

Original Article



# Human Milk Oligosaccharide Profiles and the Secretor and Lewis Gene Status of Indonesian Lactating Mothers

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## ABSTRACT

**Purpose:** Human milk oligosaccharides (HMOs) may be genetically determined based on the secretor and Lewis status of the mother. This study aims to determine the HMO profile and the secretor and Lewis gene status of Indonesian lactating mothers.

**Methods:** Baseline data of 120 mother-infant pairs between 0-4 months post-partum obtained from a prospective longitudinal study was used. The concentrations of 2'-fucosyllactose (2'FL), lacto-N-fucopentaose I (LNFP I), lacto-N-tetraose (LNT), lacto-N-neotetraose (LNnT), 3'-sialyllactose (3'SL), and 6'-sialyllactose (6'SL) were measured. Genetic analysis was performed for mothers using targeted next-generation sequencing and Sanger sequencing. Wild-type AA with the rs1047781 (A385T) polymorphism was categorized as secretor positive, while heterozygous mutant AT was classified as a weak secretor. The presence of rs28362459 (T59G) heterozygous mutant AC and rs3745635 (G508A) heterozygous mutant CT genes indicated a Lewis negative status, and the absence of these genes indicated a positive status. Subsequently, breast milk was classified into various groups, namely Group 1: Secretor+Lewis+ (Se+Le+), Group 2: Secretor-Lewis+ (Se-Le+), Group 3: Secretor+Lewis- (Se+Le-), and Group 4: Secretor-Lewis- (Se-Le-). Data were analyzed using the Mann-Whitney and Kruskal-Wallis rank tests, and a *p*-value of 0.05 indicated statistical significance.

**Results:** A total of 58.3% and 41.7% of the samples had positive and weak secretor statuses, respectively. The proportion of those in Group 1 was 85%, while 15% were Group 3. The results showed that only 2'FL significantly differed according to the secretor status (*p*-value=0.018).

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#### Conflict of Interest

The authors have no financial conflicts of interest.

**Conclusion:** All Indonesian lactating mothers in this study were secretor positive, and most of them had a Lewis-positive status.

**Keywords:** Milk; Human; Infant; Oligosaccharides

## INTRODUCTION

Human breast milk is widely recognized as the primary and optimal source of nutrition for infants because its essential nutritional contents are required for growth. Several studies have explored the various components, benefits, and mechanisms of action of human breast milk. However, the global prevalence rate of exclusive breastfeeding has remained low at only 41% [1]. Among the various molecules found in breast milk, lactose (70 g/L) and lipids (40 g/L) are the second and third most prevalent constituents, respectively. Previous studies have reported that human milk oligosaccharides (HMOs) comprise an extensive array of structures [2]. For the past few decades, extensive and continuous studies have been dedicated to exploring the intricacies and nuances of HMOs.

Based on previous studies, two genes, namely  $\alpha$ -1-2-fucosyltransferase (FUT2) and  $\alpha$ -1-3-4-fucosyltransferase (FUT3), play a significant role in determining HMO profiles. The FUT2 gene is responsible for determining secretor positive (Se+) and secretor negative (Se-) statuses, while FUT3 determines the Lewis blood group status (Le+ or Le-) [3]. Furthermore, reports have shown that approximately 20% of the world's population have inactive secretor alleles, which can be attributed to geographic and racial differences [4]. Colostrum has been reported to have a high concentration of HMOs [5], which ranges from 20 to 25 g/L but declines to 5–25 g/L over a six-month lactation period [6]. Although it is estimated that there are more than 200 HMOs, only 100 have been fully characterized to date [7,8]. HMOs can be classified into three categories [9], namely: (1) neutral fucosylated HMOs, such as 2'-fucosyllactose (2'FL) and lacto-N-fucopentaose I (LNFP I), (2) neutral non-fucosylated HMOs, such as lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNnT), and (3) acidic sialylated HMOs, such as 3'-sialyllactose (3'SL) and 6'-sialyllactose (6'SL). Genetic factors have been identified as contributors to interindividual differences in HMO profiles across various populations [10]. Thurl et al. [11] attempted to construct representative HMO profiles by conducting a systematic review and compiling data from 15 countries and regions except Indonesia. Among the eleven Southeast Asian countries, only Singapore, Malaysia, and the Philippines have conducted HMO studies, and none have been reported from the Indonesian population. The Indonesian Health Profile 2021 [12] indicated that only 56.9% of infants under six months were exclusively breastfed. Therefore, this study aims to determine the HMO profiles and the secretor and Lewis gene status of Indonesian lactating mothers.

## MATERIALS AND METHODS

This study used baseline data that were obtained from a prospective longitudinal study report. Furthermore, the study procedures were carried out in primary health centers, as well as women and children hospitals in South Jakarta from August 2021 to May 2022. The participants provided written informed consent to participate in the study after receiving an explanation as well as reading and understanding the purpose of the study. This study was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia

(No. KET-738/UN2.F1/ETIK/PPM.00.02/2020) and was registered at [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) (NCT04515264).

