, B, AB and O blood types between the controls and the HS patients:

Next, we tried to compare the frequency and the distribution of the individual blood groups between our control subjects and the patient group. An in-depth analysis of our

data showed a statistically significant increase in the percentage of HS female patients with the blood type B as compared to the percentage of B type subjects in the control group (**Figure 2**, red bar with a red arrowhead). Results were statistically significantly different at p <.01. On the contrary, we found a statistically significant reduction in the percentage of A blood type patients as compared to the controls at p <.01. We also saw a similar reduction in the percentage of AB blood type HS patients as compared to in our randomly selected control group (p <.05). However, we did not find any statistically significant changes in the distribution of O blood groups between the two groups **(Figure 2)**. Results were not significant at p < .05.

Comparative distribution of ABO-Rh^+ and ABO-Rh^- blood types in controls and the hemorrhagic stroke female patients:

To further study any possible contribution of Rh antigen (D antigen) in causing HS among women we next, analyzed and compared the percent distribution of all 8-blood types (A^+, A^-, B^+, B^-, AB^+, AB^-, O^+, and the O^-) between the two groups, separately. Overall, we found 91.5% HS females to be Rh^+ in comparison to the 83.3 Rh^+ subjects in the control group. Results are statistically significant at p <.01. In contrast, among the hemorrhagic stroke females the percentage of Rh^- blood types were significantly lower as compared to the percentage of Rh^- control subjects **(Figure 3)**. Results were significant at p <.01. Further analysis of our results revealed a noticeable decrease in the number and the percentage of A^+ female patients **(Figure 3)**. These results were again significant at p <.01. On the other hand, our results showed a significant rise in the number and percentage of the B^+ blood type HS female patients (red triangle and red bar) in comparison to the control females (**Figure 3**, blue bar). Our results were significant at p <.01. Therefore, our results suggest a possible resistance to the incidences of HS among A^+ women as compared to the women with B^+ types. As we saw in **Figure 2**, we again saw some reduction in number and percentage of AB^+ HS patients in comparison to the control females, however the results were not statistically significant at p <.05. The O blood type is the most prevalent blood type among controls as well as in the HS female patients however, we did not see any significant differences in the number and or percentage of the O blood types among HS patients in comparison to the controls **(Figure 3)**. Results were not significant at p <.05.

Although results of the current study suggest a possible resistance to the incidences of HS among the A^+ Saudi women and a low resistance to the sudden episodes of HS among the B^+ females, larger studies are needed to confirm these results.

A Comparative distribution of ABO-Rh$^+$ and the ABO-Rh$^-$ among controls and the hypercholesterolemic women:

A correlationship between blood groups and high cholesterol has previously been postulated however, results published earlier have not been consistent. To the best of our knowledge no such studies have been performed on Saudi women. Therefore, we next selected 1214 females and 307 females who had registered at a local hospital with hypercholesterolemia. Patients age ranged between 16 to 89 years (mean age 54.2 years). Among one thousand two hundred and fourteen randomly selected female control group we found 7.2% to be the smokers, 7.6% were hypertensive and 17.5% of them had diabetes type II(T_2DM). However, among hypercholesterolemia patients, 43.64% were hypertensive and 7.16% had the type 2 diabetes (T_2DM) while only 0.32% patients had the type 1 diabetes (DM1).

When we analyzed the AB and the Rh antigen distribution data separately for female patients with high LDL (3.4 mM or above) levels, high total cholesterol (5.2 mM or above) levels, and high triglyceride (1.7 mM or above) levels and compared them individually with that of the control subjects we did not see any statistically significant changes in the distributions of A^+, AB^+ or the O^+ blood groups in female HC patients as compared to the control subjects **(Figure 4)**. The results were statistically non-significant at ($p < .05$). However, we saw a statistically significant increase in the distribution of B^+ (B^+Rh^+) among HC patients as compared to its distribution among randomly selected controls. Results were statistically significant at $p < .01$.

