LAMPIRAN

Lampiran 1 *Ethical Clearance* (Kaji Etik)

KOMISI ETIK PENELITIAN KESEHATAN
HEALTH RESEARCH ETHICS COMMITTEE

POLITEKNIK KESEHATAN KEMENTERIAN KESEHATAN BANDUNG

KETERANGAN LAYAK ETIK
DESCRIPTION OF ETHICAL APPROVAL
"ETHICAL APPROVAL"

No. 75/KEPK/EC/VI/2021

Protokol penelitian yang diusulkan oleh
The research protocol proposed by

Peneliti utama : Salsabila Aulia Zahra
Principal In Investigator

Nama Institusi : Jurusan Teknologi Laboratorium Medik
Name of the Institution Poltekkes Kemenkes Bandung

Dengan judul:
Title

"HUBUNGAN STATUS SEKRETOR PADA SPESIMEN BIOLOGIS TERHADAP RISIKO PENYAKIT INFEKSI"

"RELATIONSHIP OF SECRETOR STATUS IN BIOLOGICAL SPECIMENS TO RISK OF INFECTIOUS DISEASES"

Dinyatakan layak etik sesuai 7 (tujuh) Standar WHO 2011, yaitu 1) Nilai Sosial, 2) Nilai Ilmiah, 3) Pemerataan Beban dan, 4) Risiko, 5) Bujukan/Eksploitasi, 6) Kerahasiaan dan Privacy, dan 7) Persetujuan Setelah Penjelasan, yang merujuk pada Pedoman CIOMS 2016. Hal ini seperti yang ditunjukkan oleh terpenuhinya indikator setiap standar.

Declared to be ethically appropriate in accordance to 7 (seven) WHO 2011 Standards, 1) Social Values, 2) Scientific Values, 3) Equitable Assessment and Benefits, 4) Risks, 5) Persuasion/Exploitation, 6) Confidentiality and Privacy, and 7) Informed Concent, referring to the 2016 CIOMS Guidelines. This is as indicated by the fulfillment of the indicators of each standard.

Pernyataan Laik Etik ini berlaku selama kurun waktu tanggal 28 Juni 2021 sampai dengan tanggal 28 Juni 2022.
This declaration of ethics applies during the period June 28, 2021 until June 28, 2022.

June 28, 2021
Professor and Chairperson,

KEMENTERIAN KESEHATAN RI
KOMITE ETIK PENELITIAN KESEHATAN
POLTEKKES BANDUNG

Dr. Supraman, SKM., M.Sc.

Lampiran 2 Lembar Bimbingan Skripsi

POLITEKNIK KESEHATAN BANDUNG

LEMBAR BIMBINGAN SKRIPSI

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NO	MATERI BIMBINGAN	WAKTU	TTD PEMBIMBING
1.	Konsultasi mengenai perbaikan proposal dan penelitian	18 Mei 2021	
2.	Konsultasi mengenai pengajuan kaji etik	7 Juni 2021	
3.	Bimbingan penelitian	14 Juni 2021	
4.	Konsultasi penyusunan skripsi	21 Juni 2021	
5.	Konsultasi penyusunan skripsi	23 Juni 2021	
6.	Revisi skripsi	25 Juni 2021	
7.	Konsultasi revisi skripsi	28 Juni 2021	
8.	Pengiriman draft akhir skripsi	29 Juni 2021	
9.	Konsultasi dan latihan presentasi	30 Juni 2021	

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Does Secretor Status of ABO Blood Group in Saliva Influence the Risk of Hypertension and Urinary Tract Infection in Diabetic Patients?

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Abstract
Secretor status of blood group antigens in saliva is implicated to be associated with several infectious and immune related diseases. Prospective analytical study carried out in a tertiary hospital in South India to study the association of ABO secretor status with type II diabetes mellitus and its microangiopathic complications. A total of 198 patients with type II diabetes mellitus, diagnosed and treated in the hospital were studied. 200 healthy controls were studied from healthy volunteers working in the hospital. ANOVA, Pearson's Chi Square Test and Cox Univariate and Multivariate analysis are the statistical methods used to analyse the data. 21.2% of diabetics and 33.5% of healthy controls were ABO secretors. Secretors were found to have a statistically significant risk of association with type II diabetes mellitus when adjusted for other potential confounding factors. Secretor status was in addition found to have significant association with hypertension. Non-secretors were significantly associated with increasing age of the patient and had increased risk of urinary tract infections (UTI). The risk of microangiopathic complications due to type II diabetes mellitus were significantly higher with increasing levels of glycated haemoglobin (HbA1c). In our study, we found significant association of secretors with type II diabetes mellitus and hypertension while non-secretors were associated with increased risk of urinary tract infections.
Keywords: Secretor status, Type II diabetes mellitus, Hypertension, UTI, microangiopathic Complications.

Introduction

A secretor refers to a patient who secretes blood group antigens in fluids such as saliva, sweat, tears, semen, and serum. ABH refers to 'A' and 'B' antigens of ABO blood group and 'H' the heterogenic constituent of all ABO types including type 'O'. H antigen is the indirect gene product expressed as fucose-containing glycan unit and it resides on glycoproteins or glycolipids of erythrocyte membranes or on mucin glycoproteins in secretions. H antigen is expressed by two genes FUT1(H) gene predominantly in erythroid tissues; FUT2 (Secretor) gene is expressed predominantly in secretory tissues[1]. Among all body fluids, saliva is the rich source to determine Secretor status[2].

Saliva has protein, glycoprotein, peptide and carbohydrate constituents in addition to inorganic substances. Salivary glycoproteins are in constant interaction with variety of bacteria in the oral flora through their carbohydrate moiety. Fucosyltransferase 2 (FUT2) is responsible for fucosylation of glycoforms and this influences the scaffolding of Leb/y and blood type motifs. These glycans act as receptors mediating the adhesion of several bacteria in oral cavity, few examples including sT-Ag for Streptococcal strains and Le determinants for H. pylori strains. Fucosylation of gPRP disaccharide blocks bacterial binding. Non-secretors have inactive form of FUT2 genotype and phenotype, thereby influencing salivary glycoproteins with significantly lower levels of fucosylation of the released N-glycans.

Blood group and secretor status have significant influence in inter-individual variation in salivary glycosylation and consequently on interaction with bacterial milieu in the oral cavity[3].

The ability or inability to secrete ABH blood group substances in body fluid has been studied with susceptibility to a number of pathological conditions. Previous studies have indicated that non-secretors are more prone to certain diseases such as autoimmune diseases like insulin-dependent diabetes mellitus[4], ankylosing spondylitis, reactive arthritis, psoriatic arthropathy, Sjogren's syndrome, multiple sclerosis[5], peptic ulcers[6], vaginal candidiasis[7], etc. Non-secretors have increased inflammatory response (increased C-reactive protein, erythrocyte sedimentation rate and the body temperature) to urinary tract infections (UTI) than Secretors[8].

Efforts attempting to supplement blood group substances prepared from edible food are proposed for prevention and treatment of various bacterial and viral strains but however at the moment there is little scientific evidence on the same with conflicting opinions[9].

FUT2 polymorphisms and secretor status is also proven to be one of the key drivers affecting the individual variations in gut microbiota. Association of non-secretor phenotype with various diseases have been implicated in the increased risk for Crohn's disease, type I diabetes, urinary tract infections, candidiasis and viral infections such as rota virus and norovirus infections in the GIT[10, 11]. The influence of secretor status on antibiotic treatment of enteric pathogens such as Salmonella and Clostridium difficile is also reported[12].

Type II Diabetes mellitus (DM) is a metabolic disease which has a genetic predisposition, although environmental factors do play a significant role in its genetic expression. Like many other inherited traits, ABH secretor status is also genetically pre-determined and therefore we studied an association with diabetes mellitus and its complications.

Subjects and Methods

The study design was prospective analytical and the study group was recruited from the people who came for medical and surgical check-up in the outpatient department. Patients already diagnosed to have non-insulin dependent diabetes mellitus (NIDDM) were taken as study population (type II DM). Healthy individuals who are not diabetic (non-DM) were taken as healthy control from volunteers among health care workers in the hospital. Both males and females of age ranging

from 18 to 74 years were recruited. A total of 198patients who had type II diabetes mellitus samples were subjected to secretor status identification from saliva samples. 200 healthy controls were recruited in the study and their secretor status from saliva was also determined. Ocular examination was performed and retinopathy was graded according to the modified Airlie House classification system. The moderate/severe diabetic retinopathy (DR) was defined as case: grade >=30; control: grade < 14. Physical findings as assessed by Physician were also documented in the study group. Patients who presented with symptoms of urinary tract infections were studied for routine urine examination with urine microscopy and culture and sensitivity. Majority of the patients in the study group (patients with type II diabetes mellitus) were not aware of the exact onset of the disease. Volunteers in the control group were also screened for blood pressure, urine albumin, sugar and microscopy and serum creatinine. Only those who were diagnosed in the hospital with records were included to avoid confounding factors. Ethical clearance was obtained from Institutional ethical committee. Chi-square test was performed to assess the statistical significance between the two variables.

Sample collection and processing

Collection of saliva was performed after thoroughly rinsing the mouth with water. All the saliva samples were collected 2-3 hours after the usual breakfast time. About 2ml of saliva was collected in a dry sterile tube. Saliva tube was kept for 20 minutes in a boiling water bath at 100°C to denature the salivary enzymes. It was then cooled to room temperature and centrifuged for 5 minutes at 1000g, supernatant was collected. Secretor status of the saliva samples were identified by using adsorption inhibition technique[13]. The procedure is briefly mentioned below.

Six test tubes were labelled C, 2, 4, 8, 16, 32. C refers to the control tube, to which no saliva was added. 50 µl of saliva was added into the second tube labelled 2. For the rest of the tubes labelled 4, 8, 16 and 32, saliva was titrated by doubling dilution using normal saline. 50 µl of anti A serum was added to each test tube. All the tubes were shaken well and left undisturbed for 10-15 minutes. One drop of a suspension of group A RBCs in a concentration of 5% was added to each tube. The tubes were left to stand for 5 minutes and subsequently centrifuged at 3500 rpm for 20 seconds. The sediment was decanted on a slide and studied for presence of agglutination. (Fig 1) The degree of agglutination was graded as follows,

Grade 0 - all red blood cells are discrete and evenly distributed in surrounding fluid (no agglutination)

Grade 1 - Few or very occasional clumps

Grade 2 - moderate aggregates

Grade 3 - composed of moderate to large aggregates

Grade 4- very large aggregates, very few cells remain free without agglutination.

Control tubes always showed grade 4 Agglutination. The procedure was repeated with group B RBC and anti B serum and subsequently with group O RBCs and anti H serum. This is based on the principle that when saliva has blood group antigens, the antibodies in the serum of appropriate dilution will be utilized and will result in a negative agglutination when the corresponding blood group RBC's are added. Grades 0, 1, 2, 3 agglutination are interpreted as secretor while Grade 4 agglutination is interpreted as non-secretor.

2 ml of blood was collected in ethylene diamine tetra acetic acid (EDTA) coated vaccutainer tubes and ABO blood group and Rh typing was carried out by forward and reverse grouping techniques using test tube method as per standard operating procedure in the Institute. 2 ml of serum was collected from all the controls once and 2ml serum was collected from all the patients in both fasting and post-prandial state to assess the blood sugar levels (by Glucose oxidase-perioxidase method), serum creatinine by (Modified Jaffe's method), HbA1c (by Immuno-turbidometric method) and

serum albumin (Bromocresol green dye binding method). Biochemical measurements were performed on fully authomated analyzer (Beckman Coulter) using colorimetric method. Urine examination was carried out for assessment of albuminuria (by Immuno-turbidometric method) and presence of sugar in urine by using strip test and subsequently urine was centrifuged and studied for deposits and casts by light microscopic examination. Each test was done in duplicate to ensure precision and accuracy and only concordant results were included for the analysis. 20 patients in the study population and 16 control samples had discordant results for secretor status assessment, which were excluded from the study. Duration of diabetes mellitus in the study population ranged from newly diagnosed patients to patients who had diabetes for more than 30 years.

Clean catch mid stream urine is collected in a sterile wide mouthed container of capacity 50ml. Urine samples were inoculated in MacConkey agar and Blood agar using a sterile 4 mm platinum wired calibrated loop and incubated overnight at 37ºC for 24 hours. The specimen was considered significant bacteriuria when the growth was > 10^5 colony forming unit (CFU/ml). The isolates were identified till species level using standard biochemical tests[14].Antibiotic sensitivity testing was done following the Kirby- Bauer disc diffusion method in Muller Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) guidelines[15].

Results

A total of 398 samples were processed, 198 (49.75%) of whom were from patients with type II diabetes mellitus and 200 (51.25%) were from normal healthy controls. Among the patients in the study group, 42 (21.2%) were secretors while in the control population, 67 (33.5%) were secretors. (Table 1)

Table 1 Secretor status in the study group and Control population

S.No	Secretor status in study subjects n (%)	Secretor status in healthy controls n (%)	p value (Chi square test)
1	42 (21%)	67 (33.5%)	0.008

The detailed split of the secretor status among various blood groups reflected that secretors were highest among O blood group followed by patients with A group. The picture was similar among study subjects and healthy control in the distribution of blood groups and secretors. However, non-secretors were found to be more common among study population in groups O, A and AB as highlighted in the table. (Table 2)

Table 2 Distribution of Secretor status among Blood groups and study population

Blood Group	Study Subjects			Healthy Controls		
	Total 198 (%)	Secretor 42 (21%)	Non secretor 156 (78%)	Total 200 (%)	Secretor 67 (33.5%)	Non secretor 133 (66.5%)
O	72 (36)	18 (25)	54 (75)	76 (38)	33 (43.4)	43 (56.6)
A	56 (28)	15 (26.8)	41 (73.2)	64 (32)	24 (37.5)	40 (62.5)
B	58 (29)	08 (13.8)	50 (86.2)	50 (25)	08 (16)	42 (84)
AB	12 (6)	01 (8.3)	11 (91.7)	10 (5)	02 (20)	08 (80)

A total of 52 patients presented with symptoms of lower urinary tract infections (UTI) in the study population. Among them, 47 (90.4%) patients had white blood cell (WBC) casts in urine microscopy and 40 (76.9%) of them had positive urine culture. In patients with symptoms of UTI, 44 patients (84.6%) were non-secretors. (Table 3)

Table 3 Organisms identified in Culture of Urinary Tract Infection patients in the Study group

S.No	Organism Total n=40	Number/Percentage
1	Escherichia coli	28 (70%)
2	Klebsiella pneumoniae	8 (20%)
3	Pseudomonas aeruginosa	2 (5%)
4	Enterococcus fecalis	1(2.5%)
5	Proteus mirabilis	1(2.5%)

Patients with diabetes mellitus were assessed for the duration of the disease both as continuous variables and as categorical variables. Duration of diabetes was categorized as patients who had the disease for < 3 years, 3-6, 6-9, 9-12 and >12 years and the number of patients in each category was 63, 65, 22, 18 and 30 respectively. Statistical analysis was performed to correlate the duration with various complications, age, sex, hypertension and secretor status.

Univariate analysis was performed for all the parameters. Age of the patient is significantly higher in the study (diabetes) group when compared with healthy control group. Mean age of the patients in control group is 39.8 and in the diabetes group, it is 46.63. Using Two sample t-test, the p value is <0.001. Hypertension is significantly associated with study group (p value is <0.001; Pearson's Chi square test). Non-secretors were significantly more common in study group (diabetes) when compared with control group while secretors were significantly less common in the study group (p value is <0.001; Pearson's Chi square test). Rest of the parameters were not significantly associated in Univariate analysis between the two groups. (Tables 4,5,6).

Table 4 Age – Controls vs type II Diabetes group (Two sample t test with unequal variances)

Group	Observations	Mean	Std. Err	Std. Dev	95% conf. interval	
0	200	39.805	0.5273284	7.45755	38.76513	30.84487
1	198	46.63131	0.5972146	8.403554	45.45356	47.80907
Combined	398	43.20101	0.432981	8.637088	42.34987	44.05214
Diff		-6.826313	0.796706		-8.392695	-5.259931
Diff = mean (0) – mean (1)				t = -8.5682		
Ho:diff<0		Satterthwaite's degree of freedom = 389.531				
Ha: diff < 0		Ha: diff = 0		Ha: diff > 0		
Pr(T < t) = 0.00000		Pr(T > t) = 0.00000		Pr (T>t) = 1.0000		

Table 5 Hypertension – Controls vs type II Diabetes group
(group 0= healthy control, 1= diabetes mellitus; HT 0=hypertension absent, 1=htn present)

HT	Group 0	Group 1	Total
0	193 96.50	64 32.32	257 64.57
1	7 3.50	134 67.68	141 35.43
Total	200 100.00	198 100.00	398 100.00
Pearson Chi square (1) = 179.1355			Pr = 0.000

Table 6 Secretor status in Controls and type II DM patients
(Group 0= healthy control, 1= diabetes mellitus; Secretor 0=secretor, 1= Non-secretor)

Secretor	Group 0	Group 1	Total
0	67 33.50	42 21.21	109 27.39
1	133 66.50	156 78.79	289 72.61
Total	200 100.00	198 100.00	398 100.00
Pearson Chi square (1) = 7.5545			Pr = 0.006

Multivariate logistic regression analysis was performed between the healthy volunteer group and the group with diabetes mellitus. When adjusted for other (potential confounders) significant parameters in Univariate analysis viz., age, hypertension and blood group, secretors are nearly 2.1 times (110%) at higher odds of having diabetes mellitus (odds ratio = 2.1). When

adjusted for WBC casts, age and hypertension, secretors are nearly 2.4 times at higher odds of having diabetes mellitus (odds ratio = 2.4). In multivariate analysis, Blood group B was significantly associated with secretor status (p value = <0.001). (Tables 7,8,9).