The sample population consisted of 120 mother-infant pairs within the period from the infant's birth to the first month of life were enrolled in the study. This study assessed the HMO profiles and the secretor and Lewis gene status; the relationship between HMO profiles and maternal (methods of delivery, body mass index [BMI], and the number of children) and infant (age, breastfeeding status, and sex) factors were also evaluated. The inclusion criteria were singletons with an APGAR score of  $\geq 7$  in the first minute after birth and 9/10 in the fifth minute, birth at a gestational age of  $>37$  weeks, birth weight  $>2,500$  g, exclusive breastfeeding in the first three days of life [13,14], mothers aged between 21 and 35 years, and mothers willing to breastfeed for a minimum of four months. The exclusion criteria included congenital illness or malformation that could affect growth, a history of type 1 or type 2 diabetes before and after pregnancy, a history of gestational diabetes, preexisting conditions preventing breastfeeding, and smoking. Mature breast milk was obtained from mothers at 14–30 days postpartum. Exclusive breastfeeding required the infant to receive only breast milk (expressed milk or milk from a wet nurse), oral rehydration salts, drops, and syrups (vitamins, minerals, and medications), while predominant breastfeeding required the infant to receive breast milk as the primary source of nutrition [15].

To rule out diurnal effects, milk samples were typically collected between 6:00 and 11:00 a.m. [16], approximately 1–2 hours after the last feeding [17]. The mothers were trained on the collection of breastmilk using their hands or a pump, and they were encouraged to wash their hands before the procedure. Breast skin was cleaned with warm water to reduce bacterial concentration, and the first drops were discarded [18]. Although manual expression was preferred, samples obtained using pumps were also acceptable. Milk that was fully expressed from a single breast, collected, and homogenized, and 20 mL was taken for analysis while the remaining portion was returned to the mother [19]. The breast milk was collected into a 15 mL polypropylene tube, stored at  $-18^{\circ}\text{C}$  in a box on dry ice, and transferred to the laboratory. Furthermore, it was stored at  $-80^{\circ}\text{C}$  in the laboratory before the analysis was performed [20]. 2'FL and LNFP I were measured as representatives for the FUT2-dependent HMOs, 3'SL and 6'SL for the sialylated HMOs, and LNT and LNnT as representatives of the HMO core structures that could be further modified with sialic acid or fucose. Authentic reference standards of 2'FL, LNFP I, LNT, LNnT, 3'SL, and 6'SL were obtained from Biosynth Carbosynth, England. The HMO analysis was performed using the Liquid Chromatography-Mass Spectroscopy (LC-MS/MS) method.

Targeted next-generation sequencing for the FUT2 and FUT3 genes and Sanger sequencing for confirmation were used for genotyping. White blood cells were isolated from peripheral blood collected in containers containing ethylene diamine tetraacetic acid. This study used the DNA Sequencing Library Prep Kit (CUST-SEQ FUT Library Prep), which was a customized kit, to purify blood genomic DNA, target and amplify human FUT2 and FUT3 genes, repair the ends of the amplicons, and ligate the amplicons with dual index adapters. The kit was also employed to purify and enrich the prepared amplicons library to ensure its usability in the Illumina sequencing. Secretor status was determined based on the presence of rs1047781 (A385T). Participants with wild-type AA with the rs1047781 (A385T) variant were categorized as secretor positive, while those with heterozygous mutant AT were classified as weak secretors. Lewis negative status was determined based on the presence of rs28362459 (T59G) heterozygous mutant AC and rs3745635 (G508A) heterozygous mutant CT, while their

absence indicated Lewis positive status. Based on the expression of FUT2 and FUT3, breast milk was classified into four groups, namely: Group 1: secretor and Lewis positive (Se+Le+); Group 2: secretor negative and Lewis positive (Se-Le+); Group 3: secretor positive and Lewis negative (Se+Le-); and Group 4: nonsecretor and Lewis negative (Se-Le-).

Infants were weighed using a calibrated digital baby scale, Seca 334 (Germany), by placing them in the middle of the scale without any clothes on. Furthermore, an average of two readings was taken, and measurements were read to the nearest 0.1 kg. Infant lengths were measured with a measuring rod for the baby scale, Seca 233 (Germany), which was attached to Seca 334 (Germany). An average of three readings was calculated, and measurements were read to the nearest 0.1 cm. The weight and length data obtained were then compared with the WHO growth chart for weight-for-age z score, length-for-age z score, and weight-for-length z score. The mother's weight was also measured with Seca 876 flat scales for mobile use, and the values obtained were recorded in kilograms. The height of the mothers was measured with Seca 217, and they were required to remove their footwear (shoes or sandals) and headgear (hats, caps, or hair bows) during measurement, with the values recorded in centimeters. The BMI was calculated and categorized based on the classification for the Asia-Pacific population [21].

### Statistical analysis

All of the data obtained were edited, encoded, and inputted into the computer, followed by analysis using the IBM SPSS Statistics for Windows, Version 26.0 (IBM Co.). To assess the distribution of each variable, a univariate analysis was conducted. At a significance level of  $p > 0.05$ , the Kolmogorov-Sminov test was used to determine the normality of the data. Continuous data were presented as mean and standard deviation for normally distributed data and as a geometric mean and standard deviation for non-normally distributed data, while categorical data were expressed as n (%). Furthermore, comparison of two groups of data with non-normal distributions were performed using the Mann-Whitney test. The means of three or more non-normally distributed data groups were evaluated using the Kruskal-Wallis test.