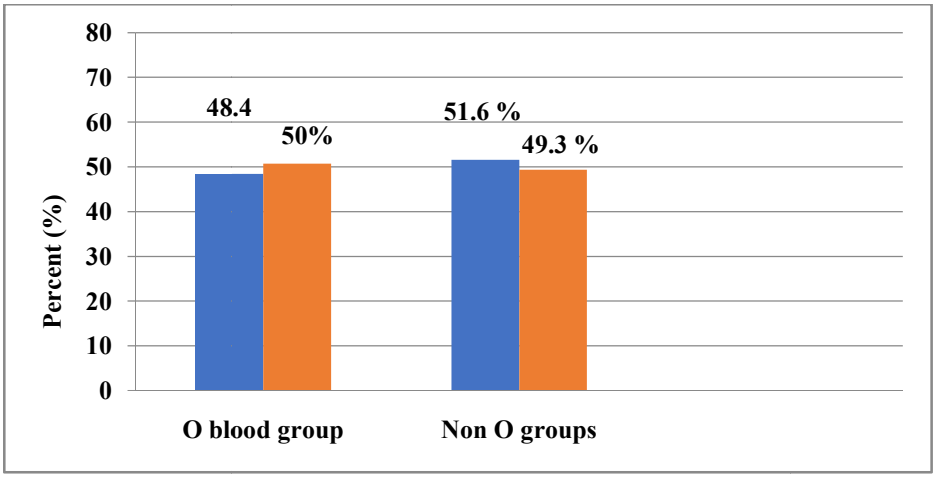


Figure 1: Distribution of O versus non-O blood type female subjects in controls (blue bars) and the HS female patients (red bars).Our results show no significant changes in distribution between the two groups. Results were not significant at p <.05