Table 7 Multivariate Logistic regression of secretors for age, diabetes, blood group and hypertension

Logistic regression				Number of obs = 398 LR Chi^2 (6) = 70.28 Prob>Chi^2= 0.0000		
Log likelihood = -198.51572				Pseudo R2 = 0.1504		
Secretor	Odds Ratio	Std. Error	Z	P > Z	95% conf. Interval	
DM	2.130969	0.6836593	2.36	0.018	1.136303	3.996321
Age	0.9941957	0.160869	-0.36	0.719	0.9631606	1.026231
HT	0.807016	0.0330899	-6.14	0.000	0.361298	0.1802596
Blood Group						
1	0.9186675	0.2598521	-0.30	0.764	0.527703	1.59929
2	0.2902595	0.0994978	-3.61	0.00	0.148253	0.5682889
3	0.4113257	0.279514	-1.31	0.191	0.108552	1.558597

Table 8 Multivariate Logistic regression of diabetes for secretors, age and hypertension

Logistic regression				Number of obs = 398 LR Chi^2 (6) = 245.52 Prob> Chi^2= 0.0000		
Log likelihood = -153.10967				Pseudo R2 = 0.4450		
DM	Odds Ratio	Std. Error	Z	P > Z	95% conf. interval	
Secretor	2.125107	0.6863043	2.33	0.020	1.128456	4.001996
Age	1.105483	0.0221705	5.00	0.000	1.062873	1.149802
HT	70.1241	31.82345	9.37	0.000	28.81232	170.6697
Blood Group						
1	0.9852747	0.3520296	-0.04	0.967	0.4891374	1.984649
2	1.313316	0.4828664	0.74	0.459	0.6388641	2.699792
3	0.4672713	0.3841435	-0.93	0.355	0.0932817	2.340677

Table 9 Multivariate Logistic regression of secretors for diabetes, WBC casts, age and hypertension

Logistic regression				Number of obs = 398 LR chi^2(7) = 71.61 Prob>chi^2= 0.0000		
Log likelihood = -197.84645				Pseudo R2 = 0.1532		
Secretor	Odds Ratio	Std. Error	Z	P > Z	95% conf. interval	
DM	2.401449	0.8120429	2.59	0.010	1.237776	4.659128
WBC cast	0.5572227	0.2888805	-1.13	0.259	0.2017164	1.539276
Age	0.9944477	0.0161011	-0.34	0.731	0.9633857	1.026511
HT	0.0814148	0.0334764	-6.10	0.000	0.0363667	0.1822648
Blood Group						
1	0.9499435	0.2703407	-0.18	0.857	0.5438231	1.65935
2	0.2890725	0.0992846	-3.61	0.000	0.1474531	0.5667084
3	0.4150275	0.2831756	-1.29	0.197	0.1089676	1.580725

Pearson Chi Square test was performed within the study group (diabetes mellitus) for association of secretor status, duration of diabetes and various complications. Non-Secretor status is significantly associated culture positive urinary tract infection (p value = 0.005). Secretor status is significantly associated with hypertension (p value < 0.001). WBC casts were significantly associated with study group when compared with healthy control group (p value <0.001).

Two sample t test with unequal variances was performed and the following parameters were found to be significant. Non-secretor status is significantly associated with increasing duration of diabetes (p value = 0.0176). Increased HbA1c level is significantly associated with both

microalbuminuria, macroalbuminuria, retinopathy and neuropathy (p value < 0.001).

Raise in HbA1c level is significantly associated with duration of diabetes (p value < 0.001, ANOVA, Barlett test for equal variances) and also with increase in serum creatinine (p value < 0 .001, ANOVA).

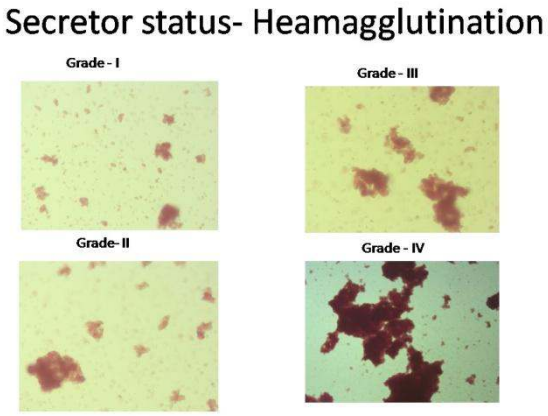

Fig 01

Discussion

Non-secretor status has been associated in the pathogenesis of diseases such as inflammatory bowel disease, rheumatic fever and increased predisposition to infections[16].

Diabetic non-secretors appear to have lower levels of complement fractions when compared to diabetic secretors[17]. In our study, Non-secretors contributed to 66.5% in the healthy controls, while in patients with type II diabetes mellitus, 79% of the patients were non-secretors. Non-secretors constituted a much higher proportion in South Indian population when compared with Caucasian population[18], where only 20% of the population were non-secretors. Our study results differ from a similar study in Pakistan19, where around 35% of their healthy population were non-secretors. The difference is significant as the method used is by adsorption-inhibition method in all the studies with serial dilutions.

However, in a recent study from Bangladesh[20], the non-secretor status ascertained by FUT2 genotyping status was 40%, these variations may be due to significant genetic variations across various geographic regions, thus highlighting the need to analyze this factor in greater detail. (Table 10)

Table 10 Comparison of Secretor status in different populations

S.No	Non-secretors inhealthy controls in our study	Study from Rajasthan (Metgud R et al 2016) [2]	Caucasian population (McGovern DP et al, 2010) [16]	Study from Karachi (Saboor M et al 2014) [19]	Study from Bangladesh (Mottram L et al 2017) [20]
1	66.5%	20%	20%	35%	40%

We also found significant increase in the percentage of non-secretors, constituting around 79% in the patients with type II diabetes mellitus. The increase in percentage of non-secretors in correlation with healthy controls was found to have strong statistical significance.

On Univariate analysis between the healthy control group and the diabetes group, age, hypertension and secretor status were significantly associated. Multivariate analysis was performed with logistic regression and when adjusted for age, hypertension and blood group, secretors are nearly 2.1 times (110%) at higher odds of diabetes mellitus. WBC casts, age and hypertension were adjusted by logistic regression and secretors are nearly 2.4 times at higher odds of diabetes mellitus (odds ratio = 2.4). Thus, though non-secretors appear to be predisposed to diabetes mellitus, when the data is analysed in comparison with healthy control and adjusted for confounding factors such as age and hypertension, secretors are at higher odds of developing diabetes mellitus. Our data is similar to the study by Smyth et al[21], who found significant association of non-secretors with type I diabetes mellitus in a study design comprising of both healthy controls and study subjects.

In our study, we also found significant association of non-secretor status with duration of diabetes mellitus and culture positive UTI, while clinically

suspected and UTI with positive WBC casts in urine did not show association. HbA1c levels were significantly associated with microangiopathic complications of diabetes mellitus which is similar to several other studies in the literature.

Non-secretors were also common in the elderly. We could not find any report in the literature with this association. Possible heterozygous non-sense and frameshift mutations in FUT2 gene, which is a fetal gene involved in innate immunity may possibly be a reason for the same. However, the exact cause and effect association of age and non-secretor status was not studied in this work.

Another interesting novel finding in the study was the significant association of hypertension with secretor status (p value<0.001). We did not find any literature with the association of secretor status and hypertension. The possible indirect association that we hypothesize is that gut microbiota is significantly associated with both systolic and diastolic hypertension[22], and at the same time secretor status is proven to have significant role in the modulation of gut microbiome[12]. However, the direct cause and effect association of hypertension with secretor status needs further study.

Association of type I diabetes has been reported with non-secretors earlier[21]. In our study too, if the analysis was confined only to the study group of patients with type II diabetes mellitus, non-secretors had significant association with type II diabetes mellitus (p value = 0.006). However, on Cox multivariate regression analysis, after eliminating the potential confounders age and hypertension, secretor status was significantly associated with type II diabetes mellitus with over 110% higher odds (Odds ratio – 2.1).

In our study, non-secretor status was significantly associated with culture positive urinary tract infections and this was similar to the results from various other studies[23, 24].All the patients with UTI included in our study group had lower urinary tract infection and we did not have any patients with pyelonephritis in our analysis.

Our results were however contradictory to the study by Smyth DJ et al[21], who had described increased resistance to infection in association with non-secretors.

We did not find any significant association of secretors with microvascular complications of type II diabetes mellitus. Raise in HbA1c levels showed significant association with retinopathy, neuropathy and diabetic nephropathy with increase in microalbuminuria, macroalbuminuria as well as serum creatinine levels (p value < 0.001). Our findings are similar to the findings from Ma et al[25].

In our study, we did not carry out genotyping for various alleles of HbA1c but however in one of the earlier studies, genome wide association studies of the HbA1c locus could not show any association with complications of type II diabetes mellitus in Asian population[26].

Another interesting observation that is highlighted in our study on multivariate analysis is the significant association of blood group B with secretor status. We could not get any literature on this association in the same population. However, in a study from Iraq, blood group O was found to be significantly associated with secretors[27].

The major limitation in the study is the cross-sectional nature of the study with lack of follow-up. However, the findings observed are novel and merit further attention and analysis in a larger population.

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Presence of ABO Antigens of Blood Types in Saliva of Women with Urinary Tract Infection

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ABSTRACT

Absence of the ABO antigen in saliva is a health disadvantage, and could increase susceptibility to a number of diseases such as urinary tract infection.

objective is to explore the influence of secretion of ABO blood group antigens into the body fluids (saliva) in women suffer from UTI.

A total of 241 women aged 18-45 years were included who complained of symptoms indicating UTIs who were attending Obstetrics and Gynecology Department of Al-Yarmouk Teaching Hospital in Baghdad during the period from March 2016 to May 2017. The secretor status of the patients was then determined using the haemagglutination inhibition assay for salivary ABO antigens. ABO antigens secretors were found in 36 women and was higher in women in the age group less than 25 and 25-29 years. Education, occupation and source of water have showed significant effect on infected women with ABO antigens secretor and non-secretor. There are significant differences between both ABO antigens secretors and non-secretor in the presence of pus cells and RBCs, and 13 women infected with *Trichomonas vaginalis* and 11 of them with negative ABO antigens secretors. Positive growth reported in399 specimens. Single Bacterial growths in 149 and 62 with more than one species. The species of bacteria is primarily *Escherichia coli* followed by *Streptococcus, Staphelococcus aureus*, *Pseudomonas, Proteus,* and *Klebseilla*. In conclusion, absence of ABO antigens in saliva increases the susceptibility to UTI with a greater tendency to increased symptoms.

Keywords: *ABO antigens, Saliva, Urinary Tract Infection*

INTRODUCTION

Urinary tract infections are amongst the commonest infections with an extensive fiscal liability to the public, particularly in women, babies and the elderly as around one in two women and one in twenty men will get the infection in their lifetime [1]. The threat of women getting Urinary tract infections in their lives is said to be above 50%, with almost 25 percent experiencing a recurrence [2]. Almost 53%of women aged more than 55 years and about 36% of younger women record a recurrence in a time within one year [3].The most common causative pathogens are Gram-negative rods "*Escherichia coli* which cause about 80% of acute infections. Other Gram-negative creatures comprise *Klebsiella pneumonia* and *Proteus mirabilis*, creatures which inhabit enteric area like Serratia, Pseudomonas, and Enterobacter are rare in the outpatient groups, and nonetheless they are very common in people with intricate Urinary tract infections [4]. A Gram positive coagulase known *Staphylococcus saprophyticus* negative Staphylococcus, results in nearly 10 percent of infections in sexually active young women". Trichomonus vaginalis also can cause UTIs, which is more common in the small group of women [5].

A Secretor can be described as "an individual who secretes their ABO antigens secretors into body fluids like saliva and mucus", while non-secretor on the other hand puts little to none of their ABO antigens secretors into these fluids. Many researchers reported the susceptibility to affect by disease increased among non-ABO antigens secretors giving reasons for these associations to be due to presence of these antigens will add a degree of protection against infectious agents [6] that

will influence pathogenic activity [7]. Non-ABO antigens secretors are at a bigger threat for recurring of Urinary tract infections and are more probable to experience renal scars [8]. Therefore, the present study aimed to determine the relationship between the ABO antigens and susceptibility of women to UTIs.

PATIENTS AND METHODS

This was a cross sectional study included 241 married women aged 18-45 years and live in Baghdad city, complaining of symptoms indicating UTIs who were attended to Obstetrics and Gynecology Department of Al-Yarmouk Teaching Hospital in Baghdad, Iraq during the period from March 2016 to May 2017. Urine specimens were collected using a clean, sterile, plastic bags from each infected woman also 1 ml of non-stimulated saliva was collected from each woman into a sterile glass jar. Questionnaire including socio-demographic and clinical data.

The collected urine samples were centrifuged, then microscopic examination was performed. Each sample was cultured aerobic and facultative anaerobic on different media (Blood agar, Mac Conkey agar, Chocolate agar, Manitol salt agar, Milk agar, Sabouroud Dextrose agar to isolate bacteria and fungi). Regarding isolates diagnosis, it was done according to the well-known established microbiological methods, principally based on morphological characters, Gram-staining method and biochemical reactions.

The salivary presence of ABO antigens was determined using haemagglutination inhibition assay using anti A, B and D sera based on a principle that if ABO antigens present in saliva they will bind with antibodies in the antisera added. The antibodies were n0t available in the mixture (Saliva & Antisera) t0 agglutinate with RBCs suspensins and the subject is a positive ABO antigen secretory and *vice versa* is none or a negative ABO antigens secretory subject [9,10].

Data analysis done using Statistical Packages for Social Sciences- version 24). and appropriate statistical tests were applied according to the variables compared.

FINDINGS

The presence of ABO antigens secretor was found in 36 women and higher levels were reported among women in the age group less than 25 years and 25-29 years (9 and 12 respectively) (Table 1).

Education, occupation and source of water have significant effect on the presence of UTI and there is a significant difference between ABO antigens secretor and non-secretor (Table 2).

The main symptom is suprapubic pain in both ABO antigen secretor and non-secretors, followed by itching and secretions. The more prevalent paired of presence of symptoms is between 7 to 13 days in both ABO antigens secretor and non-secretor (Table 3).

The microscope examination indicated that there are significant differences between both ABO antigens secretor and non-secretor in the presence of pus cells and RBCs. The patients with ABO antigens secretors have no blood cells in urine and only 8 patients have excretion of epithelial cells in urine (Table 4).

Also microscopic examinations indicated that 13 women infected with *Trichomonas vaginalis* and 11 of them (84.6%) were ABO antigens non-secretor

The results of culture in specific media indicated that 399 give positive growth and only 2(0.8%) give no growth. The bacterial growths were present as single bacterial infection is 149, while mixed infections are 62 women that infected with more than one type of bacteria. Fifty-eight women infected with two types of bacteria, while only 4 women infected with three types of bacteria. There are significant differences between ABO antigens secretor and non-secretor (Table 5).

The species of bacteria that present in urine of women included in this study is primarily *Escherichia coli* followed by *Streptococcus, Staphelococcus aureus*, *Pseudomonas, Proteus,* and *Klebseilla* which is the latest one. Also significant differences were observed between ABO antigens secretor and non-secretor (Table 6).

Table 1. Distribution ABO antigens secretor in saliva according to age group.

Age (years)	ABO antigens secretor in saliva		
	Yes (n=36) n (%)	**No (n=205) n (%)**	**Total (n=241) n (%)**
<25	9 (25.0)	28 (13.7)	37 (15.4)
25-29	12 (33.3)	55 (26.8)	67 (27.8)
30-34	4 (11.1)	62 (30.2)	66 (27.4)
35-39	8 (22.2)	44 (21.5)	52 (21.6)
≥ 40	3 (8.3)	16 (7.8)	19 (7.9)
P. value = 0.13			

Table 2. Association between socio-demographic characteristics and presence of ABO antigens secretor of the studied group.

Variable		ABO antigen secretor in saliva			P. value
		Yes(n=36) n(%)	**No (n=205) n(%)**	**Total (n= 241) n(%)**	
Education	Illiterate	1 (2.8)	3 (1.5)	4 (1.7)	0.002*
	Primary	10 (27.8)	120 (58.5)	130 (53.9)	
	Intermediate	12 (33.3)	54 (26.3)	66 (27.4)	
	Secondary	6 (16.7)	18 (8.8)	24 (10.0)	
	College & Higher	7 (19.4)	10 (4.9)	17 (7.1)	
Occupation	Housewife	30 (83.3)	194 (94.6)	224 (92.9)	0.015*
	Employed	6 (16.7)	11 (5.4)	17 (7.1)	
Source of water	Tap water	26 (72.2)	179 (87.3)	205 (85.1)	0.019*
	Filter	10 (27.8)	26 (12.7)	36 (14.9)	
*Significant at P < 0.05					

Table 3. Distribution of main presenting symptoms of the studied group in correlation to the presence of ABO antigens.

Variable		ABO antigens secretor in saliva*			P value
		Yes (n=36) n (%)	No (n=205) n(%)	Total (n= 241) n(%)	
Symptoms	Supra-pubic pain	34 (94.4)	194 (94.6)	228 (94.6)	0.992
	Itching	1(2.8)	6(2.9)	7 (2.9)	
	Secretions	1(2.8)	5(2.4)	6 (2.5)	
Period of infection (days)	< 7	5 (13.9)	24 (11.7)	29 (12.0)	0.924
	7 - 13	30 (83.3)	176 (85.9)	206 (85.5)	
	≥ 14	1 (2.8)	5 (2.4)	6 (2.5)	
	Mean ± SD (range)	7.3±1.6(4-14)	7.6±5.5(4-60)	7.5±5.1(4-60)	
*values are number and (%) unless mentioned. SD: standard deviation,					

Table 4. Results of microscope examination in correlation to the presence of ABO antigens in saliva of women infected with UTIs.

Direct microscope examination		ABO antigens secretor in saliva			P.value
		Yes (n=36) n (%)	No (n=205) n (%)	Total (n=241) n (%)	
Pus cells	Negative	24 (66.7)	38 (18.5)	62 (25.7)	0.0001*
	+	12 (33.3)	107 (52.2)	119 (49.4)	
	++	0 (0.0)	55 (26.8)	55 (22.8)	
	+++	0 (0.0)	5 (2.4)	5 (2.1)	
RBCs	Negative	36 (100.0)	177 (86.3)	213 (88.4)	0.062
	+	0 (0.0)	26 (12.7)	26 (10.8)	
	++	0 (0.0)	2 (1.0)	2 (0.8)	
Epithelial cells	Negative	28 (77.8)	117 (57.1)	145 (60.2)	0.106
	+	6 (16.7)	50 (24.4)	56 (23.2)	
	++	2 (5.6)	36 (17.6)	38 (15.8)	
	+++	0 (0.0)	2 (1.0)	2 (0.8)	
*Significant at P<0.05					

Table 5. Result of cutler in specific media in correlation with presence of ABO antigens in saliva of women infected with UTIs.

Culture finding	ABO antigens secretor in saliva		
	Yes (n=36) n (%)	No (n=205) n (%)	Total (n=241) n (%)
No growth	2 (5.6)	0 (0.0)	2 (0.8)
One type of bacteria	28 (77.8)	149 (72.7)	177 (73.4)
Two type of bacteria	6 (16.7)	52 (25.4)	58(24.1)
Three type of bacteria	0 (0.0)	4 (2.0)	4 (1.7)

Table 6. Species of bacteria that present in correlation with presence of ABO antigens in saliva of women infected with UTIs.