## RESULTS

The maternal pre-pregnancy BMI category was overweight (23.54.1) and grade 1 obesity (25.74.5) postpartum. Most of the infants were male (54.2%), with anthropometric assessments within the normal range at birth or at 2–4 weeks of age. The six HMO profiles were shown as medians because the data were not normally distributed. Total HMO concentration was 3,700 (2,097–5,878) mg/L with 2'FL having the highest level followed by LNFP I, 3'SL, 6'SL, LNT, and LNnT, in that order. Based on genotype, all the mothers were secretors, with 58.3% being positive secretors and 41.7% being weak secretors. Furthermore, 85% of the mothers in this study were secretors and Lewis positive (Group 1), and 15% were secretors and Lewis negative (Group 3) (**Table 1**).

**Table 2** shows that only 2'FL significantly differed according to the secretor status ( $p$ -value=0.018). Interestingly, the levels of LNT and 6'SL were slightly higher in weak secretors than in others, although this was not significant. The six HMOs had higher levels in

**Table 1.** Baseline characteristics of the participants (infants aged 2–4 weeks)

Characteristics	Value (n=120)	95% CI
<b>Maternal variables</b>		
Age (y)	29 (25–32)	27.8–29.4
Pre-pregnancy BMI (kg/m <sup>2</sup> )	23.5±4.1	22.7–24.2
Pregnancy weight gain (kg)	13 (10–16)	12.1–13.9
Weight (kg)	63.3 (54.7–71)	60.9–65.3
Postpartum BMI	25.7±4.5	24.9–26.6
Mode of delivery		
Vaginal	87 (72.5)	
Cesarean	33 (27.5)	
Maternal education		
High school or lower	55 (45.8)	
Undergraduate degree	22.7 (41.7)	
Postgraduate degree	15 (12.5)	
Parity		
1	48 (40.0)	
2	39 (32.5)	
3+	33 (27.5)	
Breastfeeding pattern		
Exclusive	108 (90.0)	
Predominant	12 (10.0)	
Secretor status		
Positive	70 (58.3)	
Weak	50 (41.7)	
Lewis status		
Positive	102 (85.0)	
Negative	18 (15.0)	
Secretor – Lewis status		
Secretor+Lewis+ (Group 1)	102 (85.0)	
Secretor+Lewis- (Group 3)	18 (15.0)	
<b>Infant variables</b>		
Sex (male)	65 (54.2)	
Anthropometry at birth		
Weight (g)	3,100 (2,900–3,300)	3,055–3,177
Length (cm)	48 (47.1–49)	48.1–48.7
WAZ at birth	–0.41±0.72	–0.54––0.28
WLZ at birth	0.24±0.94	0.07–0.41
LAZ at birth	–0.62 (–1.15––0.08)	–0.78––0.47
Anthropometry at 2–4 weeks of age		
Weight (g)	3,590±519	3,496–3,683
Length (cm)	52 (51–53.6)	48.3–63.9
WAZ	–0.63±0.83	–0.78––0.48
WLZ	–0.35±0.95	–0.52––0.18
LAZ	–0.78±1.18	–0.99––0.57
<b>HMO profiles</b>		
Total HMOs (mg/L)	3,700 (2,097–5,878)	3,725–6,053
2'FL	2,124 (64.4–3,314)	2,061–3,176
LNFP I	467 (135.5–1,362)	667–2,508
LNT	108 (62–211.5)	130–228
LNnT	68.3 (37.1–114.2)	72.9–147
3'SL	188 (137–248)	174–232
6'SL	186 (137–244)	172–210

Values are presented as median (interquartile range), mean±standard deviation, or number (%).  
CI: confidence interval, BMI: body mass index, WAZ: weight for age Z-score, WLZ: weight for length Z-score, LAZ: length for age Z-score, HMO: human milk oligosaccharides, 2'FL: 2'-fucosyllactose, LNFP I: lacto-N-fucopentaose I, LNT: lacto-N-tetraose, LNnT: lacto-N-neotetraose, 3'SL: 3'-sialyllactose, 6'SL: 6'-sialyllactose.

the Lewis positive group than in the Lewis negative group and in the secretor Lewis positive group (Group 1) than in the secretor Lewis negative group (Group 3).

**Table 2.** HMO profiles by secretor–Lewis status

HMO profiles (mg/L)	Secretor status <sup>†</sup>		Lewis status <sup>†</sup>		Secretor–Lewis status <sup>‡</sup>	
	Secretor positive	Weak secretor	Lewis positive	Lewis negative	Secretor+newis+	Secretor+newis–
2'FL	2,292 (809–4,891)*	1,900 (49–2,670)*	2,150 (64–3,284)	1,969 (65–5,643)	2,150 (64–3,284)	1,969 (85–5,643)
LNFP I	591 (169–1,503)	394 (59–977)	490 (150–1,398)	340 (35–994)	490 (150–1,398)	341 (35–994)
LNT	106 (50–185)	109 (65–215)	111 (65–215)	104 (25–160)	111 (65–215)	104 (25–160)
LNnT	76 (36–119)	65 (38–102)	76 (38–118)	63 (29–87)	76 (38–118)	63 (29–87)
3'SL	188 (132–261)	188 (142–225)	190 (140–249)	177 (58–209)	190 (140–249)	177 (58–209)
6'SL	185 (132–260)	188 (142–223)	188 (139–246)	178 (56–208)	188 (139–246)	178 (56–208)

Values are presented as median (interquartile range).