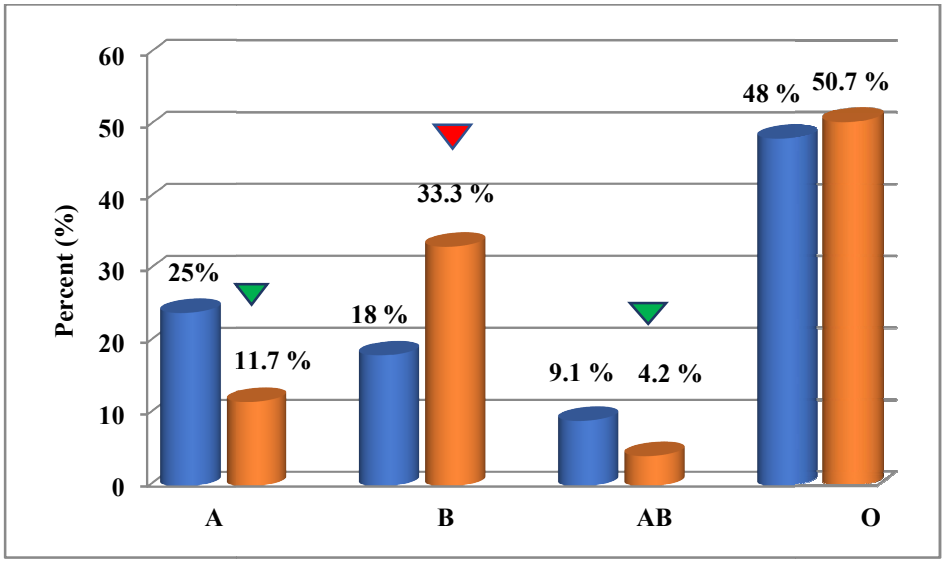
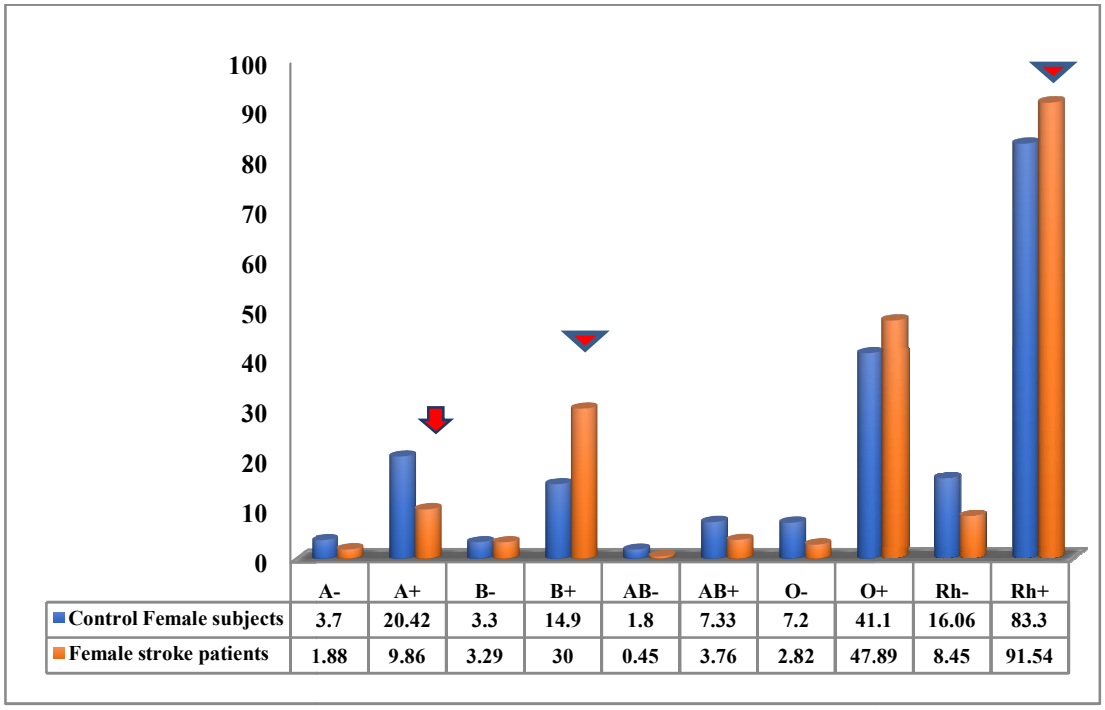
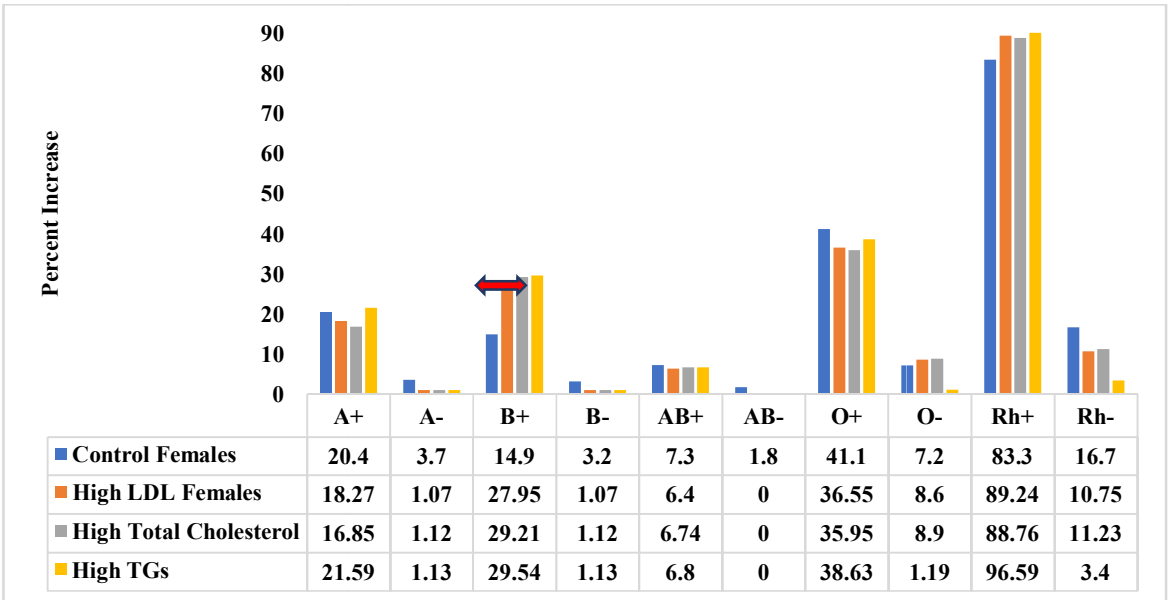


Figure 2: Distribution of four major blood types in control subjects and the HS female patients: Results show a statistically significant rise in the B blood type patients (red triangle) and a significant reduction of A and the AB blood type patients (green triangles) as compared to their respective controls (blue bars).Statistical analysis showed no significant changes in the percentage of O blood type individuals between the two groups



	A-	A+	B-	B+	AB-	AB+	O-	O+	Rh-	Rh+
Control Female subjects	3.7	20.42	3.3	14.9	1.8	7.33	7.2	41.1	16.06	83.3
Female stroke patients	1.88	9.86	3.29	30	0.45	3.76	2.82	47.89	8.45	91.54

