*Malays J Pathol 2024; 46(2) : 247 – 257*

# ORIGINAL ARTICLE

## Congenital adrenal hyperplasia testing in the Malaysian population: real-world data sourced from a national reference laboratory

**Raja Hasyidah RAJA BONGSU[1]*, Karniza KHALID[2], Wan Ahmad Dzulkarnain RAZALI[1], Nadirah ZAINAL ABIDIN[1], Noor Aleesya Imani SAIFUL NIZAM[1], Noor Aishah RAHIDIN[1], Saraswathy APPAROW[1], Anasufiza HABIB[3]**

*[1]Endocrine Unit; [2]Special Protein Unit; [3]Biochemistry and Genomics Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, Malaysia.*

*Abstract*

Congenital Adrenal Hyperplasia (CAH) is primarily caused by mutations in genes responsible to produce enzymes involved in the synthesis of cortisol, aldosterone, or both. This study aims to determine the prevalence, sociodemographic distributions, and clinical factors associated with CAH in the Malaysian population. This retrospective study reviewed laboratory records of 17-hydroxyprogesterone (17OHP) test requests received at the Institute for Medical Research, Kuala Lumpur from January 2021 to December 2021. Descriptive statistics were employed for most variables, and logistic regression analysis was conducted to determine factors associated with CAH. The dataset included a total of 775 patients (64.2%) from 1,207 test requests screened. The prevalence of newly diagnosed CAH in the year 2021 was 13.5% (n=105). The majority were Malays (15.1%), neonates (13.8%), and females (45.7%). Higher baseline 17-OHP (cOR:1.31, 95% CI:1.19, 1.45), unknown gender at birth (cOR:7.82, 95% CI:2.86, 21.37), and neonatal age group at presentation (cOR:29.3, 95% CI:12.07, 71.03) independently predict CAH. The high prevalence of CAH in our region has been speculated to be due to the cultural consanguinity norms, resulting in genetic aberrations. CAH may manifest as ambiguous genitalia, particularly in females, due to the overproduction of androgens in-utero, resulting in atypical genitalia, necessitating thorough investigation. To the best of our knowledge, the data presented are the latest report on CAH prevalence, distribution, and description of positive CAH cases in the Malaysian population. These findings are essential for further public health planning to improve the diagnostic capacity and clinical management of CAH.

*Keywords:* congenital adrenal hyperplasia, 17-hydroxyprogesterone, Malaysia, prevalence, newborn screening.

### INTRODUCTION

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder characterised by an impairment of the biosynthesis of cortisol, aldosterone, or both, as a result of the deficiency of adrenal cortex enzymes. Deficiency of 21-hydroxylase (21-OHD) resulting from CYP21A2 gene mutations or deletions is the most common form, accounting for 95% of all cases.[1] The hallmark of CAH is excessive production of 17-hydroxyprogesterone (17OHP) and its diversion to adrenal androgen synthesis, due to the defect or no conversion of 17OHP to 11-deoxycortisol and of progesterone to deoxycorticosterone.[2] Only about 5% of CAH cases are due to non-21 hydroxylase or other enzymes affecting cortisol synthesis.[3] The varying degree of enzyme deficiency has a direct impact on the clinical presentation.[4]

Salt wasting and virilization form (Classic CAH) that manifested at birth and/or in the neonatal period is the most severe clinical presentation of CAH. A total loss of enzymatic activity results in extremely low levels of cortisol and aldosterone and high amounts of androgen, the latter causing prenatal virilization of females.[5] Delayed treatment of affected babies can lead to profound consequences, including death within a few weeks after birth due to salt-wasting crisis. Non-classic CAH is a less severe form of the

**Address for correspondence:* Raja Hasyidah Raja Bongsu, MSc. Endocrine Unit, Biochemistry and Genomics Research Centre, Institute for Medical Research, National Institutes of Health, Jalan Pahang, 50588 W.P. Kuala Lumpur, Malaysia. Email: *rajahasyidah@moh.gov.my*

illness that manifests later in childhood. CAH is curable with early diagnosis and treatment.

An integrated approach consisting of both newborn screening and treatment with hormonal replacement therapy is required for the management of CAH. Despite not being available in Malaysia, screening for CAH has been incorporated as part of an extensive neonatal screening programme worldwide and has been shown to be successful in lowering the incidence of fatal salt-wasting crises and preventing delayed or misdiagnosis.[5] Dried blood spots are used in newborn screening to measure the levels of 17OHP, which is the substrate of the 21-hydroxylase enzyme. The definitive CAH diagnosis is further made based on the serum 17OHP level.[4]

The comprehensive and discrete incidence or prevalence data are essential to recognise the populations at risk and improve early detection and management of CAH in Malaysia. According to Navarro-Zambrana *et al*. (2022), countries from the Eastern Mediterranean and Southeast Asia exhibited the highest CAH incidence.[6] However, the relationship between demographic distribution and CAH has yet to be clearly demonstrated within the Malaysian population. Hence, this study aims to determine the incidence, sociodemographic distributions, and clinical factors associated with CAH in the Malaysian population.

## MATERIALS AND METHODS

*Study design and population*

This is a retrospective study reviewing laboratory records of 17OHP test requests from January 2021 to December 2021, received at the Endocrine Unit, Biochemistry and Genomic Research Centre (BGRC), Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. The data were collected from the IMR laboratory information system and the hardcopy test request forms. Data analysed from nationwide requests for 17OHP testing within the study period were included whilst rejected samples (due to technical unsuitability for analysis) and repetitive testing from the same patient were excluded.