HMO: human milk oligosaccharides, 2'FL: 2'-fucosyllactose, LNFP I: lacto-N-fucopentaose I, LNT: lacto-N-tetraose, LNnT: lacto-N-neotetraose, 3'SL: 3'-sialyllactose, 6'SL: 6'-sialyllactose.

\*p-value<0.05.

<sup>†</sup>Mann-Whitney test.

<sup>‡</sup>Kruskal-Wallis test.

**Table 3.** HMO profiles by maternal factors

HMO profiles (mg/L)	Method of delivery <sup>*</sup>		Body mass index (kg/m <sup>2</sup> ) <sup>†</sup>			Number of children <sup>‡</sup>		
	Vaginal	Caesarean	<18.5	18.5–22.9	>23	1	2	>3
2'FL	2,017 (61–3,201)	2,437 (122–3,842)	1,934 (998–4,620)	2,094 (339–3,237)	2,150 (61–3,870)	2,409 (364–3,931)	1,666 (49–4,069)	2,150 (59–5,167)
LNFP I	432 (138–1,343)	560 (123–1,398)	135 (87–19,761)	598 (207–1,076)	317 (112–1,407)	467 (136–1,452)	500 (112–1,343)	389 (166–917)
LNT	109 (64–204)	106 (53–239)	41 (27–68)	123 (81–220)	103 (60–204)	115 (57–189)	122 (57–215)	103 (71–238)
LNnT	67 (38–114)	82 (33–116)	28 (20–33)	83 (49–118)	69 (36–109)	78 (37–111)	67 (36–118)	63 (39–126)
3'SL	188 (145–238)	184 (112–259)	128 (70–144)	183 (149–233)	195 (132–261)	188 (158–223)	181 (132–251)	209 (137–261)
6'SL	188 (144–233)	182 (112–256)	128 (70–144)	183 (148–232)	192 (132–249)	186 (154–217)	181 (132–250)	208 (136–260)

Values are presented as median (interquartile range).

HMO: human milk oligosaccharides, 2'FL: 2'-fucosyllactose, LNFP I: lacto-N-fucopentaose I, LNT: lacto-N-tetraose, LNnT: lacto-N-neotetraose, 3'SL: 3'-sialyllactose, 6'SL: 6'-sialyllactose.

\*Mann-Whitney test.

<sup>†</sup>Kruskal-Wallis test.

**Table 4.** HMO profiles by infant factors

HMO profiles (mg/L)	Infants age (d)		Breastfeeding status		Sex	
	14–21	22–31	Exclusive	Partial	Male	Female
2'FL	2,124 (60–3,291)	2,084 (80–5,267)	2,183 (76–3,314)	461 (48–2,088)	1,951 (62–3,969)	2,288 (81–3,274)
LNFP I	599 (157–1,592)	341 (64–815)	440 (136–1,319)	644 (84–1,786)	430 (72–1,039)	500 (173–1,467)
LNT	111 (76–196)	97 (46–224)	106 (58–215)	136 (98–181)	110 (59–175)	104 (65–257)
LNnT	75 (40–115)	64 (30–115)	66 (36–111)	88 (54–123)	65 (36–98)	90 (38–130)
3'SL	192 (158–252)	183 (87–230)	183 (137–237)	247 (143–283)	188 (132–247)	189 (145–251)
6'SL	190 (157–248)	184 (86–230)	183 (137–233)	246 (143–282)	185 (132–244)	188 (144–250)

Values are presented as median (interquartile range).

HMO: human milk oligosaccharides, 2'FL: 2'-fucosyllactose, LNFP I: lacto-N-fucopentaose I, LNT: lacto-N-tetraose, LNnT: lacto-N-neotetraose, 3'SL: 3'-sialyllactose, 6'SL: 6'-sialyllactose.

Mann-Whitney test.

Maternal factors (methods of delivery, BMI, and number of children) did not significantly affect the levels of the six HMOs. **Table 3** shows that normal BMI was associated with higher levels of 2'FL, LNFP I, LNT, and LNnT, while overweight was associated with slightly higher levels of 3'SL and 6'SL. This table also showed that, regarding the number of children, 2'FL and LNnT tended to be higher in those with one child, LNFP I and LNT tended to be higher in those with two children, and 3'SL and 6'SL tended to be higher in those with three children. Data in **Table 4** shows that infant factors (age, sex, and breastfeeding status) did not significantly affect the levels of the six HMOs.

## DISCUSSION

### Genetic profiles

A total of thirty unique single-nucleotide polymorphisms (SNPs) have been identified, leading to diverse allelic variants of the secretor phenotype [22]. Furthermore, non-secretor phenotypes

are caused by mutations in the second exon of the FUT2 gene, with the most common cause being the presence of two alleles, namely,  $se^{428}$  (rs601338G>A) and  $se^{385}$  (rs1047781, A>T; A385T, Ile129Phe). The nonfunctional allele,  $se^{428}$  (rs601338G>A), encodes a stop codon that inactivates the FUT2 enzyme [21], leading to a non-functional protein and causing the European non-secretor phenotype [23]. Based on previous studies,  $se^{385}$  (rs1047781, A>T; A385T, Ile129Phe) is the most frequent cause of the non-secretor phenotype in East Asians [24].