Species of bacteria	ABO antigen secretor in saliva			P value
	Yes (n=36) n (%)	No (n=205) n (%)	Total (n=241) n (%)	
Escherichia coli	19 (52.8)	91 (44.4)	110 (45.6)	0.35
Streptococcus	7 (19.4)	54 (26.3)	61 (25.3)	0.38
Staphylococcus aureus	10 (27.8)	40 (19.5)	50 (20.7)	0.26
Pseudomonas	0 (0.0)	31 (15.1)	31 (12.9)	0.012*
Proteus	2 (5.6)	30 (14.6)	32 (13.3)	0.14
Klebsiella	2 (5.6)	19 (9.3)	21 (8.7)	0.47
*Significant difference.				

DISCUSSION

Urinary tract infection (UTIs) reported in almost 50% of women at some point in their lives [11], and higher morbidity rates associated with these infections. In the genetics of secretor system two options exist; a person can be either ABO antigens secretor or a non-secretor. This was found to be completely independent of person's blood type "A, B, AB, or O" . Several researches have suggested that too many diseases observed in some ABO antigens non-secretor individuals including UTI [12], *Helicobacter pylori* infection [13] and viral infections [14].

The current study revealed a non-significant association between secretor status and symptoms and the period of infection, while there was a significant association between presence of ABO antigens secretor and presence of pus, RBC, and epithelial cells in urine when examined microscopically. Regarding RBCs, all secretors positive had no RBCs in the urine while the sloughed epithelial cells reported in 8 secretors cases. These were also seen in the infected bacteria and *Trichomonas vaginalis*, the heavily bacterial infection with mixed species were present in non-secretor of ABO antigens, these findings agreed other researchers [15, 16], however, enteric bacteria; in particular, *Escherichia coli* remain the mos frequent case of UTIs. The infection with *Trichomonas vaginalis* was more prevalent in non ABO antigens secretor (84.6%). These may be due to the non-secretary people do not have the enzyme glycosyl-transferase and glyco-compounds giving a way for attachment of the organism with epithelial surface therefore resulting in an infection [15]. It is clear that non-secretor saliva n0t 0nly does not avert the connetion of candida but also stimulates the attachment to the nerves.

The virulence features of candida are as a result of host identification by the cell surface linkage [16]. Other researchers attributted this susceptibility to infections to low levels of IgG and IgA antibodies in non-secretors [17]. Antibodies seem to offer native immunity through destruction of the organism; secretors destroy attacking organisms and stop their access to the host. This description best suites current study that single and little growth seen in secretor women while mixed and heavy growth seen in none secretors. Other researches stated that the secretor status alters the carbohydrates present in the body fluids and this will influence microbial attachment and persistence [18]. The present study agreed other study on UTI that the primarily causes is *Escherichia coli*. Staplet0n et. al, [19] have stated that females with persistent UTI associated to *E. coli* are mainly n0n-secret0rs. The tendency f0r greater adherence 0f the ur0pathogenic *E.coli* was shown by uroepithelial cells of non-secretors when matched with secretors. This appears that absence of secretor substances combines to give an increased risk of recurrent UTI.

In this study, it was found that some demographic characteristics like education, occupation and source of water were associated with absence of ABO antigens and hence increased the susceptibility of UTIs. The same finding was reported by Emiru et. al, [20]; as they mentioned that UTI was high among pregnant women in the presence of associated different risk factors (anemia, low socio-demographic features, past history of UTI and sexual activity).

CONCLUSIONS

The absence of ABO antigens in saliva might increases the susceptibility to UTI in women with a greater tendency to increase symptoms, number and type of causative infectious agent and tend to present worst in low socio-demographic status.

Conflict of Interest : None

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Ethical Clearance: All official ethical agreements were approved Data of participants were collected according to the World Medical Association Declaration of Helsinki 2013, and signed consent was obtained from each participant

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Original Article

Clinical significance of the fucosyltransferase 2 (FUT2) secretor status in children hospitalized with acute gastroenteritis in Taiwan

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KEYWORDS
Fucosyltransferase 2 (FUT2);
Acute gastroenteritis;
Taiwan

Background/Purpose: The FUT2 gene is a histo-blood group antigen (HBGA) that determines the susceptibility to Norovirus (NoV) infection. This study investigated the clinical significance of the FUT2 gene profile and HBGA expression in NoV infection.
Methods: Fecal specimens were collected from children in Chang-Gung Children's Hospital with acute gastroenteritis (AGE). The medical records were reviewed for clinical data. The viral etiology of gastroenteritis was validated using molecular methods. Genomic DNA was isolated from saliva or whole blood with the Puregene B Kit, according to the manufacturers' instructions. Single-nucleotide polymorphisms (SNPs) were determined by real-time PCR assays.
Results: FUT2 gene DNA was examined in 98 children with AGE. NoV was detected by RT-PCR in 44 patients (44.8%), while 54 (55.2%) had non-NoV AGE. Of the 44 NoV patients, 38 (86.3%) were secretors (no G428A mutation) and six (13.7%) were non-secretors (G428A mutation). Of the 54 non-NoV AGE patients, 28 (51.9%) were secretors and 20 (48.1%) were non-secretors. NoV-

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infected patients who were secretors had more frequent vomiting ($P < 0.001$), longer duration of diarrhea ($P < 0.001$), and greater overall disease severity score ($P < 0.001$) compared with non-secretors. Non-NoV infection secretor AGE patients had a longer duration of diarrhea ($P < 0.001$) than non-secretors.

Conclusion: FUT2 secretor status affects NoV AGE in children. Secretor patients have prolonged diarrhea, more frequent vomiting, more severe disease, and greater infection transmissibility than non-secretors.

Introduction

Norovirus (NoV) is an enteric pathogen that is a global health burden and the leading viral cause of outbreaks of gastroenteritis worldwide. Although most symptoms of NoV infection are self-limiting, recurrent infections are not uncommon in children and the elderly. NoV outbreaks in northern Taiwan are caused by different circulating strains and we showed that the infections were associated with complications and reported several specific, uncommon presentations.[1–4] The rapid evolution and complex genetic diversity of NoV have made virus identification, classification, and surveillance difficult. Using molecular methods, the detailed genetic and molecular features of circulating NoVs have been revealed.[5,6]

Virus secretor status is controlled by the alpha-1,2-fucosyltransferase (FUT2) gene of histo-blood group antigens (HBGAs) and determines the susceptibility to NoV infection. Nonsecretors (FUT2 −/−) are highly resistant to symptomatic infections with major strains of NoV, and 20% of Europeans are nonsecretors.[7] Furthermore, the susceptibility of HBGAs to NoV infection may be strain-specific, rather than genogroup-dependent.[8]

We investigated the FUT2 gene profile and HBGA expression and antigen secretion patterns of children in Taiwan with acute gastroenteritis (AGE) to explore their effects on the clinical manifestations and complications of NoV infection.

Patients and methods

Patients enrollment and clinical assessment

Our study recruited hospitalized AGE patients aged from 3 months to 18 years in Division of Pediatric Gastroenterology, Chang-Gung Children's Hospital with the major presentation of acute nonbloody diarrhoea (passage of watery or loose stools) within 3 days. The study was approved by Chang Guan Medical Foundation Institutional Review Board 102−1437B. After obtaining informed consent from their guardians, their fecal and serum (or saliva) samples were collected. Clinical data obtained by reviewing the medical records included patient age, gender, and symptoms, including diarrhea frequency and duration, vomiting frequency and duration, dehydration status, fever, the presence of bloody stool, abdominal pain, bilious vomiting, abnormal electrolytes, and other laboratory findings. The severity of AGE was evaluated referred to Vesikari's score as previously described,[9] by scoring frequency and duration of diarrhoea or vomiting, electrolyte and dehydration status, fever severity based on the maximum temperature of fever], and the need for medical management.

Enteric viral etiology identification

The viral nucleic acids were extracted with a High Pure Viral RNA kit (Roche), following the manufacturer's recommendations. Viral nucleic acids were extracted from fecal samples using a QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). The PCR primers and conditions used for determining NoV genotypes were described previously.[10] All reference NoV sequences used for comparison were obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov).

DNA extraction and *FUT2* gene determination

Genomic DNA was isolated from saliva or whole blood with the Puregene Blood Core Kit B (QIAGEN, Hilden, Germany), according to the manufacturers' instructions. The genotype was determined using DNA extracted from peripheral blood leukocytes on treatment with 1.6 M sucrose and 10 mg/mL proteinase K. The FUT2 gene was screened by PCR for the most common inactivating mutation (G428A) using specific primers for the mutation: Se antisense (Seas) combined with Se1s sense (s) for the wild-type allele and se with Se2s for the mutant, as described previously. Exon

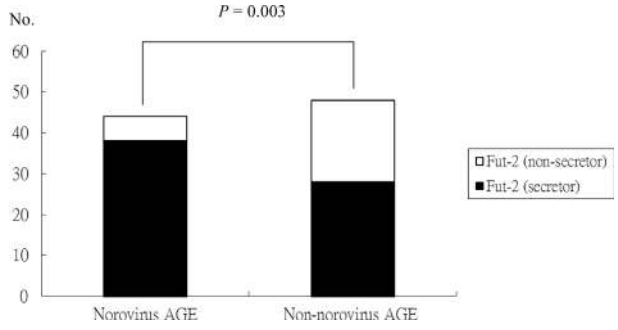

Figure 1 FUT-2 gene mutation and SNP expression profiles of 92 children hospitalized with AGEs.

2 was sequenced using primers designed for this study from the reference sequence in GenBank (NCBI). For sequencing, the PCR products were purified with ExoSAP-IT (GE/USB). The sequences were aligned using the BioEdit Sequence Alignment Editor ver. 7.0.9.0. Single-nucleotide polymorphisms (SNPs) were determined using real-time PCR assays.

Statistical analysis and clinical correlations

Continuous clinical data were analyzed using the Wilcoxon test and are expressed as the median [interquartile range (IQR)]. Binary data were analyzed using the χ^2 test. A *P*-value <0.05 was considered to indicate statistical significance. All tests were performed using SAS ver. 8 for Windows (SAS Institute, Cary, NC, USA).

Results

Sample collection and NoV identification

From January 2017 until March 2018, 108 children with AGE were enrolled. Of these, stool, serum, or saliva specimens were tested for FUT2 gene (secretor) genomic DNA in 98 children. Sequence-confirmed enteric viral pathogens were detected in 72 stool specimens from NoV and non-NoV AGE cases. Finally, a total of 92 patients fulfilled clinical, pathogen, and FUT2 gene studies and were analyzed subsequently.

NoV genotypes and non-Nov infections

Norovirus was detected by RT-PCR in 44 patients (47.8%), while 48 (52.8%) had non-NoV AGE (22 with rotavirus, 5 enteric adenovirus, 3 astrovirus, and 20 undetermined). The most commonly detected genotype, NoV GII.4, was detected in 23 (52.2%), followed by NoV GII.2 (7, 15.9%) and GII.16 (4, 9.1%). Of the GII.4 strains, GII.4 Sydney 2012 (11, 47.8%) was the most common.

FUT2 gene mutation and SNP expression

The only *FUT2* nonsecretor SNP found in our study population was at position 428, and it was used to determine secretor status. Of the 44 NoV infection patients, 38 (86.3%) had secretor status (no G428A mutation) and 6 (13.7%) were non-secretors (G428A mutation). Of the 48 AGE patients with non-NoV infection, 28 (58.3%) were secretors (no G428A mutation) and 20 (41.6%) were non-secretors (G428A mutation) (Fig. 1).

Clinical manifestations of the children with NoV infection according to secretor status

The NoV-infected patients with secretor status had a higher frequency of vomiting ($P < 0.001$), longer duration of diarrhea ($P < 0.001$), and higher overall disease severity score ($P < 0.001$) than the non-secretors (Table 1); there were no significant differences in patient age, gender, duration of vomiting, frequency of diarrhea, days hospitalized, incidence of complications, or family history. The patients with non-NoV AGE with secretor status had a longer duration of diarrhea ($P < 0.001$) than nonsecretors (Table 1); there were no significant differences in patient age, gender, frequency and duration of vomiting, frequency of diarrhea, overall disease severity, days hospitalized, incidence of complications, or family history.

Table 1 Demographic data and clinical manifestations of children with norovirus infection with different secretor status.

Characteristic	Norovirus AGE (secretor)	Norovirus AGE (non-secretor)	Non-norovirus AGE (secretor)	Non-norovirus AGE (non-secretor)	*P* value[a]	*P* value[b]
Number	38	6	28	20		
Age (months)	39 (18–54)[c]	25 (13–40)	28 (15–38)	33 (18–42)	0.817	0.937
Male to female ratio	17 to 21	4 to 2	16 to 12	11 to 9	0.316	0.217
Frequency of vomiting (times/day)	2.8* (0–4)	2 (0–3)	3 (0–5)	2.6 (0–4)	<0.001	0.984
Duration of vomiting (days)	2 (0–4)	1.8 (0–3)	3 (0–5)	3.8 (0–6)	0.33	0.814
Frequency of diarrhoea (times/day)	4.5 (3–6)	5.0 (2–7)	5.2 (3–7)	5.5 (2–8)	0.798	0.722
Duration of diarrhoea (days)	4.8* (2–7)	3.3 (1–6)	6** (3–8)	5.2 (3–7)	<0.001	<0.001
Fever > 38.5C n (%)	12* (31.5)	1 (16.6)	19 (48.7)	3 (33.3)	<0.001	0.817
Fever > 39C n (%)	5 (13.1)	0 (0)	9 (23)	2 (22.2)	0.266	0.316
Disease severity score[d]	10.8 (8–13)	9.7 (7–11)	11.5 (8–14)	10.8 (7–12)	<0.001	0.475
Hospitalization (days)	6 (4–8)	5.8 (4–7)	5.6 (4–9)	6.2 (5–9)	0.32	0.604
Complications n (%)	11 (28.9)	1 (16.6)	13 (33.3)	8 (20)	0.943	0.208
Family Hx	8 (21.1)	1 (16.6)	4 (10.3)	1 (5)	0.604	0.33

[a] Statistical comparison of norovirus AGE with secretor or non-secretor status. (*statistical significance).
[b] Statistical comparison of non-norovirus AGE with secretor or non-secretor status. (**statistical significance).
[c] Values are given as median value (interquartile range 25%–75%).
[d] The severity of AGE was evaluated refered to Vesikari's score.[9]

Discussion

The human secretor FUT2 gene, which encodes an alpha-(1,2)-fucosyltransferase synthesizing the H-type 1 antigen in saliva and the mucosa, is associated with the susceptibility to NoV infection.[11] It is a genetic population marker based on mutations occurring in populations with different evolutionary histories.[12] The frequencies of secretor and nonsecretor phenotypes are similar in different populations with different point mutations at the base of the phenotypes. A previous study showed the minor allele frequency (MAF) in Europe, sub-Saharan Africa, Central-South Asia, and East Asia of nonsynonymous SNPs, *se*385 is 0.4%, 0%, 4.10%, and 43.8%; while the frequency of SNPs, *se*428 is 22.0%, 42.9%, 32.9%, and 0.70% individually.[13] Genotyping enables the comparison of allele frequencies. Differences can arise due to protective immunity or a lack of exposure despite being close to areas where outbreaks have occurred. The classic human secretor locus (Se) FUT2 regulates expression of the Lewis ABO(H) HBGAs on the surface of epithelial cells and in body fluids and determines the secretion status of ABO antigens.[14] FUT2 secretors are at significantly greater risk of both symptomatic and asymptomatic NoV infections.

Our study investigated the FUT2 gene secretor status of pediatric patients hospitalized in Taiwan with AGE caused by NoV or non-NoV infection. We found that the FUT2 gene in NoV AGE was mostly the secretor phenotype (86.3%) as opposed to only 51.9% in non-NoV AGE. This is similar to a report that the FUT2 nonsecretor genotype is associated with resistance to NoV outbreaks.[11] A recent study showed that 91.80% of the children born in the Amazon who developed AGE or acute respiratory infection had the secretor phenotype.[12] We found that high secretor status was found only in NoV AGE in Taiwan. Another recent study suggested that secretors were also more susceptible to rotavirus infections based on elevated rotavirus-specific serum antibodies,[15] although we found no such phenotypic predominance in non-NoV infection. Further etiology studies are warranted.

We found that children with the secretor phenotype were more susceptible to NoV infections and were sicker, with more frequent vomiting, longer duration of diarrhea, and greater overall disease severity score. Therefore, the FUT2 secretor status affects not only host susceptibility but also the disease severity in children hospitalized with AGE. Patients with prolonged diarrhea and more frequent vomiting are also more likely to transmit the infection. In mutated non-secretor patients, NoV infection is less severe. The classic human secretor locus (Se) determines the secretion status of ABO antigens.[14] Furthermore, a study found that the susceptibility of HBGAs to NoV infection may be strain-specific, rather than genogroup-dependent.[8] Lindesmith et al. found that individuals who were homozygous for the FUT2 G428A allele did not express the H type-1 oligosaccharide ligand required for Norwalk virus binding.[16]

The FUT2 polymorphism appears to be a host genetic determinant of the composition of the intestinal microbiota.[17] Secretor status is associated with key drivers affecting individual variation in the human intestinal microbiota and its role in the etiology of chronic inflammatory disorders, such as primary sclerosing cholangitis, Crohn's disease and even oral cancer susceptibility.[18–22]

Our study is the first to report the FUT2 secretor status in hospitalized pediatric patients in Taiwan and its effects on the clinical manifestations of AGE. Beyond promoting good personal hygiene, such as hand washing, NoV prevention strategies depend largely on the development of effective vaccines. Given the rapid evolution of the virus, continued surveillance of emerging strain and host immunity to NoV disease is of paramount importance.

Contributors' statement

Dr. Hsin-Yeh Lin enrolled the cases, collected and analyzed the data, drafted the initial manuscript and approved the final manuscript as submitted. Dr. Hung-Hsiang Lai enrolled the cases, collected and analyzed the data, drafted the initial manuscript and approved the final manuscript as submitted. Dr. Ying Fang Elaine Chen enrolled the cases, collected and analyzed the data, drafted the initial manuscript and approved the final manuscript as submitted. Dr. Hsun-Ching Chao enrolled cases and approved the final manuscript as submitted. Dr. Chi-Neu Tsai carried out genetic experiments, drafted and approved the final manuscript as submitted. Dr. Shih-Yen Chen enrolled the cases, drafted the initial manuscript and approved the final manuscript as submitted. Dr. Yi-Jung Chang enrolled cases and approved the final manuscript as submitted.

Financial disclosure

The authors have indicated they have no financial relationships relevant to this article to disclose.

Declaration of Competing Interest

The authors have no conflicts of interest relevant to this article.