Figure 3: Comparative distribution of blood types with or without Rh antigens among controls and the HS female patients: As in figure 2, our results again show a statistically significant rise in B^+ and a comparative rise in combine Rh^+ blood type HS female patients (red triangle, red bar) in comparison to the female control group (blue bar). The results were statistically significant at p <.01. Additionally, we again saw a similar and statistically significant drop in numbers and or the percentage of A^+ HS females as compared to their respective controls (Red arrows, red bar). However, we did not find any statistically significant changes in the percentage of AB^+ and the O^+ blood types between HS and control females and the results were not found to be significant at p <.05



	A+	A-	B+	B-	AB+	AB-	O+	O-	Rh+	Rh-
Control Females	20.4	3.7	14.9	3.2	7.3	1.8	41.1	7.2	83.3	16.7
High LDL Females	18.27	1.07	27.95	1.07	6.4	0	36.55	8.6	89.24	10.75
High Total Cholesterol	16.85	1.12	29.21	1.12	6.74	0	35.95	8.9	88.76	11.23
High TGs	21.59	1.13	29.54	1.13	6.8	0	38.63	1.19	96.59	3.4

Figure 4: Percentile changes in blood types with or without the Rh antigens between the control females and hypercholesterolemia female patients: Note a significant rise in the percentage of blood type B^+ in hypercholesterolemia female patients (Left-right arrow) as compared to the B^+ blood type in control group (blue bar). Results were significant atp <.01. In contrast, we found no significant changes in the percentage of HC and control females with blood types A^+, AB^+ and O^+(p <.05)

DISCUSSION

Earlier studies have identified several major risk factors which are responsible for causing a majority of the incidences of strokes **[2]**; for example: insufficient physical activity, a poor diet selection, a high BMI or obesity, diabetes, high cholesterol levels, hypertension, smoking, stress levels and the alcohol use. Nonetheless, there has been no noticeable decrease in stroke rates in many countries, including Saudi Arabia. According to the US Department of Health and Human Services "stroke is the third leading cause of death in Saudi Arabia." A multitude of stroke risk factors are highly prevalent amongst the Saudi adult population. According to the AHA "individuals who have diabetes are 1.5 times more likely to have the stroke than the non-diabetics." In spite of these known risk factors, Saudi Arabia has a very high prevalence of obesity **[31-33]**, diabetes **[34-35]**, smoking **[36-39]**, and hypertension **[40-41]**. The preponderance of these high-risk factors amongst the Saudi population consequently puts it at an even higher risk of stroke occurrence. In accordance to a current study, out of all Saudi adult stroke patients who registered in King Khaled hospital between 01/01/2008 - 01/06/2018, we found 21.04% of patients to be HS patients. According to American Heart and the Stroke associations "a minor leak and/or a rupture in a blood vessel in the brain due to poor control of high blood pressure, can result in sudden HS in an individual".

Although in the recent past several studies have shown a correlation between the ABO blood groups and the incidences of non-communicable diseases, the results have not been very consistent. We recently showed a possible correlation between the ABO blood groups and type 2 diabetes **[9-10]**, hypertension and myocardial infarctions **[25-26]**, and the incidences of hemorrhagic stroke in Saudi adult male population **[30]**, however to the best of our knowledge to date, no such studies have been done to show a possible relationship between the incidences of hemorrhagic stroke or hypercholesterolemia and the ABO blood groups in the Saudi adult female population.

Therefore, the current study was designed to investigate a possible correlation between ABO and Rh blood groups and the incidences of hemorrhagic stroke in the Saudi adult female population. Moreover, we looked at a possible relationship between the cases of high LDL, high total cholesterol and high triglycerides amongst the Saudi adult female population and their inherited ABO blood groups.

Studies published earlier **[27]** have shown "B allele in B blood types may be associated to venous thrombosis (VT) and ischemic stroke" however, they found no association of A, AB or B alleles with any of the hemorrhagic event **[27]**. On the other hand, other studies have found absolutely no association between ABO and an ischemic stroke or any other subtypes **[42]**.