*Assay*

Two types of requests were received for 17OHP measurement - from symptomatic patients to rule out CAH and known CAH patients for treatment monitoring. The serum 17-OHP concentrations were measured by a single measurement for basal or monitoring of treatment and/or measurement of 17OHP for Cosyntropin (CST) test at 0, 30 and 60 minutes (the current gold standard for CAH confirmation testing in Malaysia besides molecular testing).

The level of 17OHP was quantitated by solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle using the IBL International GMBH kit for in-vitro determination in serum. The method is calibrated using six standards with values ranging from <1 nmol/L to 60.6 nmol/L, with some allowable variation for each lot. An unknown amount of antigen presents in the sample and a fixed amount of enzyme-labelled antigen competes for the binding sites of the antibodies coated onto the 96-well plate. The competition reactions in the wells were stopped by washing the wells after incubation. After the substrate reaction, the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. The results of samples can be determined directly using the standard curve. There was no modification or changes made to the assay procedure during the study period, and no assay drift was reported. The assay was enrolled in the UK External Quality Assurance Scheme, and reports were satisfactory throughout this period.

For this study, the 17OHP results from the basal and CST testing were interpreted using a diagnostic strategy as outlined by Speiser *et al*. (2018) (Figure 1).[7]

*Ethical clearance*

The study protocol was registered with the National Medical Research Register (NMRR-ID-23-03093-SBW(IIR)) and approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (Ref. 23-03093-SBW[1]).

*Statistical analyses*

Sample size estimation was calculated using the population proportion formula. Preliminary data indicated that the prevalence of 21-hydroxylase deficiency (21-OHD) in Malaysia which accounts for the most common cause of CAH was 0.08.[8] Therefore, a minimum sample size of 114 is required for a type I error probability and precision set at 0.05 and 0.05, respectively. Universal sampling was employed for this study. Data analysis was performed using IBM SPSS Statistics for Windows (Version 28.0). Descriptive statistics were employed for all variables. Factors associated with CAH were determined using simple logistic regression.

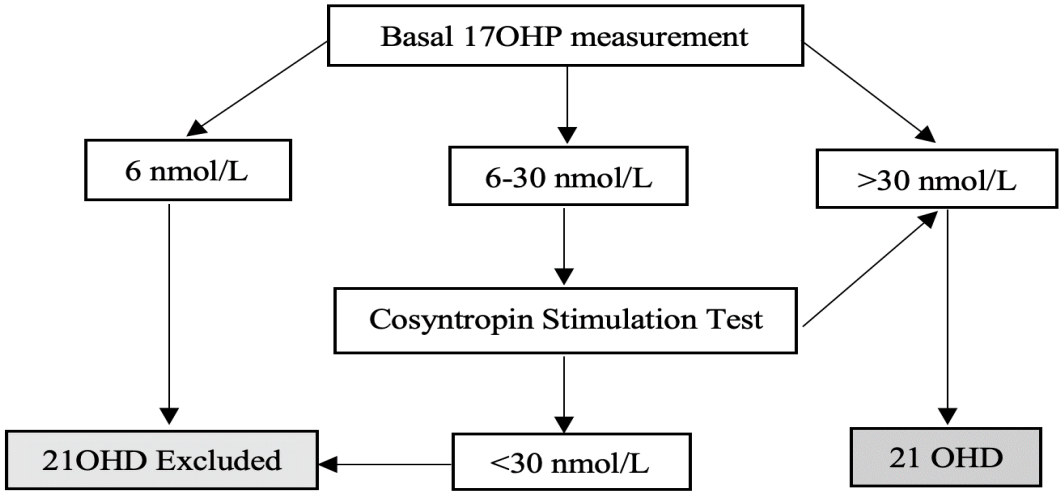


FIG. 1: Interpretation of 17-OHP results.

## RESULTS

*Dataset*

The final dataset for analysis included a total of 775 patients (64.2%) from 1,207 test requests that were screened (Figure 2).

*Demography*

The overall demographic characteristics of patients subjected to 17OHP testing are shown in Table 1. In summary, the majority of the requests (39.1%) were from patients aged less than 12 months, half of the requests were among female patients (54.5%), while 68 (8.8%) were from unknown gender. Most of the patients were Malay, 521 (67.2%) followed by Chinese, 87 (11.2%), Indian 58 (7.5%) and others (4.5%). The majority were also known CAH patients, 278 (35.9%). The interpretation of 17OHP

