Original Article

Evaluation of Predictors of Response to Ovulation Induction Using Letrozole in Women with Polycystic Ovary Syndrome: A Prospective Cohort Study

Shanti Tholiya, Archana Kumari, Reeta Mahey, Mani Kalaivani[1], Hem Chandra Sati[1], Mona Sharma[2], Aarthi K. Jayraj, Neena Malhotra

Departments of Obstetrics and Gynaecology, [1]Biostatistics and [2]Reproductive Biology, All India Institute of Medical Sciences, New Delhi, India

Abstract

Background: Ovulation induction (OI) in patients with polycystic ovary syndrome (PCOS) remains challenging, and several biomarkers have been evaluated for their ability to predict ovulation. The predictive ability of candidate biomarkers, particularly with letrozole-based therapy in infertile PCOS women, remains inconclusive as it is yet to be evaluated in a prospective study. **Aim:** To assess the role of anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinising hormone (LH)/FSH ratio, testosterone and free androgen index (FAI) as predictors of ovarian response to letrozole-based OI therapy during OI cycles in infertile women with PCOS from North India. **Settings and Design:** A prospective cohort study was conducted in a tertiary care hospital in north India. **Materials and Methods:** The study enrolled 80 infertile women with PCOS, diagnosed according to the Rotterdam criteria. OI was conducted using letrozole with or without human menopausal gonadotropin. Baseline endocrine and metabolic parameters, including serum AMH, FSH, LH, testosterone and FAI levels, were measured using ELISA or chemiluminescence methods on day 2 of the menstrual cycle. Follicular response to OI was monitored by transvaginal ultrasonography. **Statistical Analysis Used:** Descriptive and inferential statistical analyses were conducted, including Mann–Whitney, Kruskal–Wallis, Independent *t*-test, analysis of variance, Fisher's exact test and receiver operating characteristic curve analysis. Data were processed using Microsoft Excel and analysed with SPSS software, version 25.0. $P < 0.05$ was considered statistically significant. **Results:** Of 80 women enrolled, 74 responded to letrozole-based OI, while six were non-responders. Body mass index (BMI), serum testosterone and pre-treatment AMH levels significantly correlated with follicular response, with higher values linked to reduced responsiveness. The likelihood ratio+ (95% confidence interval) was 3.32 (2.45–5.06) for AMH, 1.97 (1.03–3.78) for BMI and 1.93 (1.22–3.08) for testosterone. The odds ratio for AMH was 2.88 (1.01–8.21) and 1.25 (1.02–1.53) for BMI. An AMH cut-off of ≤16.43 ng/mL predicted ovarian response with an AUC of 0.88. **Conclusions:** Pre-treatment

Address for correspondence: Dr. Archana Kumari, Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi, India.
E-mail: drarchanaaiims0312@gmail.com

Received: 21-08-2024 **Revised:** 15-10-2024
Accepted: 16-10-2024 **Published:** 23-12-2024

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/jhrs.jhrs_133_24

How to cite this article: Tholiya S, Kumari A, Mahey R, Kalaivani M, Sati HC, Sharma M, *et al.* Evaluation of predictors of response to ovulation induction using letrozole in women with polycystic ovary syndrome: A prospective cohort study. J Hum Reprod Sci 2024;17:240-5.

AMH levels, along with BMI and serum testosterone, are significant predictors of ovarian response to letrozole-based OI in infertile women with PCOS.

Keywords: *Anovulatory infertility, biomarker, gonadotropins, letrozole, ovulation induction, polycystic ovary syndrome*

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder affecting women of reproductive age, frequently resulting in infertility due to ovulatory dysfunction. While various ovulation induction (OI) therapies, including clomiphene citrate, gonadotropins and insulin sensitisers, have been employed, predicting an individual's response to treatment remains a significant clinical challenge.[1] Identifying reliable biomarkers to anticipate ovarian response could enhance the personalisation of OI therapy in women with PCOS.