The Lewis-null (le) phenotype may be caused by mutations in the FUT3 gene [25]. The most prevalent gene polymorphisms of FUT3 in Asians includes the following SNPs: rs28362459 (T59G), rs812936 (T202C), rs778986 (C314T), rs3745635 (G508A), and rs3894326 (T1067G) [26]. The substitution of amino acids due to T202C, C314T, G508A, and T1067G mutations inactivate the FUT3 enzyme, while T59G mutation reduces the availability of  $\alpha$ -(1,3,4)-fucosyltransferase [27]. Genuine Lewis-negative individuals are not entirely negative because they still express trace quantities of Lewis epitopes in other tissues.

The FUT2 rs1047781 (A385T) SNP is listed in the dbSNP and has clinical significance because of its effect on secretor/nonsecretor polymorphism with alleles A>C/A>T. Furthermore, FUT2 rs1047781 (A385T) is sometimes classified as secretor negative or a weak secretor and is more commonly found in East and South East Asians [28]. The complete secretor-negative phenotype is uncommon or completely absent in several East Asian populations [29]. Several studies have reported that any variant of rs1047781 (A385T) TT is classified as secretor negative, while other genotypes are classified as secretor. Soejima and Koda [28] stated that rs1047781-385 A>T is designated as a weak secretor. The  $se^{385}$ -encoded enzyme exhibits weak activity compared to the wild-type enzyme (2–5% or 20%), and the SNP causes a sharp decrease in the expression of ABH antigens [2]. The FUT2 385 AT mutation does not result in a secretor-negative allele but leads to the occurrence of an unstable FUT2 enzyme [30].

In our study, most genotypic secretor mothers had 2'FL concentrations ranging from 0.12 to 6.4 g/L during lactation. Meanwhile, genotypic non-secretors consistently generate concentrations less than 0.1 g/L, suggesting that 0.1 g/L of 2'FL can be used as a criterion to distinguish secretor from non-secretor mothers phenotypically [31]. All mothers with concentrations below 0.1 g/L also produce milk with moderate levels of 1,2-fucosylated HMOs, including LNFP I. In studies by Durham et al. [31] and Kunz et al. [32], the genotyping analysis of secretor status using the SNP, rs1047781, and a phenotype threshold of 0.1 g/L of 2'FL as a marker gave similar results. Based on this finding, using rs1047781 as the preferred SNP for genotyping FUT2 to determine the secretor status in the Indonesian population was reasonable.

Wang et al. [33] used 15 mg/L as the indicator of a secretor in one milk sample, while 15 mg/L served as the threshold for a negative status in all samples. Based on this cut-off level, 96.7% of participants in the current study had a positive status. Ma et al. [34] and McJarow et al. [35] employed a higher cut-off level of 50 mg/L for a positive status, and based on this, our results showed that 77.5% of the participants were secretors. Furthermore, Menzel et al. [36] used a threshold level of 53 mg/L 2'FL, leaving the secretor-positive prevalence in the current study at 77.5%.

Secretor status is reported to differ between ethnicities, with 74% of Caucasian mothers having null FUT2 mutations, compared to 60% of Asian mothers [37]. Furthermore, approximately 20% of the world's population inheriting null FUT2 mutations are classified

as secretor-negative [38]. According to another source, higher proportions of secretor-negative phenotypes were found in African (30%) than in Asian (5%) populations [39]. All samples from Mexico and Sweden and 46% from the Philippines were indicative of a positive maternal secretor status, as reported by a comprehensive study [40]. This study was the first publication to determine the secretor and Lewis status in Indonesian lactating mothers with extensive SNPs. The results obtained were also different from those of other reports [37-40], as a 100% positive secretor status was established, with 41.7% being weak secretors.

The Lewis status in this study was based on the FUT3 gene variants listed in the dbSNP and considered to have clinical significance for the Lewis phenotype in the NCBI database. This indicates that the decision was based on the following SNPs: rs28362459 allele AC and rs3745635. The combination of rs28362459 allele AC and rs3745635 allele CT SNPs led to a Lewis positivity prevalence of 85% (102 participants) and negativity prevalence of 15% (18 participants). Approximately 70–90% of populations are Lewis-positive secretors (Le+), while 5–10% are Lewis-negative secretors (Le-) [37]. In several African nations, the Lewis-negative phenotype is substantially more prevalent (20-33%) than in European and certain Asian populations (6–11%) [41].

In this study, Groups 1 and 3 consisted of 102 (85.0%) and 18 (15.0%) mothers, respectively. Human milk and blood samples used to determine the Group 1 phenotype show that between 55% and 73% of Caucasians and Asians have this phenotype. Meanwhile, Groups 2, 3, and 4 have prevalence of 20–31%, 6–11%, and 3–5%, respectively [5,42]. The frequency of the Group 4 (Se–Le–) phenotype is low in Caucasians and Asians [43]. These results were relatively distinct from those obtained in previous reports. Siziba et al. [44] demonstrated that 74%, 18%, 7%, and 1% of human milk samples belonged to milk groups I, II, III, and IV, respectively. A study by Wang et al. [33] among 116 mothers at 1–5 days, 8–14 days, four weeks, and six months showed that 76.7%, 17.2%, 4.3%, and 1.7% of mothers were in Groups 1, 2, 3, and 4, respectively. Kunz et al. [32] were also unable to identify any samples from group 4, as the frequency was 1%. Most of the aforementioned studies determined the mother's SeLe status using 2'FL, LNFP I, LNFP II, LDFT, LNDFH I, LNDFH II, and LNT. The high prevalence of Group 1 in this study was primarily due to the absence of nonsecretors, and the non-appearance of Group 4 was similar to the finding in other studies.