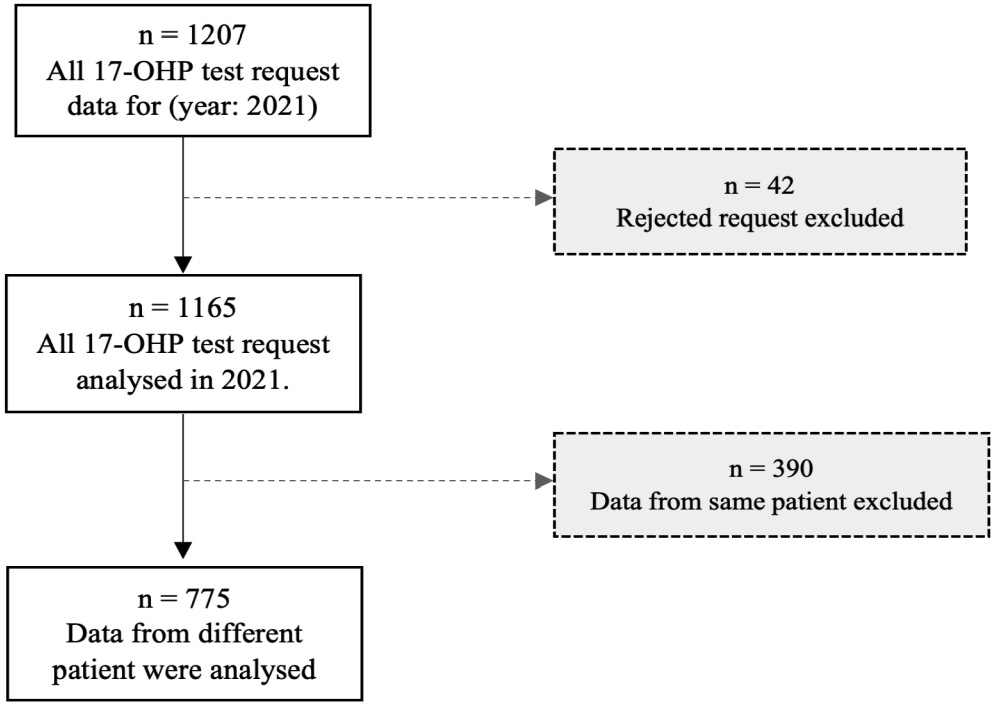


FIG. 2: Identification of the study population.

**TABLE 1: Demographic and clinical characteristics of patients tested for 17OHP (*N* = 775)**

| Demographic and 17-OHP result interpretation | | (*N* = 775) | |
|---|---|---|---|
| | | *n* | % |
| **Age group** | Newborn (0 - 30 days) | 188 | 24.3 |
| | Infancy (1-12 months) | 115 | 14.8 |
| | Childhood (1-12 years) | 229 | 29.5 |
| | Adolescence (13-18 years) | 74 | 9.5 |
| | Adulthood (≥ 19 years) | 169 | 21.8 |
| **Gender** | Female | 422 | 54.5 |
| | Male | 285 | 36.8 |
| | Unknown | 68 | 8.8 |
| **Ethnicity** | Malay | 521 | 67.2 |
| | Chinese | 87 | 11.2 |
| | Indian | 58 | 7.5 |
| | Bumiputera Sabah | 38 | 4.9 |
| | Bumiputera Sarawak | 30 | 3.9 |
| | Orang Asli | 6 | 0.8 |
| | Others | 35 | 4.5 |
| **Malaysia Region** | Central Region (Selangor, W.P. Kuala Lumpur & W. P Putrajaya) | 242 | 31.2 |
| | South Region (Johor, Melaka & Negeri Sembilan) | 167 | 21.5 |
| | North Region (Kedah, Perlis, Perak, Pulau Pinang) | 134 | 17.3 |
| | East Coast Region (Kelantan, Terengganu & Pahang) | 123 | 15.9 |
| | East Region (Sabah & Sarawak) | 109 | 14.1 |
| **17OHP results interpretation** | CAH on treatment | 278 | 35.9 |
| | Suggestive 21OHD | 105 | 13.5 |
| | Necessitates CST for confirmation | 154 | 19.9 |
| | 21OHD Excluded | 238 | 30.7 |

Abbreviation: 17-OHP; 17 Hydroxyprogesterone, 21OHD; 21 Hydroxylase Deficiency, CAH; Congenital adrenal hyperplasia, CST; Cosyntropin.

results using the algorithm by Speiser *et al.* (2018) showed that 105 (13.5%) patients were suggestive of 21-OHD.[7]

*17OHP level*

The distribution of 17OHP levels based on different clinical categories is depicted in Figure 3. Among the patients with basal 17OHP of more than 30 nmol/L, their mean 17OHP value was slightly higher than the mean value of patients with known CAH. Additionally, the mean for newly diagnosed 21OHD was 157.09

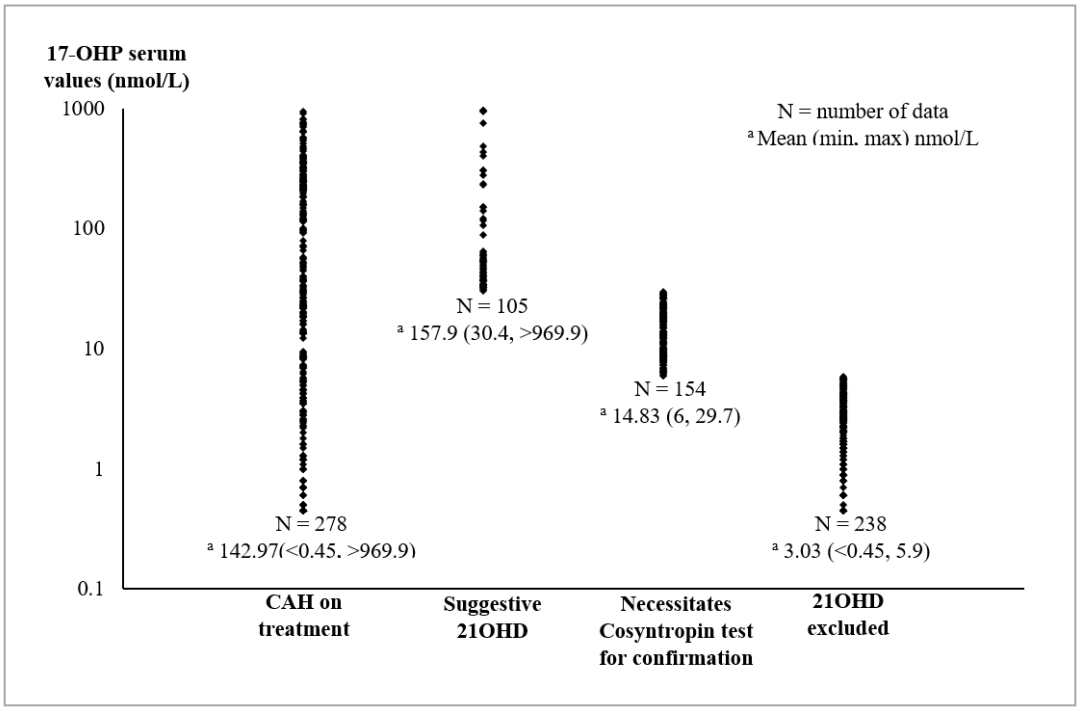


FIG. 3: Distribution of 17OHP level according to result interpretation groups.

nmol/L (Range: 30.4 to >969.9 nmol/L). Whereas the mean value for known CAH patients on treatment was 142.97 nmol/L (Range: <0.45 to >969.6 nmol/L).