Anti-Müllerian hormone (AMH) has gained attention as a promising biomarker for predicting OI outcomes, particularly in women treated with clomiphene citrate and gonadotropins.[2-4] AMH, produced by granulosa cells within developing follicles, serves as a reflection of ovarian reserve.[5] Its levels remain relatively stable throughout the menstrual cycle, in contrast to other hormonal markers like follicle-stimulating hormone (FSH) and luteinising hormone (LH), which are subject to cyclical variations. This stability makes AMH a more reliable predictor of ovarian function, especially in the context of OI.[6] Moreover, AMH correlates strongly with the antral follicle count, which is a critical determinant of ovarian response during induction therapy.[5]

The emergence of letrozole, an aromatase inhibitor, as a first-line agent for OI in women with PCOS has revolutionised treatment strategies due to its cost-effectiveness and favourable safety profile. While several studies have investigated the predictive value of AMH alongside other hormonal markers such as FSH, LH, LH/FSH ratio and androgen levels, these studies are limited by their retrospective design, and thus, conclusive evidence is lacking.[7,8]

In light of these limitations, we conducted a prospective cohort study to specifically evaluate the role of AMH in predicting ovarian response to letrozole-based OI in infertile women with PCOS from North India. By comparing AMH with other potential predictors, including FSH, LH, LH/FSH ratio, total androgen and free androgen index (FAI), our study aims to identify the most reliable biomarker for predicting OI success. This work seeks to enhance our understanding of AMH's role in guiding personalised treatment strategies for women with PCOS undergoing OI.

Materials and Methods

This prospective cohort study was conducted at a tertiary care referral centre in tertiary care setting following ethical endorsement from the Institute Ethics Committee (IECPG-114/24.02.2022, RT-30/24.03.2022). Eighty infertile women diagnosed with PCOS were recruited between March 2022 and November 2023. Written informed consent was obtained from all participants before enrolment. The inclusion criteria were infertile women aged 21–38 years who were diagnosed with PCOS according to the Rotterdam criteria.[9] The following two or more criteria were used: (a) oligo- or anovulation; (b) clinical or biochemical hyperandrogenism and (c) polycystic ovarian morphology (follicle number per ovary >12 in transvaginal scan performed on day 2). In addition, women whose husbands did not have normal semen parameters according to the WHO criteria,[10] those without patent bilateral fallopian tubes, abnormal prolactin and serum thyroid-stimulating hormone (TSH) levels, a history of previous ovarian surgery, or who had taken any hormonal oral contraceptive pill in the past three months were excluded from the study. The primary outcome was ovulation success which was defined as the development of at least one dominant follicle (>16 mm) during OI cycles. The study was conducted as per Helsinki declaration.

Fasting serum samples were collected on days 2 or 3 of the menstrual cycle to assess hormonal and metabolic profiles, including FSH, LH, prolactin, AMH, TSH, fasting insulin, glucose, lipid profile and serum testosterone. AMH levels were quantified using the ultrasensitive AMH/MIS ELISA (Roche Diagnostics), following the manufacturer's protocol. Although variability in AMH assay results is a recognised concern, our approach aimed to minimise this as much as possible. Ideally, all samples would have been stored in a deep freezer and analysed in a single batch using the same kit. However, because AMH levels were critical for clinical decisions, the assays were performed on an ongoing basis. To address potential variation, all assays were conducted in a designated laboratory by a single technician under faculty supervision, adhering strictly to quality control protocols. Standard controls and reagents were used as per the manufacturer's guidelines, and consumables were procured annually to limit variability.

By consistently following the same assay protocol for all samples, we were able to reduce inter-assay variation and ensure reliable AMH measurements.