### HMOs profiles

The median 2'FL concentration in the current study was higher than that in secretors with the same age range of 2–4 weeks postpartum in Austin's [19] study; however, it was lower than that in other studies [19,32]. The median concentrations of 2'FL, LNT, and LNnT at baseline were within the normal range compared to the results obtained by Soyylmaz et al. [45], while LNFP I, 3'SL, and 6'SL had lower concentrations. Although HMO profiles are majorly determined by genetics, which do not change throughout an individual's life, the concentrations could vary with time. This is consistent with the protective functions of colostrum and early milk when the neonate is immunologically immature and the gastrointestinal microbiota has not yet been established [16]. The stability or increase in HMO concentration during lactation suggests that they have vital biological functions extending beyond the infant's first few months.

In this study, comparison between secretor statuses based on HMO profiles was made between secretor positives and weak secretors due to the absence of secretor negative individuals. A significant difference was only observed in the 2'FL concentration



( $p$ -value=0.018). Meanwhile, other HMO profiles did not significantly differ with secretor, Lewis, or secretor-Lewis statuses. Further, all the maternal and infant factors were not significantly different between groups. No previous studies have compared of HMO profiles between strong and weak secretors. Most published reports only compared HMO profile between positive and negative secretors. This study did not compare the total HMOs between groups because it only examined secretor-negative patients. Although there was a difference between the secretor positive and weak secretor participants, the result showed that weak secretor fucosyltransferase was active in the mammary gland but expressed a minimal amount of 2'FL [46]. The mutated FUT2 gene may produce a weakly active (1,2) fucosyltransferase enzyme, but its activity is diminished [42]. Further comprehensive studies in a larger area and among various ethnic groups is required to provide more information on HMOs in Indonesian lactating mothers.

In conclusion, all Indonesian lactating mothers in this study were secretors, with 41.7% being weak secretors and 85% having a Lewis-positive status. Furthermore, 2'FL was commonly found in the breast milk of Indonesian lactating mothers and differed significantly based on secretor status.

## REFERENCES

1. UNICEF, World Health Organization/UNICEF. Global Breastfeeding Scorecard, 2017. Tracking progress for breastfeeding policies and programmes [Internet]. 2017. [cited 2023 Jan 24]. Available from: [https://cdn.who.int/media/docs/default-source/breastfeeding/global-breastfeeding-collective/global-breastfeeding-scorecard-2017.pdf?sfvrsn=d5ebb905\\_5&download=true](https://cdn.who.int/media/docs/default-source/breastfeeding/global-breastfeeding-collective/global-breastfeeding-scorecard-2017.pdf?sfvrsn=d5ebb905_5&download=true)
2. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. *Glycobiology* 2012;22:1147-62. [PUBMED](#) | [CROSSREF](#)
3. McGuire MK, Meehan CL, McGuire MA, Williams JE, Foster J, Sellen DW, et al. What's normal? Oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically. *Am J Clin Nutr* 2017;105:1086-100. [PUBMED](#) | [CROSSREF](#)
4. Saboor M, Ullah A, Qamar K, Mir A, Moinuddin . Frequency of ABH secretors and non secretors: A cross sectional study in Karachi. *Pak J Med Sci* 2014;30:189-93. [PUBMED](#) | [CROSSREF](#)
5. Gabrielli O, Zampini L, Galeazzi T, Padella L, Santoro L, Peila C, et al. Preterm milk oligosaccharides during the first month of lactation. *Pediatrics* 2011;128:e1520-31. [PUBMED](#) | [CROSSREF](#)
6. Austin S, De Castro C, Bénet T, Hou Y, Sun H, Thakkar S, et al. Temporal change of the content of 10 oligosaccharides in the milk of Chinese urban mothers. *Nutrients* 2016;8:346. [PUBMED](#) | [CROSSREF](#)
7. Urashima T, Asakuma S, Leo F, Fukuda K, Messer M, Oftedal OT. The predominance of type I oligosaccharides is a feature specific to human breast milk. *Adv Nutr* 2012;3:473S-82S. [PUBMED](#) | [CROSSREF](#)
8. Amano J, Osanai M, Orita T, Sugahara D, Osumi K. Structural determination by negative-ion MALDI-QIT-TOFMSn after pyrene derivatization of variously fucosylated oligosaccharides with branched decaose cores from human milk. *Glycobiology* 2009;19:601-14. [PUBMED](#) | [CROSSREF](#)
9. Bode L. The functional biology of human milk oligosaccharides. *Early Hum Dev* 2015;91:619-22. [PUBMED](#) | [CROSSREF](#)
10. Leo F, Asakuma S, Fukuda K, Senda A, Urashima T. Determination of sialyl and neutral oligosaccharide levels in transition and mature milks of Samoan women, using anthranilic derivatization followed by reverse phase high performance liquid chromatography. *Biosci Biotechnol Biochem* 2010;74:298-303. [PUBMED](#) | [CROSSREF](#)
11. Thurl S, Munzert M, Boehm G, Matthews C, Stahl B. Systematic review of the concentrations of oligosaccharides in human milk. *Nutr Rev* 2017;75:920-33. [PUBMED](#) | [CROSSREF](#)