*Age, gender and ethnicity*

The total number of patients with confirmed and suspected 21OHD were 537 (69.3%) (Table 2). Among the 278/537 (51.8%) cases of confirmed CAH, the highest percentage were those in groups aged between 1-12 years (147/278, 52.9%), and were equally distributed between males and females. However, for those with 17OHP levels of more than 30 nmol/L (suggestive of 21OHD), most of them were in 0-30 days old (74, 13.8%). Equally in the group of 17OHP levels of between 6-30 nmol/L (and required CST test to exclude 21OHD), the highest were those in the neonatal period (95, 17.7%) as well.

Table 3a further described known CAH patients aged less than a year while Table 3b described cases with very high levels of 17OHP (>300 nmol/L), suggestive of classical CAH (newly diagnosed). Most of the patients were less than 7 years old.

*Cosyntropin (CST) test for confirmation*

Further confirmatory testing for CAH following basal testing of 17OHP with the value of between 6-30 nmol/L is made by Cosyntropin (CST) test. Any results of more than 30 nmol/L with suggestive clinical features support the diagnosis of CAH. Figure 4 shows the results of 17OHP after ACTH stimulation at 60 minutes, where up to 50% tested positive for CAH.

*Factors associated with CAH*

A sub-analysis in a pool of subjects was also performed using logistic regression analysis to determine the factors associated with CAH (with basal 17OHP or CST test >30 nmol/L). Higher baseline 17OHP, unknown gender at birth, and neonatal age group at presentation independently predict CAH (Table 4).

## DISCUSSION

The prevalence of CAH among samples sent for testing at our centre in the year 2021 was 13.5% (n=105). However, when samples that were sent for monitoring purposes among known CAH were excluded, the incidence of newly diagnosed CAH rose to 21.1% in our one-year study period. Our findings are in agreement with a recent meta-analysis of 58 studies that concluded countries originating from the Eastern Mediterranean and Southeast Asia had the highest CAH incidence worldwide.[8] However, the incidence of CAH

**TABLE 2: Distribution of age, gender, and ethnicity among CAH-related cases**

| Variable(s) | CAH on treatment | | Suggestive 21-OHD | | CST for confirmation | | Total |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | |
| **Newborn (0 - 30 days)** | 1 | 0.2 | 74 | 13.8 | 95 | 17.7 | **170** |
| Female | 0 | 0.0 | 25 | 4.7 | 37 | 6.9 | **62** |
| Male | 1 | 0.2 | 25 | 4.7 | 33 | 6.1 | **59** |
| Unknown | 0 | 0.0 | 24 | 4.5 | 25 | 4.7 | **49** |
| **Infancy (1-12 months)** | 20 | 3.7 | 12 | 2.2 | 24 | 4.5 | **56** |
| Female | 11 | 2.0 | 8 | 1.5 | 9 | 1.7 | **28** |
| Male | 9 | 1.7 | 3 | 0.6 | 11 | 2.0 | **23** |
| Unknown | 0 | 0.0 | 1 | 0.2 | 4 | 0.7 | **5** |
| **Childhood (1-12 years)** | 147 | 27.4 | 8 | 1.5 | 7 | 1.3 | **162** |
| Female | 72 | 13.4 | 5 | 0.9 | 5 | 0.9 | **82** |
| Male | 75 | 14.0 | 3 | 0.6 | 2 | 0.4 | **80** |
| Unknown | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | **0** |
| **Adolescence (13-18 years)** | 44 | 8.2 | 1 | 0.2 | 3 | 0.6 | **48** |
| Female | 23 | 4.3 | 1 | 0.2 | 2 | 0.4 | **26** |
| Male | 19 | 3.5 | 0 | 0.0 | 1 | 0.2 | **20** |
| Unknown | 2 | 0.4 | 0 | 0.0 | 0 | 0.0 | **2** |
| **Adulthood (≥ 19 years)** | 66 | 12.3 | 10 | 1.9 | 25 | 4.7 | **101** |
| Female | 47 | 8.8 | 9 | 1.7 | 20 | 3.7 | **76** |
| Male | 18 | 3.4 | 1 | 0.2 | 5 | 0.9 | **24** |
| Unknown | 1 | 0.2 | 0 | 0.0 | 0 | 0.0 | **1** |
| **Ethnicity** | | | | | | | |
| Malay | 161 | 30.0 | 81 | 15.1 | 122 | 22.7 | **364** |
| Chinese | 48 | 8.9 | 4 | 0.7 | 10 | 1.9 | **62** |
| Indian | 24 | 4.5 | 7 | 1.3 | 3 | 0.6 | **34** |
| Bumiputera Sabah | 18 | 3.4 | 4 | 0.7 | 2 | 0.4 | **24** |
| Bumiputera Sarawak | 19 | 3.5 | 3 | 0.6 | 3 | 0.6 | **25** |
| Orang Asli | 2 | 0.4 | 0 | 0.0 | 2 | 0.4 | **4** |
| Others | 6 | 1.1 | 6 | 1.1 | 12 | 2.2 | **24** |