The induction of ovulation was started with 5 mg of the letrozole tablet for 5 days starting from day 2 or 3 of spontaneous menstruation or progesterone withdrawal (Protocol 1). The response to OI was monitored via transvaginal ultrasonography starting from day 9 until the dominant follicle (>16 mm) was reached. Ovulation was triggered with 10,000 IU of injected human chorionic gonadotropin when the dominant follicle reached >18 mm. If no dominant follicle (>16 mm) developed by day 14, the cycle was stopped, and the patient was scheduled for the next cycle. In the second treatment cycle, patients received letrozole (5 mg) for 5 days, followed by human menopausal gonadotropin (HMG; 75 IU) starting from day 9. Ultrasound monitoring was performed every 2 days. The HMG dosage was progressively increased until the dominant follicle reached 14 mm in size, after which the same dose was maintained until the follicle grew beyond 16 mm (Protocol 2). Patients who showed a follicular response to either protocol were classified as 'Responders', while those who did not respond were designated as 'Non-responders'.

A 'response to treatment' was defined as the development of at least one dominant follicle during stimulation. Conversely, cycles without follicular development, even after 14 days of stimulation, were categorised as 'Non-responders'. Women who did not respond to letrozole and HMG were offered alternative treatment options such as HMG-only cycles, laparoscopic ovarian drilling, or IVF. Patients with no response were transitioned to the next treatment protocol.

Data collection and statistical analysis

Demographic profiles, infertility details, medical history, physical examination and anthropometric measurements were recorded. Statistical analysis involved both descriptive and inferential methodologies, including Mann–Whitney tests, Kruskal–Wallis tests, independent t tests, analysis of variances, Fisher's exact tests and receiver operating characteristic (ROC) curve analysis. The data were processed using Microsoft Excel and analysed with Statistical Package for the Social Sciences (SPSS) software, (IBM Corporation, New York, USA) version 25.0. $P < 0.05$ was considered to indicate statistical significance.

Results

After applying the inclusion and exclusion criteria, eighty women were recruited in the study.

Table 1 provides the baseline characteristics of the patients recruited along with their response to OI. Of the 80 women enrolled, 74 were responders (51 showed a positive response to protocol 1, 23 responded to protocol 2) and six remained non-responders. Table 2 also reveals association between different variables (clinical features) and the response to OI protocols.

The positive likelihood ratio (LR+) with a 95% confidence interval (CI) for AMH was 3.32 (2.45–5.06), for body mass index (BMI) was 1.97 (1.03–3.78) and for serum testosterone was 1.93 (1.22–3.08). The odds ratio (95% CI) for AMH was 2.88 (1.01–8.21) and for BMI was 1.25 (1.02–1.53).

Table 3 summarises the accuracy of AMH in predicting responders at different cut-off levels. It includes sensitivity, specificity, positive predictive value and negative predictive value. Figure 1 shows the corresponding ROC curve. Based on the best combination of sensitivity, specificity and positive and negative predictive value, it was identified that AMH level of ≤16.43 ng/mL was a significant predictor of ovarian response. The discriminatory ability of AMH was notably strong, with an area under the curve of 0.88 and a 95% confidence interval ranging from 0.788 to 0.942.

Discussion

This study assessed the relationship between several clinical-biochemical markers, such as AMH, BMI, serum LH, FSH, testosterone, FAI and homeostasis model assessment of insulin resistance (HOMA-IR), and ovarian response to letrozole-based OI in women with PCOS. The findings revealed a negative correlation between pre-treatment levels of AMH, serum testosterone and BMI with follicular response, suggesting that elevated levels of these markers are

Table 1: Association between the clinical characteristics and ovarian response

Variables	Responders (*n*=74)	Non-responders (*n*=6)	*P*
Median (IQR)	25–75	25–75	
Age (years)	28 (21–37)	27.5 (25–31)	0.999
Duration of infertility (years)	4 (1–16)	3.5 (2–5)	0.4337
Primary infertility, *n* (%)	55 (75.34)	3 (50)	0.333
Secondary infertility, *n* (%)	18 (24.66)	3 (50)	0.333
Phenotype A, *n* (%)	20 (27.03)	3 (50)	0.422
Phenotype C, *n* (%)	8 (10.81)	0	
Phenotype D, *n* (%)	46 (62.16)	3 (50)	

Data of different variables are expressed as frequency (%) and median (minimum–maximum). IQR=Interquartile range