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Article

FUT2 Secretor Status Influences Susceptibility to VP4 Strain-Specific Rotavirus Infections in South African Children

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Abstract: Gastroenteritis is a preventable cause of morbidity and mortality worldwide. Rotavirus vaccination has significantly reduced the disease burden, but the sub-optimal vaccine efficacy observed in low-income regions needs improvement. Rotavirus VP4 'spike' proteins interact with FUT2-defined, human histo-blood group antigens on mucosal surfaces, potentially influencing strain circulation and the efficacy of P[8]-based rotavirus vaccines. Secretor status was investigated in 500 children <5 years-old hospitalised with diarrhoea, including 250 previously genotyped rotavirus-positive cases (P[8] = 124, P[4] = 86, and P[6] = 40), and 250 rotavirus-negative controls. Secretor status genotyping detected the globally prevalent G428A single nucleotide polymorphism (SNP) and was confirmed by Sanger sequencing in 10% of participants. The proportions of secretors in rotavirus-positive cases (74%) were significantly higher than in the rotavirus-negative controls (58%; $p < 0.001$). The rotavirus genotypes P[8] and P[4] were observed at significantly higher proportions in secretors (78%) than in non-secretors (22%), contrasting with P[6] genotypes with similar proportions amongst secretors (53%) and non-secretors (47%; $p = 0.001$). This suggests that rotavirus interacts with secretors and non-secretors in a VP4 strain-specific manner; thus, secretor status may partially influence rotavirus VP4 wild-type circulation and P[8] rotavirus vaccine efficacy. The study detected a mutation (rs1800025) ~50 bp downstream of the G428A SNP that would overestimate non-secretors in African populations when using the TaqMan®SNP Genotyping Assay.

Keywords: rotavirus; secretor status; histo-blood group antigens; VP4 genotypes; *FUT2*; susceptibility; vaccines

1. Introduction

Gastroenteritis is a preventable cause of morbidity and mortality worldwide, and the burden predominantly exists in high-risk populations such as children under the age of five years in low-income regions [1]. Rotavirus is the most frequent aetiology of diarrhoeal illness and death in children <5 years-old, and it was responsible for 29% of global diarrhoeal deaths occurring in this age group in 2016 [2].

The introduction of oral rotavirus vaccines in >100 countries worldwide has significantly reduced the burden of rotavirus diarrhoea and resulted in a 38% overall reduction in childhood diarrhoeal hospitalisations globally [3,4]. However, rotavirus vaccine efficacy appears to vary significantly between high-income (85–98%) and low-income (50–64%) countries [5]. Eliciting an adequate immune response to oral vaccines is multifactorial but may be limited in low-income settings due to impoverished living conditions and increased exposure to pathogens [3]. In addition, the passive transfer of rotavirus maternal

antibodies during breastfeeding can influence the immune response elicited by oral rotavirus vaccines in young children [4]. Understanding the factors that have contributed to an observed lower rotavirus vaccine efficacy in these settings may alleviate the burden of rotavirus-associated mortality in children.

Host genetic factors have recently been proposed to influence susceptibility to enteric pathogens. The excretion of soluble human histo-blood group antigen (HBGA) structures in gut mucosal surfaces determines a host's 'secretor status,' controlled by the human *FUT2* gene. Non-secretor phenotypes with an inability to express soluble HBGAs due to mutations in the *FUT2* gene (such as the prevalent G428A SNP; rs601338) are present globally in varying proportions. Higher proportions of non-secretor phenotypes are observed in African populations (~30%) than in Asian populations (~5%) [6,7].

Antigenic HBGA structures present in the body can act as receptors for various pathogens to bind during infection [8,9]. *FUT2* secretor status can modulate infection because it defines the presence (secretor) or absence (non-secretor) of HBGA attachment factors excreted in the gut. Susceptibility to enteric norovirus infection has been associated with secretor status, where non-secretor phenotypes have been found to display a natural resistance to GII.4 norovirus strains [10–12]. It has been proposed that variations in secretor status phenotypes and subsequent differences in host-defined susceptibility may contribute to the circulation of rotavirus strains in a similar mechanism [13].

Interactions between rotavirus particles and HBGA receptors present in the gut can occur via the VP4 (VP8* subunit) 'spike' protein on the surface of the virion [14]. Evidence of rotavirus VP4 strain-specific binding patterns between HBGAs and prevalent strains (P[8], P[4], and P[6]) has recently been noted [14]. Rotavirus P-types have distinct VP4 morphology that determines the presence or absence of HBGA-binding interfaces, allowing for different mechanisms of binding and entry of rotavirus particles to occur [13]. Studies have shown that rotavirus genotypes P[8]- and P[4]-bound complex and soluble HBGAs abundant in secretors, as well as an increased susceptibility to infection with these rotavirus strains in secretors. Non-secretors with an absence of HBGAs in the gut have been found to display a natural resistance to P[8] and P[4] strains with VP4 HBGA-binding interfaces [15–17]. Variations in host-defined secretor status can therefore influence susceptibility to infection with different rotavirus strains.

Rotavirus P[8] genotypes are responsible for more than 80% of human wild-type infections globally [15]. However, rotavirus circulation in Africa differs in strain diversity and prevalence, with more frequent cases of P[6] strains, which have reached 26% of all rotavirus strains circulating in African populations [18]. The proportions of naturally resistant non-secretors may alter the circulation of rotavirus P-types compared to that in global populations.

The Rotarix® and RotaTeq® rotavirus vaccines both contain P[8]-based strains or reassortants, and they provide protection through the replication of live-attenuated vaccine strains in the gut to induce a local immune response [4]. Associations between host-defined secretor status and susceptibility to infection with specific rotavirus strains pose interesting questions surrounding the lowered efficacy of P[8]-based rotavirus vaccines observed in some regions [19,20]. Emerging research has alluded to this idea [21–23], including the influence of the related *FUT3* Lewis host genetic factor [24–27], but further investigations are required. These data have contributed to the evidence that host genetic factors such as secretor status can influence infections by pathogens including rotavirus, as well as that strain-specific interaction mechanisms may occur [14,15,28].

The aim of this study was to investigate *FUT2*-defined secretor status in South African children <5 years-old hospitalised with diarrhoea and to examine the association between a host's genetic secretor status and rotavirus-associated hospitalisations. Understanding the relationship between pathogens such as rotavirus and the genetics of a population may identify avenues for improvements in vaccine efficacy to reduce the burden of rotavirus gastroenteritis.

2. Results

Secretor genotypes were successfully determined for all 500 children selected for the study, and the total cohort comprised 65.8% (329) secretors with at least one functional *FUT2* allele and 34.2% (171) non-secretors with both *FUT2* alleles containing the G428A SNP.

Rotavirus-positive cases (RV+) comprised 74% (185/250) secretors (Se) and 26% (65/250) non-secretors, while rotavirus-negative controls (RV-) comprised 58% (144/250) secretors and 42% (106/250) non-secretors. The distributions of secretors versus non-secretors observed amongst cases and controls were significantly different ($p < 0.001$).

Information on rotavirus genotyping from the Rotavirus Sentinel Surveillance Program (RSSP) database [29,30] showed that the rotavirus-positive cases (n = 250) comprised 124 P[8] infections, 86 P[4] infections, and 40 P[6] infections (Supplementary Material). The proportions of secretors and non-secretors were compared amongst each VP4 strain within rotavirus-positive cases (Table 1). Rotavirus P[8] infections (79% secretors and 21% non-secretors) and P[4] infections (77% secretors and 23% non-secretors) had significantly different proportions of secretor phenotypes compared to P[6] infections (53% secretors and 47% non-secretors) ($p = 0.001$ and $p = 0.006$, respectively). When considered together, rotavirus P[8] and P[4] infections (78% secretors and 22% non-secretors) had significantly different proportions of secretor phenotypes compared to P[6] infections (53% secretors and 47% non-secretors) ($p = 0.001$).

Table 1. The distribution of secretors and non-secretors amongst VP4 genotypes P[8], P[4], and P[6] of rotavirus-positive cases (RV+; n = 250).

Rotavirus Genotypes:	P[8] Infections (n = 124)	P[4] Infections (n = 86)	P[6] Infections (n = 40)
Secretors	79% (98/124)	77% (66/86)	52.5% (21/40)
Non-secretors	21% (26/124)	23% (20/86)	47.5% (19/40)
p-values for each comparison	P[8] vs. P[4]: $p = 0.693$		
	P[8] vs. P[6]: $p = 0.001$		
	P[4] vs. P[6]: $p = 0.006$		
	P[8] + P[4] vs. P[6]: $p = 0.001$		

The Sanger sequencing of the exon 2 region of the *FUT2* gene conducted for 10% of the cohort confirmed the presence of either functional *FUT2* alleles or G428A SNP alleles for 91% (48/53) of analysed specimens. Sequences of the *FUT2* exon 2 region from 12 homozygous secretors (SeSe), 24 heterozygous secretors (Sese), and 17 homozygous non-secretors (sese) were obtained and compared to RT-PCR G428A genotyping results. Five discrepant results were observed in which heterozygous secretor (Sese) individuals (one functional *FUT2* allele and one allele containing the non-functional G428A SNP) genotyped by Sanger sequencing were incorrectly genotyped by RT-PCR as non-secretors (both alleles containing the G428A SNP). A commonality between these discrepant specimens was an SNP mutation (rs1800025) ~50 bp downstream of the G428A SNP (Figure 1).

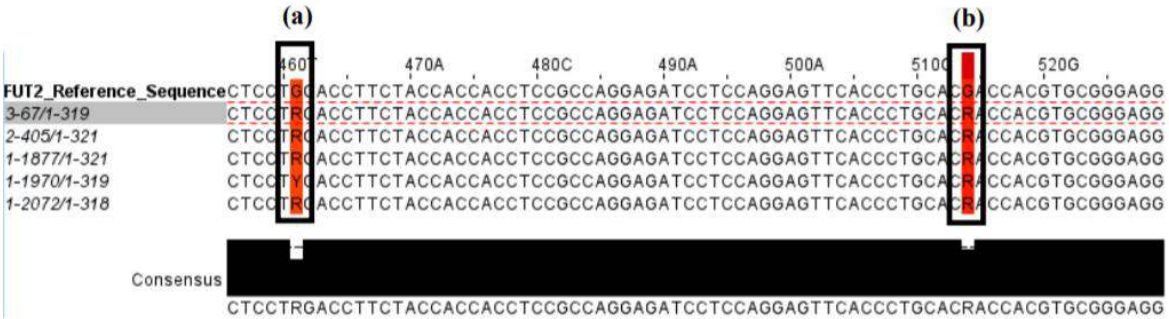

Figure 1. Sequence alignment of five participants where Sanger sequencing and RT-PCR genotyping results were discrepant. (**a**) The G428A SNP location displaying all discrepant sequences containing the two peaks 'G' and 'A,' as represented by an 'R' annotation. (**b**) The mutation site (rs1800025) located ~50 base pairs downstream of the G428A SNP, common in all discrepant results.

3. Discussion

The results from this study indicate that secretors were more susceptible to rotavirus infection, and non-secretors seemed to display a natural resistance. The absence of HBGAs in the gastric mucosa of non-secretors appeared to reduce susceptibility to rotavirus, possibly by limiting the attachment stage of binding and entry during rotavirus infection [31]. Despite this observation, non-secretors were present amongst rotavirus-positive cases, indicating that HBGA attachment may not be the only mechanism for rotavirus binding and subsequent entry. Early studies on rotavirus binding and entry described sialic acid as an attachment factor for some animal strains [32]. Alternative binding receptors such as sialic acid or yet unknown mechanisms could explain the presence of rotavirus infection in non-secretor individuals in our study.

Studies have shown that rotavirus VP4 (VP8*) binds to HBGAs in a strain-specific manner [13]. Xu and colleagues showed that P[8] and P[4] rotavirus strains similarly bound to complex HBGAs via a $\beta\beta$ binding domain, while more distantly related P[6] strains bound simple H-type 1 structures in a $\beta\alpha$ binding domain [14]. In our study, a higher proportion of secretors was observed in P[8] (78%) and P[4] (76%) rotavirus infections compared to P[6] infections (53%). This suggested that secretors were significantly more susceptible to P[8] and P[4] strains than to P[6] strains ($p < 0.01$), while non-secretors were more likely to be infected with P[6] strains. These strain-specific interactions may also influence the circulation of rotavirus strains within the South African population, as observed in other settings [15–17].

A correlation in the prevalence of rotavirus VP4 strains and HBGA genotypes suggested that the circulation of rotavirus may be partially modulated by their ability to bind to host-defined HBGA receptors. Globally, G1P[8] is the predominantly circulating rotavirus genotype, with ~74% of global strains containing the P[8] VP4 strain [18]. However, studies have shown that rotavirus strains in Africa are more diverse, with P[8] comprising 32% of rotavirus cases, P[4] comprising 13% of rotavirus cases, and P[6] comprising 26% of rotavirus cases [18]. In South Africa, P[6] strains were detected in 25% of rotavirus cases between 2003 and 2006, and they continue to circulate [30,33]. In this study, the higher proportion of non-secretors (34%), naturally resistant to P[8] and P[4] rotavirus infections, may explain the 16% detection of P[6] strains [17,34]. The *FUT2* genetics of a population may define the availability of host HBGA receptors for rotavirus infection, which could drive the epidemiology of rotavirus strain circulation in a region.

Discrepant results in Sanger sequencing revealed that five individuals were misclassified by RT-PCR as non-secretors (error rate 22.7%; 5/22), with sequencing identifying these five individuals as heterozygous secretors (Sese). The specimen sub-set comprised 58.5% secretors and 41.5% non-secretors based on RT-PCR genotyping, while the same specimens comprised 67.9% secretors and 32.1% non-secretors based on Sanger sequencing—an overall over-estimation of non-secretors of approximately 10%. This over-estimation of non-secretor genotypes is important to note for future studies, especially when using the TaqMan® SNP Genotyping Assay targeting the G428A SNP in an African population where non-secretors are frequent. The proportion of non-secretors (34%) observed in our cohort of 500 individuals correlated with other studies in African populations where higher frequencies of non-secretors were observed [35,36].

Misclassification by the commercial genotyping assay was hypothesised to be due to a mutation noted ~50 bp downstream of the G428A SNP position. The manufacturer confirmed that the mutation affected the primer binding of the reverse primer to the functional copy of the *FUT2* gene in the five heterozygous secretors, resulting in the absence of PCR product for the FAM-labelled probe (which detects the presence of the allele without the G428A SNP) to bind. Interestingly, the mutation was found in 9% of African populations compared to 2% in all populations in the 1000 genomes project [37]. Sanger sequencing remains an important tool to investigate host genetic factors such as secretor status, and further sequencing will be considered to examine the extent of the *FUT2* G514R mutation detected in this study.

Studies have indicated that secretor status can influence antibody titres to rotavirus [36], the incidence of gastrointestinal disease [38], and immune responses to rotavirus vaccines [28]. Rotarix® and RotaTeq® vaccines both contain P[8] vaccine constructs and require multiplication in intestinal cells to elicit local gut immunity [39,40]. The absence of HBGA attachment factors in non-secretors may reduce the replicative capacity of P[8] vaccine strains. The observation that non-secretors in Africa exhibit a natural resistance to wild-type P[8] strains may provide insights into the differences in vaccine efficacy across populations [5]. A study by Kazi and colleagues identified a link between the immune response to rotavirus P[8] vaccines and secretor status [28], and these associations have since been observed elsewhere [19,22,41]. Since patient sera were not collected as part of the RSSP, we could not investigate the direct effect of secretor status on rotavirus vaccine immune responses. Future studies investigating links between secretor status and variables such as vaccine immune responses, breastfeeding in young children, population genetics, and gut microbiome compositions, as well as alternative binding receptors for rotavirus entry, should be considered.

The limitations of this study include the small sample size of P[6] rotavirus cases available for further analysis (16%; 40/250). A larger sample size of rotavirus genotypes would be beneficial in confirming the relationship between specific rotavirus VP4 strains and secretor status. Another limitation of this study was the discordant results between RT-PCR genotyping and Sanger sequencing, resulting in the misclassification of heterozygous secretors by RT-PCR. Only 13% (22/171) of non-secretor genes were sequenced due to budget constraints, and additional funding will be sought to expand the sequencing of the *FUT2* gene of non-secretors in South Africa. A final limitation of this study was not including analysis of the related *FUT3* Lewis genes as it may also impact susceptibility to rotavirus infections. Future studies should consider the genetics of a cohort before utilising genotyping techniques, since alternative SNPs may be present which may skew results.

4. Materials and Methods

The South African RSSP enrolled children under the age of five years hospitalised for diarrhoea at various sites across South Africa (Protocol M091018, approved by the Human Research Ethics Committee (Medical) of the University of Witwatersrand). Diarrhoea was defined as three or more loose stools in past 24 h, with or without vomiting.

Informed consent was obtained from each child's parent or guardian prior to participation in the RSSP. Stool and dried blood spot (DBS) specimens were collected from enrolled participants, and each child's stool was screened as part of the RSSP for rotavirus group A (ProspecT™ Rotavirus Microplate Assay, Oxoid, Basingstoke, UK). Rotavirus-positive cases were genotyped using conventional RT-PCR methods and primers for G-specific and P-specific genotypes to determine the GxP[x] rotavirus strain [42].

This sub-study was conducted in accordance with the Declaration of Helsinki, and the project entitled "Investigation of secretor status, rotavirus VP4 genotypes, and gastrointestinal microbiomes in cases of diarrhoea in South Africa" (Protocol number 222/2018) was approved by the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria, in May 2018.

For this study, children enrolled in the RSSP between 2009 and 2017 with available DBS specimens were identified, and rotavirus-negative cases (n = 250) were randomly selected. Rotavirus GxP[x] genotypes were previously determined as part of the RSSP [30], and the rotavirus-positive subset (n = 250) was selected to represent the major rotavirus VP4 genotypes (P[8], P[4], and P[6]), with cases and controls selected randomly where possible.

Secretor status was investigated using DBS specimens. DNA from DBS specimens was extracted using a QIAamp DNA Mini kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions with one modification prior to extraction. The manufacturer's protocol was modified to improve lysis by incubating DBS cards (~1 cm diameter) in a 200 µL buffer ATL overnight at 37 °C, instead of at 85 °C for 10 min. Following extraction, DNA was stored at −40 °C at the Centre for Enteric Diseases (Virology), National Institute for Communicable Diseases.

Secretor status was determined by detecting the presence or absence of the *FUT2* G428A SNP using a Predesigned TaqMan® SNP Genotyping assay (Life Technologies Corporation, CA, USA, supplied by Thermo Fisher Scientific, Carlsbad, CA, USA) in a 10 µL reaction volume according to the manufacturer's instructions [27,43].