Abbreviation: CAH; Congenital Adrenal Hyperplasia, 21OHD; 21-Hydroxylase Deficiency, CST; Cosyntropin test

in Asia Pacific populations varied between countries and populations; 1:594 (Indonesia),[9] 1:22,321 (Singapore),[10] 1:5,771 (Thailand),[11] 1:19,859 (Japan).[12] The last published case survey in Malaysia reported an overall incidence of CAH of 1:3,000.[13] The relatively higher prevalence of CAH in the region has been speculated to be due to the cultural consanguinity norms, resulting in less genetic diversity, hence genetic aberrations.[6,8]

The majority of the subjects tested were females. Similarly, female preponderance of CAH was also identified in a nationwide population-based study in Denmark with a

**TABLE 3a: Known CAH patients on treatment, aged <12 months (Classical CAH)**

| No | Gender | Race | Age | Clinical characteristics | Type of CAH |
|---|---|---|---|---|---|
| 1 | Male | Not Mentioned | 26d | Poor weight gain, polyuria | CAH |
| 2 | Female | Malay | 2m 28d | Ambiguous genitalia | SW CAH |
| 3 | Male | Malay | 3m 0d | Hyponatremia | CAH |
| 4 | Male | Malay | 3m | Salt Wasting Crisis | CAH |
| 5 | Female | Malay | 3m 15d | Anaemia | CAH |
| 6 | Female | Malay | 3m 18d | Salt Wasting Crisis | Classic CAH |
| 7 | Male | Malay | 5m 11d | Hyponatremia-hyperkalemia | CAH |
| 8 | Female | Malay | 5m 20d | Simple virilising | SV CAH |
| 9 | Female | Malay | 6m 28d | Salt losing | SW CAH |
| 10 | Female | Bumiputera Sarawak | 7m 9d | Simple virilising | SW CAH |
| 11 | Male | Indian | 7m 23d | NA | CAH |
| 12 | Female | Chinese | 8m 19d | Ambiguous genitalia | SV CAH |
| 13 | Female | Malay | 9m 25d | Ambiguous genitalia | CAH |
| 14 | Female | Chinese | 10m 15d | NA | CAH |
| 15 | Male | Malay | 10m 20d | Salt Wasting Crisis | SW CAH |
| 16 | Male | Malay | 11m 18d | NA | CAH |
| 17 | Female | Bumiputera Sabah | 11m 21d | Ambiguous genitalia | SV CAH |

Abbreviation: d; day/s, m; month/s, NA; not available, SW; salt wasting, SV; simple virilizing

**Table 3b: Patients with 17OHP>300 nmol/L for single/basal measurement/ Cosyntropin test at 60 minutes (Suggestive of newly diagnosed Classical CAH)**

| No | Gender | Race | Age | 17-OHP level (nmol/L) | Clinical characteristics |
|---|---|---|---|---|---|
| 1 | Unknown | Malay | 1d | >969.6 | Ambiguous genitalia |
| 2 | Male | Malay | 2d | 308.8 | Sibling with CAH |
| 3 | Unknown | Malay | 5d | 312.7 | Ambiguous genitalia |
| 4 | Unknown | Malay | 5d | 463 | Ambiguous genitalia |
| 5 | Male | Bumiputera Sabah | 12d | 940.5 | Ambiguous genitalia, Salt losing |
| 6 | Male | Indian | 16d | >969.6 | Weight loss, Hyper-kalaemia, vomiting |
| 7 | Unknown | Malay | 24d | >969.6 | Salt Wasting Crisis |
| 8 | Male | Malay | 1m 13d | 407.4 | Sibling with CAH |
| 9 | Female | Not Mentioned | 2m | >969.6 | Ambiguous genitalia |
| 10 | Female | Not Mentioned | 2m | >969.9 | Ambiguous genitalia |
| 11 | Female | Bumiputera Sabah | 11m | >969.9 | Hyponatremia-hyper-kalemia |
| 12 | Female | Malay | 3y 7m 22d | 480.8 | Salt Wasting crisis |
| 13 | Female | Malay | 3y 7m 22d | 764.8 | Salt losing |
| 14 | Female | Malay | 3y 9m 17d | 303.7 | Ambiguous genitalia |
| 15 | Male | Malay | 4y 5m 18d | 306.1 | Salt wasting crisis |
| 16 | Female | Malay | 6y | 751.2 | Clitoromegaly |
| 17 | Female | Malay | 24y 7m 29d | 435.1 | Hirsutism, primary amenorrhea |

Abbreviation: d; day/s, m; month/s, y; year/s

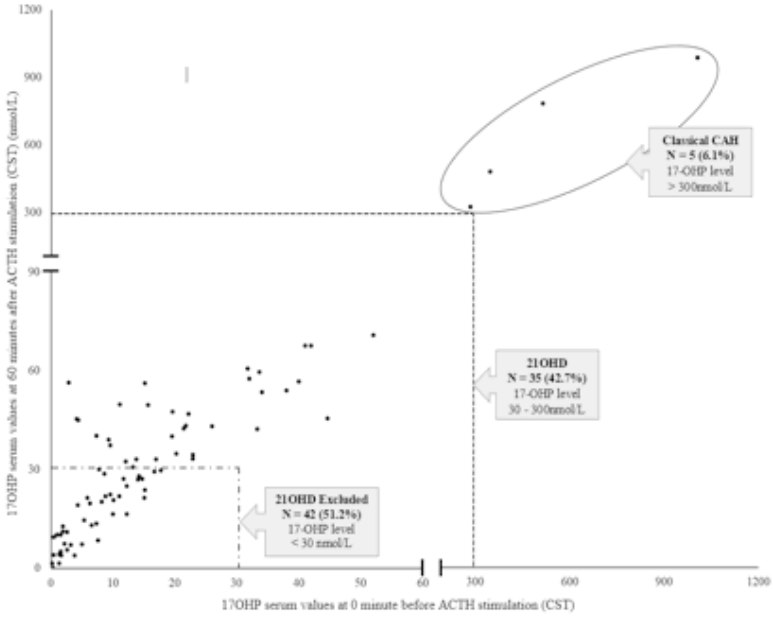


FIG 4: 17OHP nomogram for the diagnosis of steroid 21-hydroxylase deficiency (21OHD) (at 60-minute CST stimulation test). The data points represent 82 samples sent from CST testing during the study period.