12. Kemenkes RI. Profil Kesehatan Indonesia 2021 [Internet]. 2021. [cited 2023 Feb 5]. Available from: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/profil-kesehatan-indonesia/Profil-Kesehatan-2021.pdf>
13. Liben ML. Colostrum: the golden milk for Infants' health. *Glob J Intellect Dev Disabil* 2017;1:555566. [CROSSREF](#)
14. Wagner EA, Chantry CJ, Dewey KG, Nommsen-Rivers LA. Breastfeeding concerns at 3 and 7 days postpartum and feeding status at 2 months. *Pediatrics* 2013;132:e865-75. [PUBMED](#) | [CROSSREF](#)
15. WHO. Infant and young child feeding [Internet]. [cited 2019 Nov 2]. Available from: <https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding>
16. Thurl S, Munzert M, Henker J, Boehm G, Müller-Werner B, Jelinek J, et al. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. *Br J Nutr* 2010;104:1261-71. [PUBMED](#) | [CROSSREF](#)
17. Fields DA, Demerath EW. Relationship of insulin, glucose, leptin, IL-6 and TNF- $\alpha$  in human breast milk with infant growth and body composition: analytes in human breast-milk. *Pediatr Obes* 2012;7:304-12. [PUBMED](#) | [CROSSREF](#)
18. Cabrera-Rubio R, Kunz C, Rudloff S, García-Mantrana I, Crehuá-Gaudiza E, Martínez-Costa C, et al. Association of maternal secretor status and human milk oligosaccharides with milk microbiota: an observational pilot study. *J Pediatr Gastroenterol Nutr* 2019;68:256-63. [PUBMED](#) | [CROSSREF](#)
19. Austin S, De Castro CA, Sprenger N, Binia A, Affolter M, Garcia-Rodenas CL, et al. Human milk oligosaccharides in the milk of mothers delivering term versus preterm infants. *Nutrients* 2019;11:1282. [PUBMED](#) | [CROSSREF](#)
20. Azad MB, Robertson B, Atakora F, Becker AB, Subbarao P, Moraes TJ, et al. Human milk oligosaccharide concentrations are associated with multiple fixed and modifiable maternal characteristics, environmental factors, and feeding practices. *J Nutr* 2018;148:1733-42. [PUBMED](#) | [CROSSREF](#)
21. Steering Committee. The Asia-Pacific perspective: Redefining obesity and its treatment. International Diabetes Institute, 2000:11-2.
22. Koda Y, Tachida H, Pang H, Liu Y, Soejima M, Ghaderi AA, et al. Contrasting patterns of polymorphisms at the ABO-secretor gene (FUT2) and plasma alpha(1,3)fucosyltransferase gene (FUT6) in human populations. *Genetics* 2001;158:747-56. [PUBMED](#) | [CROSSREF](#)
23. Kelly RJ, Rouquier S, Giorgi D, Lennon GG, Lowe JB. Sequence and expression of a candidate for the human Secretor blood group alpha(1,2)fucosyltransferase gene (FUT2). Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. *J Biol Chem* 1995;270:4640-9. [PUBMED](#) | [CROSSREF](#)
24. Kudo T, Iwasaki H, Nishihara S, Shinya N, Ando T, Narimatsu I, et al. Molecular genetic analysis of the human Lewis histo-blood group system. II. Secretor gene inactivation by a novel single missense mutation A385T in Japanese nonsecretor individuals. *J Biol Chem* 1996;271:9830-7. [PUBMED](#) | [CROSSREF](#)
25. Corvelo TC, De Loiola Rdo S, Aguiar DC, De Matos Gde C, De Brito DC. The Lewis histo-blood group system: molecular analysis of the 59T>G, 508G>A, and 1067T>A polymorphisms in an Amazonian population. *PLoS ONE* 2013;8:e69908. [PUBMED](#) | [CROSSREF](#)
26. Liu TC, Chang JG, Lin SF, Chang WC, Yang TY, Lin CL, et al. Lewis (FUT3) genotypes in Taiwanese, Thai, and Filipino populations. *Ann Hematol* 2000;79:599-603. [PUBMED](#) | [CROSSREF](#)
27. Koda Y, Soejima M, Liu Y, Kimura H. Molecular basis for secretor type alpha(1,2)-fucosyltransferase gene deficiency in a Japanese population: a fusion gene generated by unequal crossover responsible for the enzyme deficiency. *Am J Hum Genet* 1996;59:343-50. [PUBMED](#)
28. Soejima M, Koda Y. Rapid genotyping of 508G>A (rs3745635) and 1067T>A (rs3894326) of FUT3 by a duplex Eprobe-mediated melting curve analysis. *Vox Sang* 2022;117:741-5. [PUBMED](#) | [CROSSREF](#)
29. Chang JG, Yang TY, Liu TC, Lin TP, Hu CJ, Kao MC, et al. Molecular analysis of secretor type alpha(1,2)-fucosyltransferase gene mutations in the Chinese and Thai populations. *Transfusion* 1999;39:1013-7. [PUBMED](#) | [CROSSREF](#)