The Sanger sequencing of 10% of the cohort *FUT2* genes was performed to ensure that alternative non-secretor-causing SNPs, which may be undetected by this assay, were absent. The specimens were selected to include all secretor genotypes, with a slight selection bias towards heterozygous secretors (n = 19) and non-secretors (n = 22) compared to homozygous secretors (n = 12), as well as a range of cycle threshold values (Ct range of 10–39) obtained during RT-PCR. The coding exon 2 region of the *FUT2* gene was amplified using the FUT2Ex2F and FUT2Ex2R primers [7], cleaned using an ExoSAP-IT™ PCR Product Cleanup protocol (Thermo Fisher), and sequenced using a BigDye™ Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Life Technologies, Waltham, MA, USA) on an Applied Biosystems 3500xL Genetic Analyzer instrument (Applied Biosystems). Sequences were aligned to a *FUT2* protein-coding reference sequence (NG_007511.1:11987-13018 *Homo sapiens* fucosyltransferase 2 (*FUT2*), RefSeqGene on chromosome 19) (NCBI) using Molecular Evolutionary Genetics Analysis software version 7.0.26 (MEGA7).

The sequences of the FUT2 exon 2 region of 10% of the cohort were submitted to BankIt (National Center for Biotechnology Information, Bethesda, MD, USA), and the accession numbers are as follows: MW036696, MW036697, MW036698, MW036699, MW036700, MW036701, MW036702, MW036703, MW036704, MW036705, MW036706, MW036707, MW036708, MW036709, MW036710, MW036711, MW036712, MW036713, MW036714, MW036715, MW036716, MW036717, MW036718, MW036719, MW036720, MW036721, MW036722, MW036723, MW036724, MW036725, MW036726, MW036727, MW036728, MW036729, MW036730, MW036731, MW036732, MW036733, MW036734, MW036735, MW036736, MW036737, MW036738, MW036739, MW036740, MW036741, MW036742, MW036743, MW036744, MW036745, MW036746, MW036747, MW036748.

Statistical analyses using Chi-squared tests and univariate logistic regression models were performed using STATA version 14.0, where $p < 0.05$ was considered significant (StataCorp College Station, TX, USA).

5. Conclusions

Rotavirus susceptibility appeared to be influenced by secretor status in this study of South African children hospitalised with acute diarrhoea. Secretors expressing HBGAs in gut mucosal surfaces were more likely to be infected with rotavirus, specifically the P[8] and P[4] strains, compared to non-secretors. Non-secretors, with an absence of HBGAs in the gut, appeared to be less susceptible to rotavirus P[8] and P[4] infections compared to secretors—thus, the P[6] genotype was more frequent in these individuals. Interactions between rotavirus and secretor status could provide insights into the circulation of rotavirus strains amongst genetically diverse populations. Insights into the potential causes of altered rotavirus susceptibility and subsequent vaccine efficacy will aid in minimising the burden of disease. Diarrhoeal deaths are preventable, and secretor status may be an important host genetic factor to help understand and improve rotavirus disease prevention. Finally, the choice of assay for detecting or classifying secretor status in different populations should be carefully considered because the tools currently available all have pros and cons associated with their use.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-0817/9/10/795/s1, File: Final DBS Cohort Results_7.9.2020.

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The Journal of Infectious Diseases

BRIEF REPORT

Rotavirus Vaccine Take in Infants Is Associated With Secretor Status

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Rotaviruses bind to enterocytes in a genotype-specific manner via histo-blood group antigens (HBGAs), which are also detectable in saliva. We evaluated antirotavirus immunoglobulin A seroconversion ("vaccine take") among 166 Ghanaian infants after 2–3 doses of G1P[8] rotavirus vaccine during a vaccine trial, by HBGA status from saliva collected at age 4.1 years. Only secretor status was associated with seroconversion: 41% seroconversion for secretors vs 13% for nonsecretors; relative risk, 3.2 (95% confidence interval, 1.2–8.1; $P = .016$). Neither Lewis antigen nor salivary antigen blood type was associated with seroconversion. Likelihood of "take" for any particular rotavirus vaccine may differ across populations based on HBGAs.

Keywords. secretor; rotavirus; vaccine; *FUT2*; Lewis antigen.

Recent laboratory advancements have transformed the understanding of host–pathogen interaction for rotaviruses, elucidating the required expression of specific histo-blood group antigens (HBGAs) on the gastrointestinal mucosa for rotavirus binding [1–4]. Rotaviruses are designated by genes encoding the VP7 (termed G genotype) and VP4 (P genotype) proteins. VP4 is cleaved to VP8*, which appears to bind to particular HBGAs in a genotype–specific manner. Translational epidemiological studies support the concept that there are differential disease risks from specific P genotypes, by host HBGAs [5–9]. Both rotavirus vaccines in wide use have the human rotavirus P[8] genotype, and understanding whether HBGAs restrict vaccine take and contribute to the differences observed in rotavirus vaccine performance measured across different settings is an important goal. We previously evaluated the immunogenicity of Rotarix vaccine (GSK Biologics, Rixensart, Belgium; RV1) in Ghanaian infants [10]. Here we investigated whether HBGAs of trial participants, measured in saliva, predicted take of RV1.

METHODS

In the original trial (NCT015751) conducted in 2012–2013, healthy infants were consented and randomized into 3 arms to receive RV1: arm 1 (RV1 at ages 6 and 10 weeks); arm 2 (RV1 at 10 and 14 weeks); arm 3 (RV1 at 6, 10 and 14 weeks). Serum samples were obtained just before RV1 dose 1 and 1–2 months after the last dose [10]. Infants who were negative for serum antirotavirus immunoglobulin A (IgA) antibody just before dose 1 were included in per-protocol results. The follow-on study with saliva collection was approved by institutional review boards of participating institutions. Parents of trial participants were approached August 2016–February 2017 for their child's participation (median age, 4.1 years [interquartile range, 4.0–4.4]); saliva was collected from the child if consent was given (Supplementary Materials). Saliva study enrollment was prioritized by arm (arm 3 > arm 2 > arm 1) because the higher seroconversion rates in arms 3 and 2 provided greater power to detect an association with HBGA status if one existed [10].

The child's HBGA phenotype was determined by testing saliva for H antigen, Lewis a and b antigens, and A and B blood group antigens using enzyme immunoassays and lectin and anti-HBGA antibodies (Supplementary Materials) [11]. Initial phenotype categories were secretor positive, low, or negative; Lewis positive, low, or negative; and blood group O, A, B, or AB. Phenotype results were used to select a subset of samples for DNA extraction and genotyping. Twelve initial samples had fucosyltransferase 2 (*FUT2*, the so-called secretor gene) genotyping based on G428A nonsense single-nucleotide polymorphism, and then 76 additional samples were selected for *FUT2* genotyping: all secretor negatives (n = 26), all low secretors (n = 18), samples with inconsistent HBGA results (n = 3), and secretors from arm 2 and 3 that were Lewis low (n = 10) or were Lewis negative and blood type O (n = 19). The 21 samples selected for *FUT3* (the so-called Lewis gene) genotyping were those from arm 2 and arm 3 that were negative for all HBGAs assessed (n = 4), Lewis low (n = 11), a random selection of those Lewis negative and blood type O (n = 2), and a random selection of those Lewis positive and blood type O (n = 4) (Supplementary Materials).

Except for testing of the initial 12 samples, the laboratory that performed genotyping was different from the laboratory that performed phenotyping, and laboratory staff that performed

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genotyping were blinded to phenotype results and the reasons for requesting genotyping. Laboratory staff testing saliva were blinded to serum results.

During the original trial, antirotavirus IgA antibody concentrations in serum had been determined using enzyme immunoassay [10]. Preexisting antirotavirus immunoglobulin G (IgG) antibody concentration, presumed to be maternally derived, had been measured in serum collected just before RV1 dose 1 [10]. Seroconversion was defined as antirotavirus IgA antibodies ≥20 U/mL after RV1 doses (at 18 weeks in arm 3 and arm 2 infants; at 14 weeks in arm 1 infants) among infants seronegative (IgA <20 U/mL) just before dose 1.

Relative risks (RRs) of seroconversion, stratified by secretor (positive vs negative), Lewis (positive vs negative), and blood group by salivary antigen, were estimated using binomial log-linear regression. For the main analysis, the child's status was categorized based on all available information (HBGA phenotype, plus *FUT2* and/or *FUT3* genotype); genotype was used over phenotype if results were discrepant. Subjects whose Lewis antigen status could only be based on phenotype and were in the Lewis low category were classified as Lewis negative. Secondary analyses were performed using only phenotype results. Variables planned a priori to assess for possible confounding were randomization arm (arm 3 or 2 vs arm 1), antirotavirus IgG antibody concentration before dose 1 (by quartile), nutritional status at age 14 weeks by *z* scores, and exposure to season of higher wild-type rotavirus circulation (mid-December 2012–February 2013, as determined by local diarrhea surveillance and original trial data) after the serum collection pre-RV1 dose 1 and before serum collection post-RV1. Confounding was assessed by backward elimination; factors whose elimination changed the risk ratio of the variable of interest by ≥10% were retained.

Genotyping data from surveillance demonstrated that genotypes G1P[8] and G2P[4] predominated during late 2012–early 2013; P[6] strains were not detected (unpublished data).

RESULTS

Saliva was collected from 166 children: 57% (82/143) of arm 3, 28% (39/139) of arm 2, and 32% (45/142) of arm 1 participants. The phenotype/genotype concordance was 87% (73/84) for secretor status and 62% (8/13) for Lewis status (Supplementary Materials). Overall, 81% (135/166) of infants were secretors and 19% (31/166) were nonsecretors; 57% (95/166) were Lewis positive, 43% (71/166) were Lewis negative. Among secretors, by salivary antigen assay, 52% (70/135) were blood type O, 30% (40/135) type B, 16% (22/135) type A, and 2% (3/135) type AB.

Overall, 41% of secretors seroconverted compared with 13% of nonsecretors (Table 1). In the model that included only secretor status, secretors were 3.2 (95% confidence interval [CI], 1.2–8.1; $P = .016$) times more likely to seroconvert than nonsecretors (Table 2). Neither arm, Lewis status, nor any other factor evaluated were confounders (RR for Lewis positive compared with Lewis negative, 0.9 [95% CI, .6–1.4]; $P = .65$); Lewis status was also not an effect modifier (interaction term $P = .37$). Results were similar when secretor and Lewis status were based only on phenotype for all infants (RR secretor vs nonsecretor, 3.8 [95% CI, 1.3–11.2]; RR Lewis positive vs negative, 1.0 [95% CI, .7–1.5]; interaction term $P = .11$) and when all information was used but the 16 phenotypically "Lewis low" subjects were excluded rather than categorized as Lewis negative, or categorized as Lewis positive (Supplementary Materials). Exposure to period of higher wild-type rotavirus circulation was not found to be a confounder or effect modifier. Using only the 84 children that had *FUT2* genotype results available (note: most selected for genotyping had low *Ulex europaeus* agglutinin I (UEA-1) assay optical densities), the RRs for seroconversion for secretors vs nonsecretors were similar when those subjects were classified as secretor or nonsecretor based only on genotype result, or when based only on phenotype results (Supplementary Materials).

Including only secretors in the model, there was no statistically significant difference in seroconversion by salivary blood group antigen. However, the lower likelihood for seroconversion among type O vs type B just missed statistical significance (RR, 0.7 [95% CI, .4–1.0]) (Table 2). In these models, the RR for Lewis positive vs negative ranged from 1.0 to 1.2 and was not statistically significant. The RR for seroconversion was not statistically different among phenotypic "high" vs "low" secretors (RR, 1.3 [95% CI, .7–2.3]; $P = .38$); few children were "low" secretors.

Table 1. Proportion of Infants That Seroconverted by Secretor Status, Lewis Status, Salivary Antigen Blood Group Type, and Study Arm

Category	Seroconverted/Total, No. (%)
Arms combined (n = 166)	
Nonsecretor (n = 31; 19% of total)	4/31 (13)
Lewis positive	2/22 (9)
Lewis negative	2/9 (22)
Secretor (n = 135; 81% of total)	55/135 (41)
Lewis positive	29/73 (40)
Lewis negative	26/62 (42)
Type O	25/70 (36)
Type B	20/40 (50)
Type A	9/22 (41)
Type AB	1/3 (33)
Arms 2 + 3 (n = 121)	
Nonsecretor (n = 23; 19%)	4/23 (17)
Secretor (n = 98; 81%)	46/98 (47)
Arm 1 (n = 45)	
Nonsecretor (n = 8; 18%)	0/8 (0)
Secretor (n = 37; 82%)	9/37 (24)

Table 2. Relative Risks of Seroconversion by Secretor/Histo-Blood Group Antigen Status, From Regression Models

	Using Phenotype Plus Available Genotype Results (n = 166)		Using Phenotype Results Only (n = 166)		Using Secretors Only[a] (n = 135)	
Secretor/HBGA Status	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value
Secretor positive vs negative	3.2 (1.2–8.1)[b]	.016	3.8 (1.3–11.2)	.016	…	
Lewis positive vs negative	0.9 (.6–1.4)	.65	1.0 (.7–1.5)	.95	0.9 (.6–1.4)	.64
Salivary ABO blood group						
O vs non-O	…		…		0.8 (.5–1.2)	.22
O vs A	…		…		0.8 (.4–1.4)	.40
O vs B	…		…		0.7 (.4–1.0)	.055
O vs AB	…		…		0.8 (.2–4.4)	.84

Abbreviations: CI, confidence interval; HBGA, histo-blood group antigen; RR, relative risk.

[a]Using phenotype plus available genotype results.

[b]RR, 3.1 (95% CI, 1.2–7.9), *P* = .018, in model adjusted for Lewis status, study arm, exposure to period of higher wild-type rotavirus circulation, antirotavirus immunoglobulin G pre-RV1 dose 1, and height for age at 14 weeks.

Among subjects who seroconverted, serum antirotavirus IgA concentrations were not statistically different between different subject groups (ie, secretor positive vs negative; Lewis positive vs negative overall; Lewis positive vs negative among secretors only).

DISCUSSION

We investigated the correlation between HBGAs and rotavirus infection (defined by seroconversion) shortly after a standardized exposure to G1P[8] vaccine strain among rotavirus-naive infants, while accounting for other factors. Our data support the hypothesis that susceptibility to G1P[8] infection, and therefore take of RV1, is specifically associated with secretor status. Differences in HBGA distributions across populations may contribute to the differences in results on vaccine take and efficacy across different regions.

Our findings provide additional in vivo evidence supporting the hypothesis that susceptibility to P[8] infection is associated with secretor status. In a similar study among Pakistani infants, with the same laboratories performing the serologic and salivary phenotyping assays as in this study, infants who were nonsecretors also were not absolutely restricted from seroresponse but had the lowest rate of seroconversion (19%) following RV1 exposure [11]. Also similar to our Ghana findings, in the Pakistan evaluation, Lewis status was not independently associated with seroconversion at a statistically significant level, although the numbers of Lewis-negative children in that study were low. In a study of Nicaraguan infants aged approximately 8 weeks, most of whom had detectable antirotavirus IgA at time of vaccination, increased antirotavirus titer or seroconversion following RV1 dose 1 was detected in a statistically higher proportion of secretors (24%) vs nonsecretors (8%), in univariate assessment [12]. Other studies, with some population diversity, compared secretor status of children with rotavirus *disease* from P[8] strains with the general population and found that those with P[8] disease were significantly more likely to be secretors [5–8]. Based on analysis of saliva samples collected from 275 Bangladeshi children aged 1–2 years who had been under active surveillance as the unvaccinated cohort in a vaccine trial, researchers found an overall increased risk of rotavirus disease to age 1 year among phenotypic secretors vs nonsecretors [13]. This difference, however, was due to differences in risk of P[4] disease and there was no difference in risk of P[8] disease [13]. Among the unvaccinated in that trial, Lewis-negative infants tended to be at lower risk of P[8] disease, and were at significantly increased risk of P[6] disease (these associations have also been described for children from Burkina Faso [5]). Within the RV1-vaccinated arm in Bangladesh, those similar associations were found between the infants' HBGA status and postvaccination risk of rotavirus disease by specific P-genotypes. As the authors comment, their finding of similar risk of P[8] disease in nonsecretors vs secretors may suggest that unique strains of P[8] may differ in their ability to infect nonsecretors (eg, the G9P[8] strain in their study) [13]. In addition to human rotavirus infectivity and glycan binding investigations using MA104 cells [14], the auspicious work with human intestinal enteroids may further reveal if there are differences in host restriction, via HBGAs and beyond, between wild-type and attenuated human rotavirus vaccine strains of the same genotype, as well as mechanisms of vaccine strain attenuation [15].

In Ghanaian infants, we did not find an association with salivary ABO status; as with other evaluations that have reported results on this aspect, our study was not specifically powered to examine this possibility. Our results, however, are different from the Pakistan findings, where secretors of blood group type O (by salivary antigen) had statistically higher likelihood of seroconversion compared with secretors of non-blood group O (RR, 1.7 [95% CI, 1.1–2.7]). In Nicaraguan infants, among secretors, those of blood type B (by hemagglutination) were the

group with the lowest frequency of increased antirotavirus IgA titer after RV1 dose 1 [12]. In vitro data have suggested that the type B epitope may interfere with the binding of P[8] strains [1].

Our study has limitations. HBGA phenotype in saliva, as an indicator of that expressed at the gastrointestinal mucosal surface, was determined from saliva collected at age 4 years and not at time of vaccine receipt in early infancy, which could be relevant if there are phenotype changes during this time. We did not perform genotyping for all subjects to allow full comparison of results using phenotype only vs genotype only. However, we had high concordance between secretor phenotype and genotype and our findings were consistent when we incorporated available genotype results or when phenotype only was used. Inherent in nearly all rotavirus vaccine trials, seroconversion in some of our infants may reflect wild-type infection rather than vaccine response.

Our data support the theory that secretor status plays a role in host susceptibility to rotavirus infection, specifically from P[8] genotypes. Continued laboratory advancements and vaccine studies that incorporate HBGA assessments will be important to understand the extent to which such host factors impact our measurements of rotavirus vaccine performance as well as risk for possible adverse events (ie, intussusception) in different populations.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The findings and conclusions of in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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RESEARCH ARTICLE

REVISED *FUT2* secretor genotype and susceptibility to infections and chronic conditions in the ALSPAC cohort [version 2; referees: 2 approved]

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Abstract

Background: The *FUT2* (fucosyltransferase-2) gene determines blood group secretor status. Being homozygous for the inactive "non-secretor" rs601338(A) allele confers resistance to certain infections (e.g. *Norovirus*, *Rotavirus*) and susceptibility to others (e.g. *Haemophilus influenza*, *Streptococcus pneumonia*). Non-secretors also have an increased risk of type 1 diabetes and inflammatory bowel disease. We examined *FUT2* genotype, infections and chronic conditions in a population-based cohort.

Methods: We studied 7,582 pregnant women from the ALSPAC pregnancy cohort. Infections (measles, mumps, chicken pox, whooping cough, meningitis, herpes, gonorrhea and urinary infections) and chronic conditions (kidney disease, hypertension, diabetes, rheumatism, arthritis, psoriasis, hay fever, asthma, eczema and allergies) were self-reported. *FUT2* secretor status was determined from the rs601338 genotype. ABO blood type was obtained from clinical records.