**Table 4: Factors associated with CAH**

| Variable(s) | CAH *n* (%) | Non-CAH *n* (%) | Crude OR (95% CI) | p-value |
|---|---|---|---|---|
| 17-OHP (nmol/L)[a] | 50.3 (74.4) | 2.7 (2.20) | 1.31 (1.19, 1.45) | <0.001* |
| Gender | | | | <0.001* |
| Male | 32 (28.8) | 79 (71.2) | 1.00 (Ref.) | |
| Female | 48 (24.5) | 148 (75.5) | 0.80 (0.47, 1.35) | |
| Unknown | 19 (76.0) | 6 (24.0) | 7.82 (2.86, 21.37) | |
| Age group | | | | <0.001* |
| Neonate | 62 (69.5) | 16 (20.5) | 29.3 (12.07, 71.03) | |
| Infancy | 15 (20.8) | 57 (79.2) | 1.99 (0.81, 4.88) | |
| Children | 10 (12.0) | 73 (88.0) | 1.04 (0.40, 2.70) | |
| Adolescence | 0 (0.0) | 19 (100.0) | 0.00 (0.00, 0.00)[b] | |
| Adults | 9 (11.7) | 68 (88.3) | 1.00 (Ref.) | |
| Prematurity | | | | 0.758 |
| Yes | 16 (53.3) | 14 (46.7) | 1.00 (Ref.) | |
| No | 20 (57.1) | 15 (42.9) | 1.17 (0.44, 3.11) | |
| Sibling with CAH | | | | 0.361 |
| Yes | 4 (80.0) | 1 (20.0) | 3.0 (0.29, 31.63) | |
| No | 12 (57.1) | 9 (42.9) | 1.00 (Ref.) | |
| Symptoms | | | | 0.083 |
| Ambiguous genitalia | 47 (57.3) | 35 (42.7) | 2.32 (0.98, 5.49) | |
| Amenorrhea | 0 (0.0) | 14 (100.0) | 0.0 (0.0, 0.0) | |
| Hirsutism | 1 (6.7) | 14 (93.3) | 0.12 (0.01, 1.07) | |
| Hyperpigmentation | 3 (33.3) | 6 (66.7) | 0.86 (0.18, 4.16) | |
| Infertility | 0 (0.0) | 6 (100.0) | 0.00 (0.00, 0.00)[b] | |
| Precocious puberty | 0 (0.0) | 19 (100.0) | 0.00 (0.00, 0.00)[b] | |
| Salt-wasting crisis | 11 (36.7) | 19 (63.3) | 1.00 (Ref.) | |

Note: [a]Described in median (IQR), [b]Values considerably less than 0.001. * Statistically significant. OR; odd ratio

combined prevalence of 15.1 and 9.0 per 100,000 newborn females and males with CAH, respectively.[5] However, CAH is not inherently more common among females; rather, it is an autosomal recessive disorder, meaning that it can affect both males and females equally.[14,15] Female newborns with classical CAH are virilised at birth, presenting with slight clitoromegaly to complete masculinisation, hence would naturally prompt investigation. In contrast, male infants may present with no apparent physical signs at birth, hence are particularly at risk for dehydration and death from salt-wasting crisis. This is further supported by prior data that suggested potential missed cases of CAH male babies before the introduction of universal screening.[16] For example, about half of the patients with CAH were missed before mass population newborn screening was introduced in Japan and most of them were male and had the salt-wasting form of CAH at presentation.[17] Additionally, 8.8% of subjects tested were from an unknown gender. Most were from the neonatal age group (24.3%) and children (1-12 years old) (29.5%). Earlier and correct gender assignments are important in patients with CAH for the clinical management of hormonal balance and control, determining gender identity, sexual behaviour, and aiding in discernment of familial, social, and religious beliefs.[18]

The distribution of CAH cases among the Malaysian ethnicities in our study aligned with two previous studies reported by Wu *et al*. in 1994 whereby Malay, Chinese and Indian, comprising 57%, 16%, and 13% of all cases, respectively, and by Shubashini *et al*. in 2015 with Malay patients at 58.8%, Chinese at 29.4% and Indian at 7.8%.[13,19] We were not able to further divide the geographic distribution of CAH cases according to the various states in Malaysia as the samples were mostly referred to and sent from tertiary or quaternary health centres with specialised endocrine paediatrics services.

We also found that a higher baseline 17-OHP, unknown gender at birth, and neonatal age group at presentation independently predicts CAH. Each 1 nmol/L increment in basal 17-OHP increases the odds of CAH by 1.31 times. Similarly, an unknown gender at presentation increases the odds of CAH by 7.82 times. CAH can cause ambiguous genitalia, particularly in females, due to the overproduction of androgens in utero. In females with CAH, the high levels of androgens may cause the clitoris to become enlarged and resemble a small penis. Additionally, the labia may fuse, resembling a scrotum. These changes in genital development can result in ambiguous or atypical genitalia that may not clearly align with a typical male or female anatomy (1,20–22). We also found that neonates carried 29.3 times higher odds of CAH, as compared to infants (cOR: 1.99) and children (cOR: 1.04).