30. Henry S, Mollicone R, Fernandez P, Samuelsson B, Oriol R, Larson G. Molecular basis for erythrocyte Le(a+ b+) and salivary ABH partial-secretor phenotypes: expression of a FUT2 secretor allele with an A->T mutation at nucleotide 385 correlates with reduced alpha(1,2) fucosyltransferase activity. *Glycoconj J* 1996;13:985-93.  
[PUBMED](#) | [CROSSREF](#)
31. Durham SD, Robinson RC, Olga L, Ong KK, Chichlowski M, Dunger DB, et al. A one-year study of human milk oligosaccharide profiles in the milk of healthy UK mothers and their relationship to maternal FUT2 genotype. *Glycobiology* 2021;31:1254-67.  
[PUBMED](#) | [CROSSREF](#)
32. Kunz C, Meyer C, Collado MC, Geiger L, García-Mantrana I, Bertua-Ríos B, et al. Influence of gestational age, secretor, and lewis blood group status on the oligosaccharide content of human milk. *J Pediatr Gastroenterol Nutr* 2017;64:789-98.  
[PUBMED](#) | [CROSSREF](#)
33. Wang M, Zhao Z, Zhao A, Zhang J, Wu W, Ren Z, et al. Neutral human milk oligosaccharides are associated with multiple fixed and modifiable maternal and infant characteristics. *Nutrients* 2020;12:826.  
[PUBMED](#) | [CROSSREF](#)
34. Ma L, McJarrow P, Jan Mohamed HJB, Liu X, Welman A, Fong BY. Lactational changes in the human milk oligosaccharide concentration in Chinese and Malaysian mothers' milk. *Int Dairy J* 2018;87:1-10.  
[CROSSREF](#)
35. McJarrow P, Radwan H, Ma L, MacGibbon AKH, Hashim M, Hasan H, et al. Human milk oligosaccharide, phospholipid, and ganglioside concentrations in breast milk from United Arab Emirates mothers: results from the MISC cohort. *Nutrients* 2019;11:2400.  
[PUBMED](#) | [CROSSREF](#)
36. Menzel P, Vogel M, Austin S, Sprenger N, Grafe N, Hilbert C, et al. Concentrations of oligosaccharides in human milk and child growth. *BMC Pediatr* 2021;21:481.  
[PUBMED](#) | [CROSSREF](#)
37. Rudloff S, Pohlentz G, Borsch C, Lentze MJ, Kunz C. Urinary excretion of in vivo <sup>13</sup>C-labelled milk oligosaccharides in breastfed infants. *Br J Nutr* 2012;107:957-63.  
[PUBMED](#) | [CROSSREF](#)
38. Bergström A, Skov TH, Bahl MI, Roager HM, Christensen LB, Ejlerskov KT, et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. *Appl Environ Microbiol* 2014;80:2889-900.  
[PUBMED](#) | [CROSSREF](#)
39. Parker EP, Ramani S, Lopman BA, Church JA, Iturriza-Gómara M, Prendergast AJ, et al. Causes of impaired oral vaccine efficacy in developing countries. *Future Microbiol* 2018;13:97-118.  
[PUBMED](#) | [CROSSREF](#)
40. Erney RM, Malone WT, Skelding MB, Marcon AA, Kleman-Leyer KM, O'Ryan ML, et al. Variability of human milk neutral oligosaccharides in a diverse population. *J Pediatr Gastroenterol Nutr* 2000;30:181-92.  
[PUBMED](#) | [CROSSREF](#)
41. Selma-Royo M, González S, Gueimonde M, Chang M, Furst A, Martínez-Costa C, et al. Maternal diet is associated with human milk oligosaccharide profile. *Mol Nutr Food Res* 2022;66:e2200058.  
[PUBMED](#) | [CROSSREF](#)
42. Guo M, Luo G, Lu R, Shi W, Cheng H, Lu Y, et al. Distribution of *Lewis* and *Secretor* polymorphisms and corresponding CA19-9 antigen expression in a Chinese population. *FEBS Open Bio* 2017;7:1660-71.  
[PUBMED](#) | [CROSSREF](#)
43. Elwakiel M, Hageman JA, Wang W, Szeto IM, Van Goudoever JB, Hettinga KA, et al. Human milk oligosaccharides in colostrum and mature milk of Chinese mothers: lewis positive secretor subgroups. *J Agric Food Chem* 2018;66:7036-43.  
[PUBMED](#) | [CROSSREF](#)
44. Siziba LP, Mank M, Stahl B, Gonsalves J, Blijenberg B, Rothenbacher D, et al. Human milk oligosaccharide profiles over 12 months of lactation: the Ulm SPATZ health study. *Nutrients* 2021;13:1973.  
[PUBMED](#) | [CROSSREF](#)
45. Soyylmaz B, Mikš MH, Röhrig CH, Matwiejuk M, Meszaros-Matwiejuk A, Vignsæs LK. The mean of milk: a review of human milk oligosaccharide concentrations throughout lactation. *Nutrients* 2021;13:2737.  
[PUBMED](#) | [CROSSREF](#)
46. Lefebvre G, Shevlyakova M, Charpagne A, Marquis J, Vogel M, Kirsten T, et al. Time of lactation and maternal fucosyltransferase genetic polymorphisms determine the variability in human milk oligosaccharides. *Front Nutr* 2020;7:574459.  
[PUBMED](#) | [CROSSREF](#)