Results: Overall, 1920 women (25.3%) were homozygous for the non-secretor allele (AA). Secretor status was associated with mumps, with 68% of non-secretors experiencing this infection, compared to 48% of secretors (RR, 1.40; 95% CI, 1.34–1.46). A weaker association was observed for measles infection (76% vs. 72%; RR, 1.05; 95% CI, 1.02–1.09). Non-secretors also experienced an increased risk of kidney disease (5.4% vs. 3.9%; RR, 1.39; 95% CI, 1.11–1.75). Independent of secretor status, AB blood type was a risk factor for mumps (RR 1.15; 95%CI, 1.03, 1.28 compared to type O). We found no evidence of interaction between secretor status and blood type. For some conditions, including asthma and arthritis, *FUT2* heterozygosity (GA) appeared to confer an intermediate phenotype. There was no strong evidence of association between secretor status and other infections or chronic conditions, although statistical power was limited for rare outcomes.

Conclusion: Our results identify an association between *FUT2* secretor status and self-reported kidney disease, and confirm a recently reported association with susceptibility to mumps infection. The clinical implications of these associations warrant further investigation.

Open Peer Review

Referee Status: ✓ ✓

	Invited Referees 1	2
REVISED version 2 published 25 Sep 2018	✓ report	✓ report
version 1 published 30 May 2018	? report	? report

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Comments (0)

Keywords
FUT2, infection, chronic disease, secretor status, ALSPAC, mumps, kidney disease

This article is included in the Avon Longitudinal Study of Parents and Children (ALSPAC) gateway.

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REVISED **Amendments from Version 1**

We have revised our manuscript taking into account the reveiwers' suggestions. The main updates include: 1) incorporation of ABO blood group data; 2) expanded discussion of limitations related to self-reported data; 3) discussion of possible mechanisms for the observed association between FUT2 secretor status and kindey disease; 4) re-analysis accounting for two alternative null FUT2 alleles. These changes are detailed in our posted responses to the reviewers' comments.

See referee reports

Introduction

The *FUT2* (fucosyltransferase 2) gene encodes the alpha (1,2) fucosyltransferase, which determines blood group secretor status. About 20% of Caucasians are homozygous for the nonsense mutation W143X (rs601338G>A), encoding a stop codon that inactivates the *FUT2* enzyme[1]. Individuals who are homozygous for this "non-secretor" allele (AA) are unable to secrete histo-blood group antigens into bodily fluids, or express them on mucosal surfaces.

Non-secretors have a lower risk of diarrheal illness[2] and ear infections in childhood[3]. The non-secretor phenotype also confers resistance to specific pathogens that require *FUT2*-dependent antigens to infect host cells, including *Norovirus*[4–7], *Rotavirus*[8–11] and *Helicobacter pylori*[12,13]. By contrast, the non-secretor phenotype has been associated with increased susceptibility to other pathogens, including *Candida*[14–16], *Haemophilius influenza*[17], *Neisseria meningitis*[18] and *Streptococcus pneumonia*[18]. Most recently, in a genome-wide association study (GWAS) of common infections, Tian *et al*. reported an increased susceptibility to mumps in non-secretors[3]. In addition, non-secretors appear to be at increased risk for certain autoimmune diseases, including type 1 diabetes[19], psoriasis[20,21] and inflammatory bowel disease[22,23].

The above associations have not been simultaneously examined in a single population and several have not been independently replicated. Moreover, the association of *FUT2* secretor status with other infectious and chronic diseases has not been widely studied. Finally, previous studies have typically only considered the secretor phenotype as dichotomous, assuming the non-secretor allele to be recessive. In this study, we characterized the association of *FUT2* secretor status with a variety of infectious and chronic diseases in the population-based Avon Longitudinal Study of Parents and Children (ALSPAC), and examined the impact of heterozygosity for the non-secretor allele.

Methods

Study design and population

This study accessed data from the ALSPAC cohort. ALSPAC recruited 14,541 pregnant women (98% Caucasian) resident in the former county of Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992[24,25]. The current analysis included a subset of 7,582 Caucasian women who selected and provided written informed consent for genotyping analysis, and reported their personal medical history during pregnancy. ABO blood group was collected from clinical records for the majority of participants (N=6,757). The ALSPAC website contains details of all the data that is available through a fully searchable data dictionary at http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Genotyping

ALSPAC mothers were genotyped using the Illumina human660W-quad array at Centre National de Génotypage (CNG) (Evry, France) and genotypes were called with Illumina GenomeStudio. PLINK (v1.07) was used to carry out quality control (QC) measures on an initial set of 10,015 participants and 557,124 directly genotyped single nucleotide polymorphisms (SNPs). SNPs were removed if they displayed more than 5% missingness or a Hardy-Weinberg equilibrium P value of less than 1.0×10^{-6}. Additionally, SNPs with a minor allele frequency of less than 1% were removed. Samples were excluded if they displayed more than 5% missingness, had indeterminate X chromosome heterozygosity or extreme autosomal heterozygosity. Samples showing evidence of population stratification were identified by multidimensional scaling of genome-wide identity by state pairwise distances using the four HapMap populations as a reference, and then excluded[26]. Cryptic relatedness was assessed using an identity by descent (IBD) estimate of more than 0.125, which is expected to correspond to roughly 12.5% alleles shared IBD or relatedness at the first cousin level. Related participants that passed all other QC thresholds were retained during subsequent phasing and imputation. In total, 9,048 mothers and 526,688 SNPs passed these QC filters.

Imputation

A total of 477,482 SNP genotypes in common between the sample of mothers described above and a second sample of 9,115 children were combined. SNPs with genotype missingness above 1% due to poor quality (N=11,396 SNPs) were removed and a further 321 participants were removed due to potential ID mismatches. This resulted in a dataset of 17,842 participants, containing 6,305 duos and 465,740 SNPs (112 were removed during liftover and 234 were out of HWE after combination). Haplotypes were estimated using ShapeIT (v2.r644), which utilizes relatedness during phasing. The phased haplotypes were then imputed to the Haplotype Reference Consortium (HRC) panel of approximately 31,000 phased whole genomes using Impute V3. For this study, we excluded the mothers who had removed consent, leaving 8,698 eligible mothers. We further excluded those who did not provide personal medical history, leaving 7,582 for analysis.

Exposure: FUT2 genotype

FUT2 secretor status was defined based on the rs601338 SNP[1], where G is the wild-type "secretor" allele and A is the nonsense W143X "non-secretor" allele. Following previous studies[7,11,19,23], we considered the A allele to be recessive and dichotomized secretor status, combining the GA and GG genotypes as secretors and comparing them to the homozygous AA non-secretors. In addition, we explored the impact of GA heterozygosity at this locus. Two other commonly reported non-secretor alleles

were considered. The missense variant at rs1047781, described in Asian populations[27] was not detected in our Caucasian population. The non-synonymous S258G variant at rs602662[28] was highly correlated with rs601338; incorporating this SNP to define secretor status had no impact on 98% of participants' phenotype classification, and did not materially change our results.

Outcomes: infections and chronic conditions

Infections and chronic conditions were self-reported using a standardized questionnaire during pregnancy. Women were asked if they had ever had various infections (measles, mumps, chicken pox, whooping cough, cold sores, meningitis, genital herpes, gonorrhea and urinary infections) or chronic conditions (diabetes, hypertension, kidney disease, rheumatism, arthritis, psoriasis, hay fever, asthma, eczema, and any allergies, including cat, dust, pollen, insect bites or 'other').

Statistical analysis

Demographic characteristics were summarized with descriptive statistics and compared between non-secretors (AA) and secretors (GA or GG combined) using t-tests for continuous variables or chi-squared tests for categorical variables. For each outcome, the relative risk (RR) and 95% confidence interval (95% CI) was calculated for non-secretors versus secretors. A multivariable model was used to determine whether the association of *FUT2* secretor status and kidney disease was independent of measles, mumps and urinary tract infections. Multivariable models were also used to mutually adjust for *FUT2* secretor status and ABO blood group and to formally test for interaction between these two factors. To explore the potential impact of *FUT2* heterozygosity, a three group analysis was also conducted, considering the AA, GA and GG genotypes separately and using homozygous secretors (GG) as the reference group. All statistical analyses were performed in SAS (version 9.4, Carey, NC, US).

Results

Overall, 1920 women were homozygous for the *FUT2* non-secretor allele (AA, 25.3%), 1906 were homozygous for the secretor allele (GG, 25.1%) and 3756 were heterozygous (GA, 49.5%). Almost half (46%) were first-time mothers, 21% were unmarried, 21% smoked, and 14% had a university degree. The mean (± standard deviation) age was 26.9 (± 5.9) years and the mean body mass index was 22.9 (± 3.7) kg/m^2. These demographic characteristics were not associated with *FUT2* secretor status (Table 1). The lifetime incidence of infections ranged from <1% for meningitis to 87% for chicken pox, while the incidence of chronic conditions ranged from 1% for diabetes to 43% for allergies.

Dichotomous FUT2 secretor status and infections

The homozygous AA non-secretor genotype was associated with mumps infection, with 68% of non-secretors experiencing this infection, compared to 48% of secretors (RR, 1.40; 95% CI, 1.34–1.46; p<0.0001) (Table 2). Weaker associations were observed for measles infection (76% vs. 72%; RR, 1.05; 95% CI, 1.02–1.09; p=0.0008) and urinary infections (57% vs. 55%; RR, 1.05; 95% CI, 1.00–1.10; p=0.05). There was no strong evidence of association between *FUT2* secretor status and whooping cough, chicken pox or cold sores (Table 2).

Table 1. Demographics of mothers in the ASLPAC cohort according to *FUT2* secretor status.

	***FUT2* Secretor Status (rs601338 genotype)**				
	Non-Secretors (AA)		**Secretors (GG or GA)**		**P value**
	N=1920		**N=5662**		
Age, years	26.9	± 5.9	26.9	± 5.8	0.94
BMI, kg/m^2	22.8	± 3.6	23.0	± 3.7	0.05
Married					
No	423	(22.4)	1173	(21.2)	0.28
Yes	1468	(77.6)	4364	(78.8)	
Parity					
0	861	(46.3)	2539	(46.2)	0.67
1	641	(34.4)	1957	(35.6)	
2	264	(14.2)	738	(13.4)	
3 or more	95	(5.1)	259	(4.7)	
Smoking					
No	1480	(78.8)	4324	(77.9)	0.37
Yes	397	(21.2)	1229	(22.1)	
Education					
<O level	457	(24.6)	1418	(25.8)	0.44
O level	642	(34.5)	1946	(35.4)	
A level	461	(24.8)	1306	(23.8)	
University degree	276	(14.8)	762	(13.9)	

Values are mean ± standard deviation or n (%). AA, homozygous for non-secretor alleles; GG, homozygous for secretor alleles; GA, heterozygous; ALSPAC, Avon Longitudinal Study of Parents and Children; BMI, body mass index. Comparisons by t-test for continuous variables or chi-squared test for categorical variables.

Dichotomous FUT2 secretor status and chronic conditions

Homozygous AA non-secretors experienced a 39% increased risk of self-reported kidney disease compared to secretors (5.4% vs. 3.9%; RR, 1.39; 95% CI, 1.11–1.75; p=0.004) (Table 2). This association was essentially unchanged in a multivariable model controlling for mumps, measles and urinary infections (adjusted RR, 1.39; 95% CI, 1.10–1.75; p=0.005). Directionally consistent results were also observed for diabetes (RR, 1.23; 95% CI, 0.76–2.00; p=0.40), rheumatism (RR 1.19, 95%CI: 0.94–1.51, p=0.14) and arthritis (RR, 1.21; 95% CI, 0.93–1.57; p=0.15), although power was lacking for these relatively rare outcomes. There was no strong evidence of association between *FUT2* secretor status and hypertension, hay fever, asthma or allergies (Table 2).

ABO blood group

Since secretor status determines the ability to secrete blood group antigens, we also explored the impact of ABO blood group on associations observed for mumps infection and kidney disease (Table 3). For both conditions, the effect estimate for *FUT2* secretor status was essentially unchanged following adjustment for ABO blood group and there was no significant interaction

Table 2. Lifetime incidence and relative risk of infectious and chronic conditions among mothers in the ALSPAC cohort according to dichotomized *FUT2* secretor status.

Condition*	*FUT2* Secretor Status (rs601338 genotype)				Relative Risk Non-Secretors vs. Secretors	P value
	Non-Secretors (AA)		Secretors (GA or GG)			
	N=1920		N=5662			
	cases	(%)	cases	(%)	RR (95% CI)	
Infections						
measles	1458	(75.9)	4076	(72.0)	**1.05 (1.02, 1.09)**	**0.0008**
mumps	1299	(67.7)	2734	(48.3)	**1.40 (1.34, 1.46)**	**<0.0001**
chicken pox	1656	(86.3)	4925	(87.0)	0.99 (0.97, 1.01)	0.41
whooping cough	222	(11.6)	638	(11.3)	1.03 (0.89, 1.18)	0.73
cold sores	843	(43.9)	2458	(43.4)	1.01 (0.95, 1.07)	0.71
meningitis	15	(0.8)	61	(1.1)	0.73 (0.41, 1.27)	0.26
genital herpes	45	(2.3)	108	(1.9)	1.23 (0.87, 1.73)	0.24
gonorrhea	29	(1.5)	70	(1.2)	1.22 (0.80, 1.88)	0.36
urinary infection	1095	(57.0)	3085	(54.5)	**1.05 (1.00, 1.10)**	**0.05**
Chronic conditions						
kidney disease	103	(5.4)	218	(3.9)	**1.39 (1.11, 1.75)**	**0.004**
hypertension	272	(14.2)	804	(14.2)	1.00 (0.88, 1.13)	0.94
diabetes	23	(1.2)	55	(1.0)	1.23 (0.76, 2.00)	0.40
rheumatism	93	(4.8)	230	(4.1)	1.19 (0.94, 1.51)	0.14
arthritis	77	(4.0)	188	(3.3)	1.21 (0.93, 1.57)	0.15
psoriasis	59	(3.1)	213	(3.8)	0.82 (0.62, 1.08)	0.16
hay fever	573	(29.8)	1742	(30.8)	0.97 (0.90, 1.05)	0.45
asthma	215	(11.2)	652	(11.5)	0.97 (0.84, 1.12)	0.71
eczema	469	(24.4)	1271	(22.4)	1.09 (0.99, 1.19)	0.07
any allergies	837	(43.6)	2412	(42.6)	1.02 (0.97, 1.09)	0.42

AA, homozygous for non-secretor alleles; GG, homozygous for secretor alleles; GA, heterozygous; ALSPAC, Avon Longitudinal Study of Parents and Children; RR, relative risk; CI, confidence interval.
*Self-reported during pregnancy: "Have you ever had…?"

Table 3. Mutually-adjusted associations of *FUT2* secretor status and ABO blood group with mumps infection and kidney disease in the ALSPAC cohort

	Mumps				Kidney Disease			
	n/N	%	RR (95% CI)	p	n/N	%	RR (95% CI)	p
***FUT2* Genotype**								
Non-Secretor (AA)	1299/1920	67.7	**1.39 (1.33, 1.46)**	**<.0001**	103/1920	5.4	**1.32 (1.04, 1.69)**	**0.02**
Secretor (AG or GG)	2734/5662	48.3	1.00 (ref)		218/5662	3.9	1.00 (ref)	
Blood Group								
A	1522/2917	52.2	1.00 (0.95, 1.05)	0.93	135/2917	4.6	1.09 (0.56, 2.11)	0.14
B	316/608	52.0	0.99 (0.91, 1.07)	0.78	26/608	4.3	1.10 (0.73, 1.67)	0.64
O	1595/3015	52.9	1.00 (ref)		117/3015	3.9	1.00 (ref)	
AB	129/217	59.4	**1.15 (1.03, 1.28)**	**0.01**	9/217	4.1	1.09 (0.56, 2.11)	0.80

RR, relative risk; CI, confidence interval. Models are mutually adjusted for *FUT2* genotype and blood group.

between *FUT2* and ABO blood group (p for interaction: 0.60 for mumps, 0.57 for kidney disease). Independent of *FUT2* secretor status, women with type AB blood had an increased risk of mumps infection (59.4%) compared to women with type A, B, or O blood (52.2%, 52.0%, 52.9%, respectively; adjusted RR 1.15, 95% CI: 1.03, 1.28 for AB vs O, p=0.01). ABO blood group was not associated with kidney disease.

FUT2 heterozygosity

Compared to homozygous GG secretors, GA heterozygotes experienced a similar risk of mumps infection (RR, 0.97; 95% CI, 0.91–1.02; p=0.24) and kidney disease (RR, 0.99; 95% CI, 0.75–1.30; p=0.93), suggesting that increased susceptibility to these conditions (as described above) is likely to be a recessive trait experienced only in homozygous AA non-secretors. Similar evidence was found for measles, urinary infections and eczema, where disease risk was comparable for individuals with GG and GA genotypes. However, this pattern was not consistent across all conditions. For example, the risk of asthma was similarly reduced in GA heterozygotes (11.1%) and AA non-secretors (11.2%) compared to GG secretors (12.2%), and the risk of arthritis was lower in GA heterozygotes (3.0%) compared to either homozygous genotype (4.0% in AA, 3.8% in GG), although statistical evidence of association was weak for these relatively rare outcomes (Figure 1).

Discussion

Our findings from the population-based ALSPAC cohort confirm and extend previous research associating the *FUT2* genotype with susceptibility to infections and chronic diseases. Specifically, we confirmed a recently reported association with mumps infection[3] and identified an association with self-reported kidney disease. We also evaluated a number of other common conditions (e.g. whooping cough, chicken pox and asthma) but found no strong evidence of association with *FUT2* secretor status, indicating that *FUT2* influences pathogen- or disease-specific processes, rather than overall innate or adaptive immunity. Finally, our results suggest that *FUT2* heterozygosity may confer an intermediate phenotype for certain conditions, although further research is required to replicate these findings.