From this study, most of the CAH cases were diagnosed at an early age. This is crucial as salt-wasting crises that are associated with the severe form of CAH may lead to death and are associated with learning disabilities and behavioural problems.[3] Therefore, universal newborn screening for CAH may help diagnose potential missed cases early on and may reduce overall infant mortality, as reported by Suwa (1994).[17] A late CAH diagnosis leads to high mortality and morbidity rates, notably increasing public health costs, and may result in physical and psychological damage that is not easily measurable.[23] For example, Miranda *et al*. (2021) performed a study to determine the cost-effectiveness between screened and unscreened CAH cases in newborns which showed that the mortality rate from CAH among unscreened infants was at 11%, while zero death was reported among the screened cohort. Therefore, CAH-newborn screening (CAH-NBS) is important in preventing CAH mortality and morbidity, hence reducing overall costs associated with adverse outcomes, prolonged hospitalisation and critical care treatment.[24]

Early detection is also important in the context of gender assignment since most of the CAH cases presented with ambiguous genitalia. From our study, 68/775 requests had unknown gender, hence constituted the disorders of sex development (DSD) that are undoubtedly challenging to all involved parties.[25] There were also a significant number of adolescents (48/537) and adult cases (101/537) with CAH in our study. CAH participants have an increasing rate of co-morbidities with increasing age, rising rates of requiring other medications and reduced rates of perceived self-compliance to their daily medications.[26] Apart from the pathological issues, there are also psychosocial issues that may be difficult to handle as a result of abnormal steroid hormone production. A local study performed in two paediatric endocrine clinics in Malaysia also found that CAH patients' attitudes and compliance towards related treatment have shifted over time making it challenging to achieve targeted treatment outcomes, particularly in those who were diagnosed late.[20] All of these issues

are potentially avoidable with early detection by universal newborn screening and good clinical follow-up that may improve long-term outcomes for patients with CAH.[27]

CAH in Malaysia has been studied in various aspects. One study investigated the health-related quality of life (HRQOL) of female patients with CAH in Malaysia and found that their HRQOL was comparable to age-matched diabetic controls.[26] Another study identified various mutations affecting the CYP21A2 gene among 21-OHD Malaysian patients using the MLPA (Multiplex Ligation-dependent Probe Amplification) technique.[28] Another survey was conducted by the Lawson Wilkins Paediatric Endocrine Society (LWPES) that provided insights into the current management of CAH.[29] A comprehensive review highlighting the recent developments in CAH, including advancements in understanding the steroidogenic pathways, diagnostic measurements, genotyping methods, and clinical trials of alternative medications and treatments was also published.[30] The treatment of CAH in Malaysia involves hormonal replacement with synthetic glucocorticoids and mineralocorticoids and suppressing adrenal androgen production, while the treatment for nonclassical CAH is somewhat intricate and there is a degree of flexibility as physicians may opt for different approaches based on their experience and preferences.[31]

To the best of our knowledge, the data presented are the latest report on CAH incidence and prevalence, distribution of cases based on biochemical testing, and description of positive CAH cases in a Malaysian population. The findings are essential for further public health planning and clinical research initiation to improve the management of CAH.

*Acknowledgements:* The authors would like to thank the Director General of Health Malaysia for his permission to publish the paper. We are also expressing our gratitude to all staff in the Endocrine Laboratory, Biochemical and Genomic Research Centre, Institute for Medical Research (IMR), Kuala Lumpur, Malaysia for their unwavering support and technical assistance throughout this project.

*Conflicts of Interest:* The authors declare no conflict of interest.

## REFERENCES

1. Güran T, Tezel B, Çakır M, Akıncı A, Orbak Z, Keskin M, et al. Neonatal screening for congenital adrenal hyperplasia in Turkey: Outcomes of an extended pilot study in 241,083 infants. JCRPE J Clin Res Pediatr Endocrinol. 2020;12(3):287-94.
2. Livadas S, Bothou C. Management of the female with non-classical congenital adrenal hyperplasia (NCCAH): A patient-oriented approach. Front Endocrinol (Lausanne). 2019;10(JUN):1-11.
3. Pearce M, Demartino L, McMahon R, Hamel R, Maloney B, Stansfield DM, et al. Newborn screening for congenital adrenal hyperplasia in New York State. Mol Genet Metab Reports. 2016 Jun 1;7:1-7.
4. Binay C, Simsek E, Cilingir O, Yuksel Z, Kutlay O, Artan S. Prevalence of nonclassic congenital adrenal hyperplasia in Turkish children presenting with premature pubarche, hirsutism, or oligomenorrhoea. Int J Endocrinol. 2014;2014.
5. Berglund A, Ornstrup MJ, Lind-Holst M, Dunø M, Bækvad-Hansen M, Juul A, et al. Epidemiology and diagnostic trends of congenital adrenal hyperplasia in Denmark: a retrospective, population-based study. Lancet Reg Heal - Eur [Internet]. 2023;28:100598.
6. Navarro-Zambrana AN, Sheets LR. Ethnic and National Differences in Congenital Adrenal Hyperplasia Incidence: A Systematic Review and Meta-Analysis. Horm Res Paediatr. 2023;96(3):249-58.
7. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: An endocrine society* clinical practice guideline. Vol. 103, Journal of Clinical Endocrinology and Metabolism. 2018. 4043-4088 p.
8. Gong LF, Gao X, Yang N, Zhao JQ, Yang HH, Kong YY. A pilot study on newborn screening for congenital adrenal hyperplasia in Beijing. J Pediatr Endocrinol Metab. 2019;32(3):253-8.
9. Pulungan AB, Soesanti F, Utari A, Pritayati N, Julia M, Annisa D, et al. Preliminary Study of Newborn Screening for Congenital Hypothyroidism and Congenital Adrenal Hyperplasia in Indonesia. 2020;8(2).
10. Loke KY, Tan IT, Lee WRW, Lee YS. Epidemiology of 21-hydroxylase deficiency in Singapore. J Pediatr Endocrinol Metab. 2002;15(4):397-403.
11. Somboonnithiphol K, Panamonta O, Kiatchoosakun P, Jirapradittha J, Panamonta M, Lumbiganon P, et al. Newborn screening for congenital adrenal hyperplasia in Srinagarind Hospital, Khon Kaen University, Thailand. Asian Biomed. 2011;5(6):855-9.
12. Tsuji A, Konishi K, Hasegawa S, Anazawa A, Onishi T, Ono M, et al. Newborn screening for congenital adrenal hyperplasia in Tokyo, Japan from 1989 to 2013: A retrospective population-based study. BMC Pediatr. 2015;15(1).
13. Loo L WU, Gabi Krob*, Janine Pekarak*, Ben S Hasri, Ursula Kuhnle. Department of Pediatrics, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia, and *Universitaetskinderklinik, Muenchen G. High Frequency Of Classical. 21-Hy-