In comparison, our ABO blood group distribution analysis between the controls and the HS female patients showed a very high prevalence of B blood type amongst the HS female patients as compared to the randomly selected female control subjects. Results were statistically and significantly different at $p<.01$. Be that as it may, we saw a statistically significant reduction ($p<.01$) in the frequency of distribution of blood group A in HS patients when compared to our control subjects. Such results suggest a possible correlation between the ABO blood groups and the incidences of HS in the Saudi adult female population. Blood type A individuals show some resistance to episodes of HS, while the B blood type Saudi female population shows very high incidences of HS, suggesting a possible low resistance to HS. However, we saw no significant differences in the distribution of O blood type in both blood groups **(Figure 2)**.

To further analyze our results, we compared the prevalence of Rh^+ individuals between the two groups. As expected, we found the distribution of Rh^+ patients to be statistically and significantly higher in the HS female patients as compared to the control subjects while the distribution of Rh^- HS female patients was significantly lower in comparison to the female control subjects ($p<.01$) **(Figure 3)**. When we further investigated the cause of significant increase in the Rh^+ blood types in the HS females we found, a statistically significant increase in the distribution of the B^+ or BRh^+ blood groups, as compared to the female control population. However, once again, we did not see this increase in any other non-O blood groups; on the contrary, a statistically significant decrease was found in the distribution or prevalence of the blood group A^+ in HS female patients when compared to the control. We did not find statistically significant variations in the distributions of AB^+ and the O^+ blood groups, in terms of comparison between controls and the HS female patients **(Figure 3)**. Therefore, an increase in percentage of Rh^+ blood types among the HS patients that we see could reflect the statistically significant rise that we see in the HS patients with blood type B^+**(Figures 2 and 3)**.

Since there is a high prevalence of hypertension in Saudi adult population **[40-41]** and it has affected more than one fourth of the Saudi population **[40]** it puts them at much higher risk of HS. Since having a high cholesterol can also put people at higher risk of having a stroke and since result of our findings suggest some association between the distribution of ABO blood groups with the incidences of stroke, we next looked for female patients with high or uncontrolled cholesterol levels (uncontrolled LDL, total cholesterol and triglyceride levels) and tried to analyze if there was any possible correlation between the distribution of ABO blood groups with hypercholesterolemia. Yet again, our results showed significantly higher levels of total cholesterol (TC), LDL, and TGs amongst female patients with blood type B^+**(Figure 4)**. However, our results do not agree with the earlier studies which found higher concentrations of total cholesterol (TC) and LDL in blood type A subjects **[20]**. These differences in our ABO distributions could be a result of genetic variations in varying populations and/or due to the different geographical regions where these studies were conducted. Larger studies are underway to confirm our findings.

In summary, results of our current study suggest that the Saudi adult female population with blood type B^+ have a high prevalence of high LDL, high cholesterol and high triglyceride levels when compared to other blood types. Moreover, our findings show that Saudi females with blood type B^+ tend to have a higher incidence rate of hemorrhagic stroke. Among HS female patients, we saw a significant decrease in the distribution of A blood type in comparison to the distribution in randomly selected control females. These results are completely different from our recently published finding of high prevalence of A blood group in hemorrhagic stroke Saudi adult male patients **[30]**. Results of our current study suggest differences in the prevalence of high incidences of HS and their blood groups depending on gender.

CONCLUSIONS

In summary, in regards to HS female patients, we saw a dramatic and statistically significant increase in the distribution of B^+ blood groups and a statistically significant reduction in the distribution of A^+ blood groups as compared to the female control population. AB^+ and O^+ blood groups showed non-significant variations between the two groups. Additionally, there was a high prevalence of hypercholesterolemia amongst B^+ females. Our findings suggest a possible correlation between the ABO blood groups

and the incidences of hemorrhagic stroke and hypercholesterolemia in the Saudi adult female population.

ACKNOWLEDGEMENTS

First and foremost, the authors of this study would like to thank the Dean of the College of Dentistry at the University of Hail, Professor Hazza Abdullah Alhobeira, for providing his help and guidance to the medical students in collecting patient data from King Suleiman Hospital. We would also like to thank the administrators of King Khaled and King Suleiman Hospitals for allowing the fifth-year medical students as well as the medical interns to retrieve the relevant patient data in spite of difficulties during Covid lockdown. Last but not least authors would like to thank Mrs. Saman Assad for her untiring efforts in final editing of parts of this manuscript.

Conflict of Interest:

This study was self-supported and the authors of the current study have no conflict of interest.

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