Our results confirm the association reported in a recent GWAS for common infections among 23andMe research participants by Tian *et al.*[3], where the *FUT2* rs516316(C) allele was associated with mumps infection (odds ratio, 1.25; 95% CI, 1.24–1.27). This risk allele is in complete linkage disequilibrium with the non-secretor rs601338(A) allele evaluated in our study, where a strong association was also observed (RR, 1.40; 95% CI, 1.34–1.46). Tian *et al.* hypothesized that non-secretors are more susceptible to mumps infection because binding of the mumps virus to host cell sialic acid receptors is enhanced in the absence

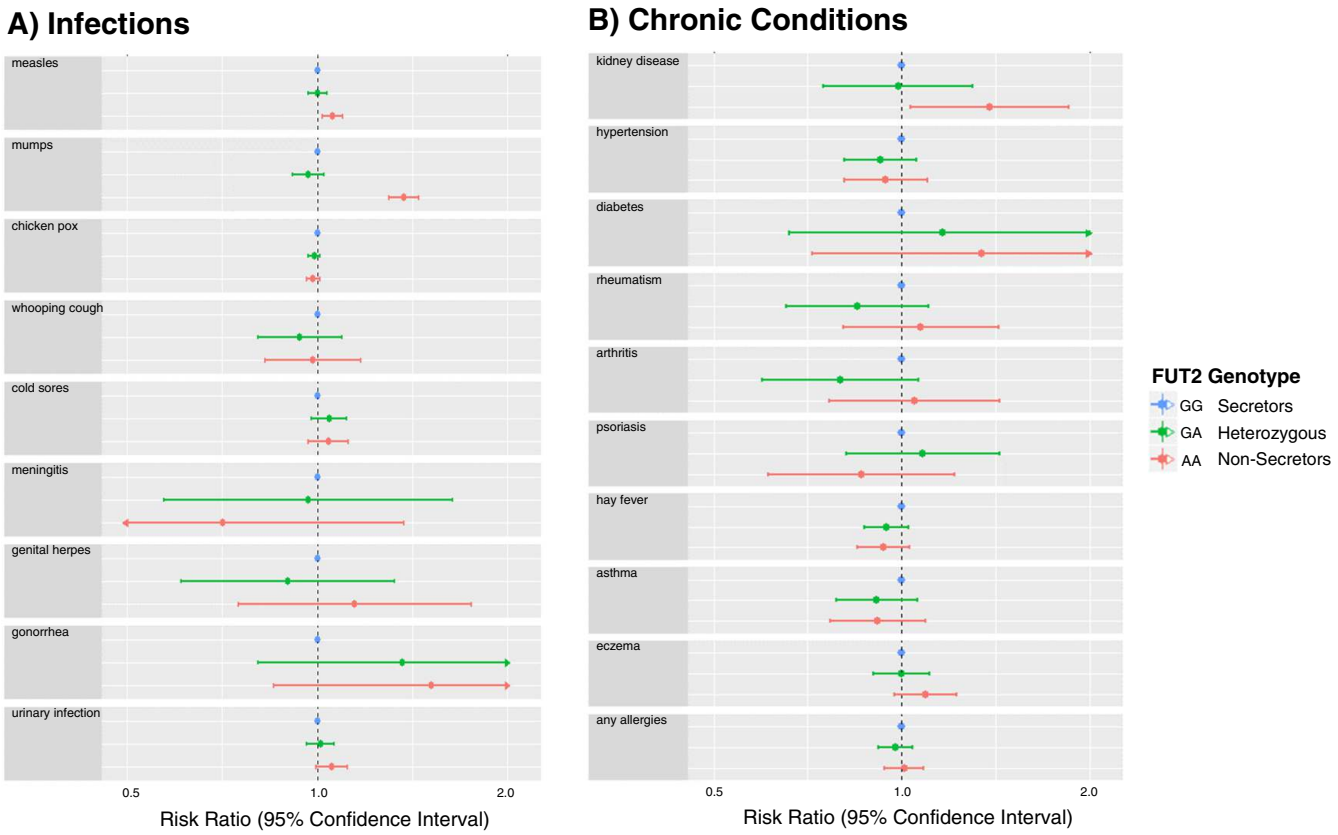

Figure 1. Relative risk of (**A**) infectious and (**B**) chronic conditions among 7,582 mothers in the ALSPAC cohort according to *FUT2* genotype at rs601338.

of *FUT2*-dependent antigens on the cell surface. Indeed, using x-ray crystallography and functional assays, Kubota *et al.*[29] recently showed that mumps virus preferentially uses a trisaccharide containing α2,3-linked sialic acid in unbranched sugar chains as a receptor. Our results provide further evidence that susceptibility to mumps infection is modulated by *FUT2* secretor status.

Tian *et al.*[3] also reported an association between the ABO gene and mumps infection, and suggested that ABO antigens may disrupt binding of the mumps virus to host cell receptors. Our results using clinical ABO blood group data support and extend this finding by confirming an association and specifically identifying blood type AB as a risk factor for mumps infection. Since *FUT2* secretor status determines whether ABO antigens are secreted into body fluids and onto cell surfaces, we hypothesized that secretor status and blood type may interact to influence susceptibility to mumps infection; however, we found no evidence of this interaction. Thus, our study suggests that *FUT2* genotype and ABO blood group are independently associated with mumps infection, with increased risk among non-secretors and blood type AB.

Our study also provides new evidence that non-secretors may be predisposed to kidney disease (RR, 1.39; 95% CI, 1.11–1.75), although we lacked clinical information to confirm and classify this self-reported diagnosis. To our knowledge, the *FUT2* genotype has not previously been associated with kidney disease in the general population, although some studies have used traditional blood group assays to evaluate secretor status in patients with pyelonephritis (kidney inflammation, typically due to bacterial infection). One study of women with acute uncomplicated pyelonephritis found that non-secretor status was significantly more common in these patients than in the general population[30], and another found that renal scarring in girls with recurrent pyelonephritis was more common in non-secretors than secretors[31]. It has been hypothesized that these associations reflect an increased susceptibility to uropathogenic *Escherichia coli* infection among non-secretors, resulting from the enhanced expression of preferred binding receptors in the vaginal epithelium and kidneys of non-secretor women[32,33]. Notably, in our study, the association between *FUT2* genotype and self-reported kidney disease appeared to be independent of self-reported urinary infections. However, there are multiple clinically-distinct causes of "kidney disease" and "urinary infections", and we lacked clinical information to define the etiology of these conditions in our study. Thus, additional research is needed to replicate our observations with confirmed and clinically-defined kidney disease and urinary infections, and to examine the possible relationship between these conditions and *FUT2* genotype.

Consistent with previous studies[19–21], we observed a trend towards an increased risk of arthritis, rheumatism and diabetes among non-secretors, although we lacked statistical power for the analysis of these relatively uncommon autoimmune disorders.

Finally, we examined disease risk among GA heterozygotes, who are typically considered secretors because the non-secretor rs601338(A) allele is assumed to be recessive. Our results for mumps and kidney disease support this assumption, as increased susceptibility was only seen in homozygous AA non-secretors. However, we observed different patterns of association for some other conditions, including a potentially increased risk of gonorrhea and reduced risk of arthritis among GA heterozygotes, although our effect estimates were imprecise for these relatively rare conditions. Further research is warranted to replicate these observations in larger populations, and explore whether heterozygosity may impart an intermediate risk or unique protection from certain conditions.

Limitations of this work include the reliance on self-reported medical histories and low power for rare outcomes (such as meningitis, diabetes and other autoimmune diseases). Power was also limited for interaction analyses. Also, we could not identify the specific pathogens responsible for urinary infections, and we lacked clinical data to confirm, classify and define the etiology of multifactorial disorders (such as allergies and kidney disease). Finally, our analysis of the ALSPAC pregnancy cohort was limited to women, so the results may not be generalizable to men, and potential sex differences could not be investigated.

In conclusion, our results identify a novel association between *FUT2* non-secretor status and increased risk of kidney disease, and confirm a recently-reported association with increased susceptibility to mumps infection. The clinical implications of these associations warrant further investigation.

Data availability

The ALSPAC data management plan (http://www.bristol.ac.uk/alspac/researchers/data-access/documents/alspac-data-management-plan.pdf) describes in detail the policy regarding data sharing, which is through a system of managed open access. The steps below highlight how to apply for access to the data included in this paper and all other ALSPAC data. The datasets used in this analysis are linked to ALSPAC project number B3047; please quote this project number during your application.

1. Please read the ALSPAC access policy (PDF, 627kB) which describes the process of accessing the data and samples in detail, and outlines the costs associated with doing so.
2. You may also find it useful to browse the fully searchable ALSPAC research proposals database, which lists all research projects that have been approved since April 2011.
3. Please submit your research proposal for consideration by the ALSPAC Executive Committee. You will receive a response within 10 working days to advise you whether your proposal has been approved.

If you have any questions about accessing data, please email alspac-data@bristol.ac.uk.

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Frequency of ABH Secretors/Non Secretors and Its Clinical Significance: A Cross Sectional Study in Gwalior

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Abstract: The ABO blood group and secretor status of individuals is inherited independently. ABO blood group antigens are inherited by A, B & H genes and gene responsible for secretor state is Se (Se/Se & Se/se) gene. If recessive gene se/se is inherited person is non secretor. These group specific substances, ABH may be detected in most body fluid as soluble form in secretors except cerebrospinal fluid (CSF). One of the richest and most available source is saliva. Secretor status of a person can be quite useful to determine certain doubtful cases of ABO blood grouping and also has clinical significance. A total number of 1001 blood donors were randomly registered and studied for secretor/ non-secretor status in the present study. From the study, we conclude that secretors are more prevalent in our region (72.4%) and probable frequency Se and se gene was .475 and .525 while frequency of SeSe, Sese and sese allele genes was .226, .498 and .276 respectively. Secretors have an added degree of protection against the environment, particularly with respect to microorganisms and lectins. Non secretors are more prone to different varieties of auto-immune diseases and TTIs infections. Alcoholism has been associated with the Non-secretor blood type. Secretor status of an individual is also helpful to identify weaker variants of ABO group.

I. Introduction

The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who identified the O, A and B blood types in 1900[1]. Interestingly, the antigens comprising this blood group system were among the first human genetic markers identified [2, 3]. Later on, AB blood group was added to the ABO blood system by Alfred Von Decastello and Adriano Sturli in 1902 [4]. This major blood group system consists of four blood types: A, B, AB and O [5]. These antigens are genetic markers inherited as Mendelian characteristics in a co-dominant autosomal fashion. In 1930, Putkonen noted that a person could be either secretor or non-secretor with respect to his/her genetic ability to secrete ABH blood group substances in secretions [5]. Weiner in 1943 discovered that A & B substances are present in saliva of most A & B individuals (secretors) [6]. The ABO blood group and secretor status of individuals is inherited independently. ABO blood group antigens are inherited by A, B & H genes and gene responsible for secretor state is Se (Se/Se & Se/se) gene. If recessive gene se/se is inherited person is non secretor. These group specific substances, ABH may be detected in most body fluid as soluble form except cerebrospinal fluid (CSF). One of the richest and most available sources is saliva [7]. The H, Fucosyltransferase 1 (FUT 1) gene codes for the ABO blood group. The secretor, Fucosyltransferase 2 (FUT 2) gene interacts with FUT 1 gene to determine the ability to secrete blood group antigens into body fluids and secretions. Absence of the blood group antigen in secretions is a health disadvantage, as this appears to increase the susceptibility to a number of diseases. There are certain diseases which show evidence of association with non secretor status [8]. ABH non secretors also have a higher prevalence of different varieties of auto-immune diseases including ankylosing spondylitis, Sjogren's syndrome, multiple sclerosis, reactive arthritis, psoriatic arthropathy and grave's disease. Non-secretors have high incidence of diseases of mouth, esophageal cancer, and epithelial dysplasia as compared to secretors [9].
Secretor status of a person can be quite useful to determine certain doubtful cases of ABO blood grouping by conventional method, especially the subgroups of ABO system [10]. Present study was aimed to evaluate the prevalence of secretor status of blood donors in Gwalior region and its clinical significance.

II. Materials And Methods

This work was carried out in Blood Bank, Department of Pathology, at a tertiary care hospital of central India from 1st February 2015 to 31st January 2016. Donor's questionnaire form was filled in accordance of standard protocol for blood donation and relevant past medical history of illness was also taken from the donors, those were registered for the project. For ABO and Rh-D blood grouping, with all aseptic precautions 5 ml blood was collected either from ante-cubital vein of blood donor by disposable syringe, or procured from the

blood unit of the donor. To know the secretor status of donor, 3-5 ml of saliva was collected in a sterile tube. Prior to collection, donors were asked to rinse their mouth thoroughly with distilled water and for increasing salivation they were requested to chew wax, paraffin, or a clean rubber band. After collection, Saliva tube was kept for 8-10 minutes in boiling water bath to denature the salivary enzymes, cooled and centrifuged for 5 minutes at 1000 rpm and then supernatant was collected and diluted with equal volume of normal saline. Denatured diluted saliva was used to know the secretor status of donors. For ABO grouping, Red Cell suspension: 20% in normal saline for conventional tube method and 0.8% in low-ionic strength saline (LISS) for column agglutination method (Gel Technology) was prepared. ABO blood grouping of the donors was done by conventional tube methods/ column agglutination method. For conventional method, commercial monoclonal Antisera; Anti-A, Anti-B, Anti-AB, Anti Rh-D make Tulip were used. For detection of ABO sub-groups, lectins Antisera (Anti-H, Anti-A1), extended ABO grouping Gel card and results of saliva grouping were used.
For the saliva grouping, 1: 32 dilution of anti-sera A and B while 1:8 dilutions of anti-sera H was prepared. Agglutination inhibition test along with positive and negative control was performed, using Neutral gel cards of make- Tulip to know the secretor/ non-secretor status of an individual. Card was well allowed to stand at room temperature for 15 minutes before use. Test procedure is as follows. (Table no.1)

Table no 1-Agglutination Inhibition Saliva Test for secretor status.

S.No	Substance (Antigen)	Test	Positive Control	Negative Control
1.	Test for A substance	0.025 ml of anti-A + 0.05 ml of denature diluted saliva	0.025 ml of anti-A + 0.05 ml of known A substance saliva	0.025 ml of anti-A + 0.05 ml of saline
2.	Test for B substance	0.025 ml of anti-B + 0.05 ml of denature diluted saliva	0.025 ml of anti-B + 0.05 ml of known B substance saliva	0.025 ml of anti-B + 0.05 ml of saline
3.	Test for H substance	0.025 ml of anti-H + 0.05 ml of denature diluted saliva	0.025 ml of anti-H +0.05 ml of known H substance saliva	0.025 ml of anti-H + 0.05 ml of saline

After completing the above test procedure the labelled test cards were incubated at room temperature for 20 minutes. Then, 0.025 ml of A, B and O red cells were added to micro-well marked A, B, H and controls. Further cards were incubated at room temperature for 15 minutes and then Centrifuged at 1000 rpm for 10 minutes. Results were observed as follows-

- In Positive control, if there is agglutination inhibition (No agglutination) and in Negative control there is agglutination, it means test procedure is correct.
- If in the test's tubes there is no agglutination (i.e. agglutination inhibition), person is Secretor for ABH substance.
- If in the test's tubes there is agglutination, person is Non-Secretor for the corresponding substance.

Probable Gene frequency of Se/se and frequency of allele genes: SeSe, Sese and sese were calculated by using Hardy-Weinberg Theorem for probabilities and possibilities. All data was collected, compiled and compared statistically by frequency distribution and percentage proportion. Chi-square ($\chi 2$) test was applied to know the statistically significant difference (p value) of the data. Epicalc version 2000 software was used for the same.

III. Observation & Results

A total number of 1001 blood donors were randomly registered and studied for secretor/ non-secretor status in this region of central India. Out of 1001 cases, 725 (72.4%) were secretors and 276 were non-secretors (27.6%); statistically significant (p= .000001). Male: female ratio of donors was 96.8% (969n): 3.3% (32n); statistically significant (p= .000004). Out of 32 females, 24 (75%) were secretors and 08 (25%) were non-secretors and for 969 males, 701 (72.3%) were secretors and 268 (27.7%) were non-secretors. Prevalence of transfusion transmitted infection (TTI's) i.e. HIV 1 & 2, HCV, HBsAg, VDRL, Malaria in the present study was 3.8% (38) reactive and 96.2% (963) non-reactive. Among 1001 cases, most prevalent TTI was HBsAg 2.6% (26) followed by HCV 0.79% (08), HIV 1 & 2 0.19% (02), Malaria 0.9% (01) and Syphilis 0.9% (01). We observed that TTI positive cases were more in non-secretors. Out of 26 HBsAg positive cases; 11 were secretors and 15 were non-secretors, in 08 HCV positive cases; 03 were secretors and 05 non-secretors and the entire cases positive for HIV 1 & 2, malaria and syphilis belonged to non-secretors.
Age group wise distribution of donors in the present study was- 50 (4.9%) were below 20 years, 604 (60.3%) between 21-30 years, 279 (27.8%) between 31-40 years, 62 (6.2%) between 41-50 years and 06 (0.6%) between 51-60 years which is statistically significant (p= .000001). Among different age groups, secretor and non-secretor status was shown in the figure no. 1.