droxylase Deficiency (CAH) In Malaysia. Nature. 1994;118.
14. Antal Z, Zhou P. Congenital adrenal hyperplasia: diagnosis, evaluation, and management. Pediatr Rev. 2009;30(7):e49-57.
15. Carvalho B, Marques CJ, Santos-Silva R, Fontoura M, Carvalho D, Carvalho F. Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency: An Update on Genetic Analysis of CYP21A2 Gene. Vol. 129, Experimental and Clinical Endocrinology and Diabetes. Exp Clin Endocrinol Diabetes; 2021. p. 477-81.
16. Van Der Kamp HJ, Wit JM. Neonatal screening for congenital adrenal hyperplasia. Eur J Endocrinol Suppl. 2004;151(3):71-5.
17. Suwa S. Commentary on neonatal mass-screening for congenital adrenal hyperplasia in Japan. Screening. 1993;2(4):229-30.
18. Razzaghy-Azar M, Karimi S, Shirazi E. Gender identity in patients with congenital adrenal hyperplasia. Int J Endocrinol Metab. 2017;15(3):10-4.
19. Thambiah SC, Ahmad Z, Hambali Z, Osman M. Clinical presentation of congenital adrenal hyperplasia in selected multiethnic paediatric population. Article in Malaysian Journal of Medicine and Health Sciences. 2015.
20. Zainuddin AA, Grover SR, Soon CH, Nur Azurah AG, Mahdy ZA, Wu LL, Rasat R, Harun F, Chia WY, Shamsuddin K. Malaysian Females With Congenital Adrenal Hyperplasia: Surgical Outcomes and Attitudes. Front Pediatr. 2019;7:144.
21. V RD, R JM, Silambanan S, Jamir S. Neonatal screening for congenital adrenal hyperplasia: 17-hydroxyprogesterone cut-off values based on birth weight. Int J Clin Biochem Res. 2020;5(2):321-3.
22. Shafie AA, Supian A, Hassali MAA, Ngu LH, Thong MK, Ayob H, et al. Rare disease in Malaysia: Challenges and solutions. PLoS One. 2020;15(4):1-17.
23. Mirela Costa de Miranda M.D.1, Luciana Bertocco de Paiva Haddad PhD2, Guiomar Mafdureira PHD BB de M. Adverse outcomes and economic burden of congenital adrenal hyperplasia late diagnosis in the newborn screening absence. Journals Gerontol Ser A Biol Sci Med Sci. 2019;6:1-11.
24. Miranda MC de, Haddad LB de P, Trindade E, Cassenote A, Hayashi GY, Damiani D, et al. The Cost-Effectiveness of Congenital Adrenal Hyperplasia Newborn Screening in Brazil: A Comparison Between Screened and Unscreened Cohorts. Front Pediatr. 2021;9:1-12.
25. Zainuddin AA, Mahdy ZA. The Islamic Perspectives of Gender-Related Issues in the Management of Patients With Disorders of Sex Development. Arch Sex Behav. 2017;46(2).
26. Zainuddin AA, Grover SR, Abdul Ghani NA, Wu LL, Rasat R, Abdul Manaf MR, et al. Health-related quality of life of female patients with congenital adrenal hyperplasia in Malaysia. Health Qual Life Outcomes. 2020;18(1).
27. Lajic S, Karlsson L, Zetterström RH, Falhammar H, Nordenström A. The success of a screening program is largely dependent on close collaboration between the laboratory and the clinical follow-up of the patients. Int J Neonatal Screen. 2020;6(3).
28. Balraj P, Lim PG, Sidek H, Wu LL, Khoo ASB. Mutational characterization of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Malaysia. J Endocrinol Invest. 2013;36(6):366-74.
29. Clark PMS, Gordon K. Challenges for the endocrine laboratory in critical illness. Best Pract Res Clin Endocrinol Metab. 2011;25(5):847-59.
30. Yau M, Nimkarn S, New MI. Congenital Adrenal Hyperplasia. Second Edi. Genetic Diagnosis of Endocrine Disorders: Second Edition. lsevier Inc.; 2015. 203-210 p.
31. Coleman MA, Honour JW. Reduced maternal dexamethasone dosage for the prenatal treatment of congenital adrenal hyperplasia. BJOG An Int J Obstet Gynaecol. 2004;111(2):176-8.