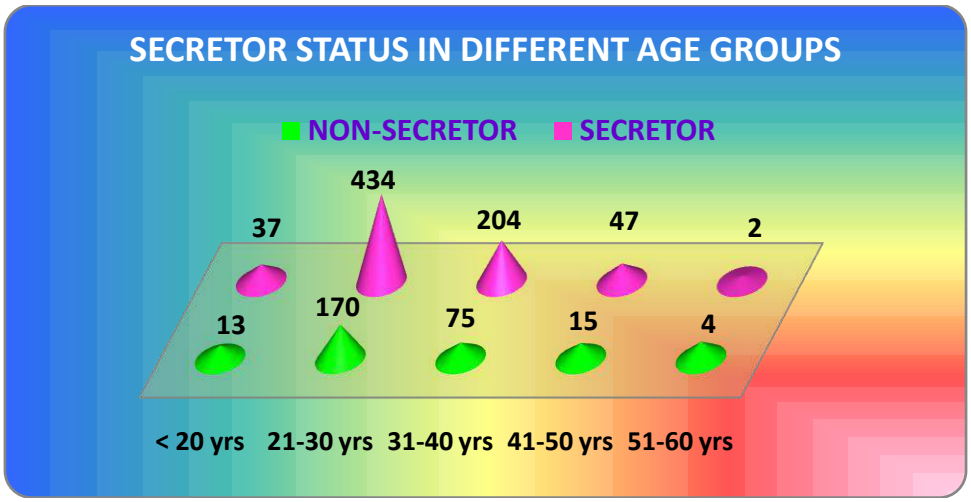

Figure No.1- Secretor Status in Different Age Groups

ABO group distibution of donors were 223 (22.3%) of A group, 340 (33.9%) of B group, 313 (31.2%) of O group and 125 (12.5%) of AB group; statistically significant (p= .000001). Distribution of secretor and non secretor state among different ABO group are summarised in fig. no.2 . In the study, 912(91.1%) cases were Rh positive and 89 (8.9%) Rh negative; statistically significant (p= .000001)

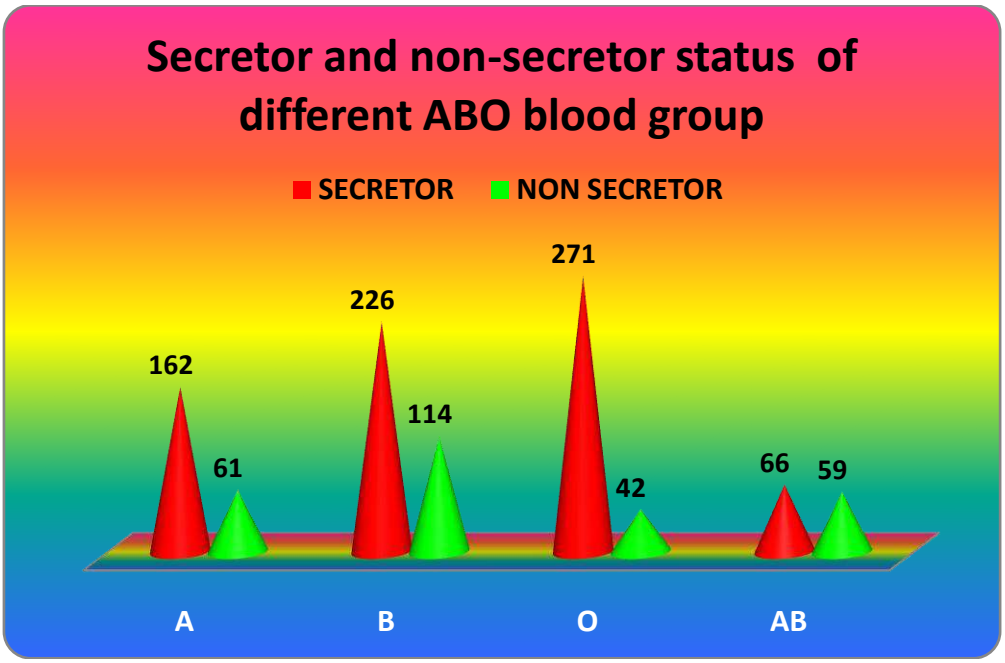

Figure No.2- Secretor and Non-Secretor Status of Different ABO Blood Group

At the time of donation, donors were fit for donation as per standard questionnaire. A total number of 1001 cases in the study were enquired for the history of past discomfort and illness. Out of 1001 cases 51.9% (520) donors were in good health till date and there no history of clinical illness, 45.1% (451) donors gave the history of their past illness, which is summarized in table no.2 and 03% (30) of donors didn't respond to the questionnaire. Out of 45.1% donors with relevant history of past illness, maximum reported multiple system disorder history i.e. two system involvement 36.8% and three system involvement 30.2%, whereas 33.3% donors gave history of only one system disorder. The overall incidence in 1001 cases for single, double and triple system involvement were 14.9%, 16.6% and 13.6% respectively. Also, 39.9% donors had a history of alcohol intake while rest 59.9% was non-alcoholic. Higher incidence of alcohol intake was reported in non-secretors 63% while in secretors it was 37 %. (Table No.2)

Table No.2- Common Health Problems in Blood Donors and Its Relation with Secretor Status.

System Disorders	Prevalence of symptoms P=0.000004	Secretor Cases P=0.000003	Non-Secretor Cases P=0.000003	Comment & p value
Digestive discomfort Gastritis, Dyspepsia Loose Motion, etc.	321 (32.1%)	148 (46.1%)	173 (53.9%)	Non secretors are more prone to digestive disorders. P=0.1629
Respiratory disorders- Recurrent Cough & Cold Recurrent Viral infections Breathlessness	185 (18.5%)	102 (55.1%)	83 (44.9%)	Secretors are more prone to respiratory disorders. P=0.16244
Autoimmune- Joint Pain Swelling Of Joints Restriction Of Movement	80 (7.9%)	26 (32.5%)	54 (67.5%)	Non secretors are more prone to autoimmune disorders. P=0.00174
Cardiovascular-Hypertension Breathlessness Angina	92 (9.2%)	42 (45.7%)	50 (54.3%)	Non secretors are more prone to cardiovascular disorders. P=0.4042
Metabolic – High Blood Sugar Low Blood Sugar Thyroid disorder, etc.	20 (1.9%)	08 (40%)	12 (60%)	Non secretors are more prone to metabolic disorders. P=0.3710
Renal- UTI Fungal Infection	110 (10.9%)	49 (44.5%)	61 (55.5%)	Non secretors are more prone to renal disorders. P=0.2525
Oral Ulcers/ Dental Hygiene	82 (8.2%)	40 (48.8%)	42 (51.2%)	Oral / dental hygiene is poor in non-secretors. P=0.82519
Alcoholism	400 (39.9%)	147 (37%)	253 (63%)	Non secretors are more comfortable with alcohol consumption. P=0.000001

The subgroup of A & B were encountered in the study is summarized in table no 3 &4. Out of 223 cases of 'A' group 190 A_1 positive, 26 A_2 and 07 weak variants of 'A' group were detected in the study. They were 02 of A_3, 02 of A_{int} and 01 each case, A_{end}, A_x and A_m. Out of 340 cases of B group 337 were B, and 03 weaker variants i.e. B_X, B3 and B_M one each were reported in the study. Out of 125 AB group, 112 were A_1B, while 13 were A_2B. Weaker variant of A & B in AB group was not evaluated in the study.

Table 3-Serological Reactions of Weak Subgroups of A Phenotype

ABO Phen-otype	Total No.	Forward Grouping			Reverse Grouping				Lectin		Saliva Test			Adsorption Elution	
		Anti-A	Anti-B	Anti-AB	A_1 Cells	A_2 Cells	B Cells	O Cells	Anti-A_1	Anti-H	A	B	H	Group O sera	Group B Sera
A_3	2	2+	-	1+	0	0	4+	-	-	4+	1+	-	2+	1+	1+
A_{int}	2	4+	-	4+	0	0	4+	0	1+	4+	1+	-	1+	2+	2+
A_{end}	1	1+	-	1+	-	-	4+	-	-	4+	-	-	1+	1+	1+
A_x	1	wk	-	1+	2+	-	4+	-	-	4+	-	-	1+	1+	-
A_m	1	0	0	Wk	0	0	4+	0	1+	4+	3+	-	4+	-	-

Table 4- Serological Reactions of Weak Subgroups of B Phenotype

ABO Phen-otype	Total No.	Forward Grouping			Reverse Grouping				Lectin	Saliva Test			Adsorption Elution	
		Anti-A	Anti-B	Anti-Ab	A1 Cells	A2 Cells	B Cells	O Cells	Anti-H	A	B	H	Group O	Group A Sera
B_x	1	-	wk	2+	4+	4+	-	-	4+	-	-	1	1	-

												+	+	
B_3	1	-	2+	Wk	4+	4+	-	-	4+	-	1+	-	1+	1+
B_m	1	-	-	-	4+	3+	-	-	4+	-	1+	-	2+	2+

Probable frequency of Se and se gene and frequency of SeSe , Sese and sese allele genes in the study was calculated by using Hardy-Weinberg Theorem for probabilities and possibilities as shown in table no.5

Table no. 5-Phenotype, Genotype and Gene Frequency in present study

Phenotype			Gene		Genotype	
	No.	Frequency	Calculated Frequency		Calculated Frequency	
Secretors	725	.724	Se	.475	SeSe	.226
					Sese	.498
Non-secretors	276	.276	se	.525	sese	.276

IV. Discussion

It is a universal fact that blood is man's absolute and unchangeable identity [11]. Although almost 400 blood group antigens have been reported, the ABO and RhD are recognized as clinically significant and dominant blood group antigens. ABO blood group system derives its importance from the fact that A and B are strongly antigenic and anti A and anti B occur naturally in the serum of persons lacking the corresponding antigens [12]. ABO Blood group antigens (substances) are secreted by the secretors into various body fluids. Non-secretors secrete out very minor or none of their blood group antigens into different body fluids. Increased degree of protection against bacterial and fimbrial lectins may be associated with the secretion of these antigens into saliva and mucus. However, secretors are more prone to hemolytic anemia and viral infections that have been cited by Raza MW et al 1991 [13]. Whereas, non secretors have a higher prevalence of autoimmune diseases including ankylosing spondylitis, reactive arthritis, sjogren's syndrome, psoriatic multiple sclerosis, grave's disease, peptic ulcer, metabolic syndromes, oral ulcers etc .[14, 15]

In our study out of 1001 cases, 725 (72.4%) were secretors and 276 were non-secretors (27.6%). These findings were similar to the study done by Igbeneghu C et al 2014 in which out of 740 cases; 78.1% were secretors and 21.9% non-secretors [16]. In our study prevalence of secretor was higher in comparison to studies done by Saboor M et al (64.4% secretor and 35.6% non secretor) [17] and Akhter S et al (60% secretor and 40 % non secretor)[18] while lower to study done by Sikander et al (93% secretor and 07% non secretor) [19]. A lower incidence of secretor status was also reported by Rahil A et al (31.8% secretor and 68.2% non secretor)[20] and Sylvia Devi A. et al (49.5% secretor and 50.5% non secretor) [21]. Frequency of ABH secretor status in the world population is about 80% secretors and 20% non secretors [22]. Wide variation of secretor status in different studies may be due to geographical distribution and racial differences.

In our study, out of 1001 cases, 223 (22.3%) were of A group, 340 (33.9%) of B group, 313 (31.2%) of O group and 125 (12.5%) of AB group. Predominence of B group was also reported by Nasim F.H et al 1987 from Bahawalpur, Pakistan 36.6%[23] and by Saboor M et al [17] from karachi,Pakistan 35.6% while predominence of O group was reorted by Akhter S et al [18] from Dhaka, Fridpur 36%. It may/ may not be related to the distribution of ABO group in the population beecause of random sampling of the cases.

In the present study, 912 (91.1%) cases were Rh positive and 89 (8.9%) Rh negative. Distribution of secretor and non secretor state among different ABO group was : In A group 72.6% were secretors and 27.4% non-secretors, in B group 66.4%were secretors and 33.6% non secretors, in AB group 52.8% Secretors and 47.2% non secretors lastly, in O group 86.3% were secretors and 13.7% non secretors. While in a study done in

southwestern nigeria by Igbeneghu C et al 2014, secretor and non secretor status was 65.9% and 34.1% in A group, 70.4% and 29.6% in B group, 70% and 30% in AB group and 86.2% and 13.8% in O group [16] . Secretor and non secretor status in study done by sylvia A. et al was 51.1% and 45.9% in A group, 46.4% and 53.6% in b group, 40.5% and 59.5% in AB group and 54.1 and 45.9% in O group [21]**.** In our study and study done by ibeneghu et al, uniformly in all the groups, secretors were more prevalent, while in the study by A.Sylvia devi et al non-secretors were reported predominantly in blood group B and AB.

In present study with the contemplation of Rh status along with ABO group secretor status of the donors i.e. out of 223 cases of A group; A positive were 215 and A negative were 08 . In A positive 74.4% were secretors and 25.6% non secretors, in A negative 25% were secretors and 75% non-secretors. Out of 340 cases of B group; Rh positive were 312 and negative were 28. In B positive 71.1% were secretors and 28.8% non secretors, in B negative 14.2% were secretors and 85.7% non-secretors. Out of 125 cases of AB group; Rh positive were 94 and negative were 31. In AB positive 54.2% were secretors and 45.7% non secretors, in AB negative 48.3% were secretors and 51.6% non-secretors. Out of 313 cases of O group; Rh positive were 291 and negative were 22. In O positive 89.6% were secretors and 10.3% non secretors, in O negative 45.5% were secretors and 54.5% non-secretors. With this in our study, we observed that in Rh negative cases non-secretors are more prevalent than secretors, while in other similar studies this type of distribution were not reported; in best of our knowledge.

In present study of 1001 cases prevalence of transfusion transmitted infection (TTI's) i.e. HIV 1 & 2 , HCV, HBsAg, VDRL, Malaria were 3.8% (38) reactive and 96.2% (963) non-reactive. Among the total 1001 cases, most prevalent TTI was HBsAg 2.6% (26) followed by HCV 0.79% (08), HIV 1 & 2 0.19% (02), Malaria 0.9% (01) and Syphilis 0.9% (01) respectively. Higher incidence of HBsAg positive cases was because of higher prevalence of HBsAg in our region as reported by Sharma D.C et al [24]. In the present study, we observed that TTIs infections are more common in non secretors as compare to secretors. Prevalence of males and females donors in the present study was 96.8% (969) and 3.3% (32) respectively, mimic with our previous study on "female contribution in blood donation…" in this area [25]. In present study out of 32 females; 24 (75%) were secretors and 08 (25%) were non-secretors and out of 969 males; 701 (72.3%) were secretors and 268 (27.7%) were non-secretors. A study conducted by Sherwani SK, et al [19] indicated that secretor and non-secretor ratio among the young population were tested which showed that out of 300 females 278 were secretors and out of 250 males 234 were secretors. No remarkable distribution was observed in secretor status among males and females in the present study.

Age group wise distribution in the present study was 50 (4.9%) cases were below 20 years of age, 604 (60.3%) between 21-30 years, 279 (27.9 %%) between 31-40 years, 62 (6.1 %%) between 41-50 and 06 (0.6%) between 51-60 years correspondingly. The study revealed that the most common age group of the donors was between 21-30 years, followed by age groups 31-40, 41-50, <20 and lastly 51-60 with the prevalence of 60.3%, 27.8%, 6.2%, 4.9% and 0.6%. Among different age groups secretor and non-secretor status are as follows- In donors <20 years among 50 cases 37 (74%) were secretors and 13 (26%) non-secretors, in 21-30 years out of 604 cases, 434 (71.9%) secretors and 170 (28.1%) non-secretors, in 31-40 years total 279 cases out of which 204 (73.2%) were secretors and 75 (26.8%) non-secretors, in 41-50 years total 62 cases with 47 (75.8%) secretors and 15 (24.2%) non-secretors and in 51-60 years total number of donors were 6 in which 02 (33.3%) were secretors and 04 (66.7%) non-secretors. In all the groups secretor status is similar as seen in entire study except in the age group of 51-60 years where non-secretors were 66.7% but in this group sample size is too small to draw any relevant observation.

At the time of donation, donors were fit for donation as per standard questionnaire. A total number of 1001 cases in the study were enquired for the history of past discomfort and illness, out of which 51.9% donors were in good health till date, 45.1% donors gave the history of his/her past illness which included signs and symptoms related to - digestive problems, gastritis, breathlessness, metabolic disorders, joint pain, alcoholism, urinary tract infection, oral ulcers poor dental hygiene etc., whereas 03% of donors didn't respond to the questionnaire. Out of 45.1% donors with relevant history of past illness maximum reported multiple system disorder history i.e. two system involvement 36.8% and three system involvement 30.2%, whereas 33.3% donors gave history of only one system disorder. The overall incidence in 1001 cases for single, double and triple system involvement were 14.9 %, 16.6% and 13.9% respectively. Also, 39.9% donors had a history of alcohol intake while rest 59.9% was non-alcoholic. Higher incidence of alcohol intake was reported in non-secretors 63% while in secretors it was 37%. From the above related findings we have drawn following observations that secretors are on the brighter side and have a protection from the following diseases such as gastric problems including ulcers, gastritis, metabolic disorders, Sjogren's syndrome, oral ulcers, poor dental hygiene, respiratory disorders etc. but are more prone towards viral infections, and hemolytic anaemia [13]. This is also reported by M.Saboor et al, S Akhter et al, Abdel Rahim et al and A.Sylvia Devi et al. It is said that every cloud has a silver lining; likewise in cases of non-secretors they possess protection against norvovirus infection causing acute gastroenteritis as reported by Nordgren J et al [26].

Not only secretor/ non secretor status but also certain blood groups make somebody prone towards communicable and non-communicable diseases [27]. The prevalence of coronary heart diseases in blood group A was found higher than in all other ABO blood groups; from England [28], from other parts of Europe [29] and from USA [30]. The women who are not secretors of blood group antigens have 2-3 fold higher risk of developing UTI's [31]. Individuals of blood group O and those who are non-secretors of their ABO blood group antigens are over-represented among patients with gastric or duodenal ulcers [32]. In short, non-secretors are associated with susceptibility to a number of infectious diseases as indicated by few research manuscripts [33, 34]. In our study alcoholism is more common in non-secretors; statically significant (p=0.000001). Alcohol consumption can causes a reduction in the ability to secrete ABH blood group substances in the saliva of alcoholics quoted by Egesie UG et al 2005[35]. Alcoholism has been associated with the Non-secretor blood type. On the positive side, alcohol consumption appears to exert a protective effect on lung function and to lower the risk of heart disease more in Non-secretors than in Secretors. The key principle with the use of alcohol is for Non-secretors (and everybody actually) is moderation [36]. With the help of secretor status of donor, we have identified few weaker blood groups of A & B (table no 3 & 4) as also reported by Thakral B et al [37]. In our study phenotype frequency secretor and non-secretor was .724 and .276 respectively, gene frequency of Se/se gene was .475/.525 and genotype frequency of SeSe, Sese and sese alleles were .226, .498 and .276 respectively, which is almost similar with the frequencies reported in the study of Liverpool mentioned in textbook of human blood groups by Geoff Daniels, 3rd edition[38]. Table no.8

Table no.5 - Comparative Study of Phenotype, Genotype and Gene Frequency

Phenotype			Gene		Genotype	
	No.	Frequency	Calculated Frequency		Calculated Frequency	
Study from Liverpool						
Secretors	864	.772	Se	.523	SeSe	.273
					Sese	.498
Non-secretors	254	.227	se	.476	sese	.227
Present study						
Secretors	725	.724	Se	.475	SeSe	.226
					Sese	.498
Non-secretors	276	.276	se	.525	sese	.276

It is hypothesized that ability to secrete A, B and H substances in saliva, mucus and other glandular secretions was, perhaps, of paramount adaptive significance at a stage in the early history of man when human ancestors subsisted on raw food [39]. The non-human primates who feed mostly on wild plants (fruits, leaves, tubers etc.), and consume un-tempered lectins in bulk, are invariably secretors [39]. The hunter-gatherers, with a high content of lectin-rich raw food in their diet, likewise, are mostly secretors and the non-secretors are rare (zero to 3%) in these human isolates [32]. The modern human societies, on the other hand, show a sustained high frequency of non-secretors (over 20%) [32]. A frequent occurrence of non-secretors in modern human societies is perceived as the consequence of '**relaxation of selection'** pressure on the secretor gene (Se) which has a selective advantage in a lectin-rich dietary environment [40].

V. Conclusion

From the study we conclude that secretors are more prevalent in our region and have an added degree of protection against the environment, particularly with respect to microorganisms and lectins. Non secretors are more prone to TTIs infections. Alcohol is useful for non secretors. Secretor status is also helpful to identify weaker variants of ABO group.

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Consent

The authors declare that written informed consent was obtained from the Donors before being recruited for this research.

Ethical Approval

All author(s) hereby declare that all procedure have been examined and approved by the appropriate ethics committee of Gajra Raja Medical College, Gwalior, India and research have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

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Competing Interests

Authors have declared that no competing interests exist.

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