Table 2: Association between the hormonal profiles with ovarian response

Investigations	Responders, median (minimum–maximum)	Non-responders, median (minimum-maximum)	*P*
LH (IU/L)	6.90 (1.56–48.77)	9.4 (4.25–1.41)	0.4111
FSH (IU/L)	5.76 (3.11–9.1)	5.22 (4.62–5.96)	0.1794
Triglyceride	110 (42–312)	125.5 (82–189)	0.3855
LH/FSH ratio	1.21 (0.26–9.38)	1.84 (0.79–2.37)	0.2351
BMI	25.72 (17.8–38.11)	31.015 (23.48–34.6)	0.0455
Waist hip ratio	0.89 (0.72–1.154)	0.894 (0.86–0.96)	0.4668
AMH	12.745 (1.48–1.75)	17 (16.5–19.84)	0.0023
Testosterone	0.395 (0.054–0.9)	0.54 (0.377–1.07)	0.0360

AMH=Anti-Müllerian hormone, BMI=Body mass index, FSH=Follicle-stimulating hormone, LH=Luteinising hormone

Table 3: Sensitivity, specificity, positive predictive value and negative predictive value of anti-Müllerian hormone (ng/mL) for predicting responders at different cut-offs

Cut off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
≤15.97	71.62 (59.9–81.5)	100 (54.1–100.0)	100 (93.3–100.0)	22.2 (8.6–42.3)
≤16	72.97 (61.4–82.6)	100 (54.1–100.0)	100 (93.4–100.0)	23.1 (9.0–43.6)
≤16.2	74.32 (62.8–83.8)	100 (54.1–100.0)	100 (93.5–100.0)	24 (9.4–45.1)
≤16.43	75.68 (64.5–84.9)	100 (54.1–100.0)	100 (93.6–100.0)	25 (9.8–46.7)
≤16.5	78.38 (67.3–87.1)	66.67 (22.3–95.7)	96.7 (88.5–99.6)	20 (5.7–43.7)
≤16.6	79.73 (68.8–88.2)	50 (11.8–88.2)	95.2 (86.5–99.0)	16.7 (3.6–41.4)
≤16.67	81.08 (70.3–89.3)	50 (11.8–88.2)	95.2 (86.7–99.0)	17.6 (3.8–43.4)

CI=Confidence interval, PPV=Positive predictive value, NPV=Negative predictive values

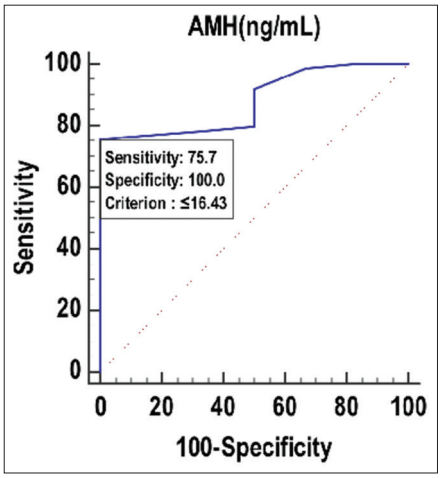

Figure 1: Receiver operating characteristic curve of anti-Müllerian hormone (ng/mL) for predicting responders. AMH: Anti-Müllerian hormone

associated with poorer responses to letrozole-based OI therapy.

Our results demonstrated that higher AMH levels were associated with a poorer response to letrozole-based OI, with an identified AMH cut-off of ≤16.43 ng/mL serving as a significant predictor of ovulation success. Notably, this cut-off is higher than those found in studies evaluating other OI agents, such as clomiphene citrate and HMG, where AMH cut-offs for predicting ovarian response ranged between 4.7 ng/mL and 6.25 ng/mL.[11,12] Besides, the higher threshold in our study may be influenced by ethnic and regional variations, consistent with previous research suggesting that Indian women tend to have higher AMH cut-offs compared to Western populations.[13,14] Elevated AMH levels, reflecting a higher antral follicle count in women with PCOS, reduce follicular sensitivity to FSH, thereby impairing folliculogenesis and contributing to anovulation.[15,16]

We found a statistically significant association between higher BMI and poor ovulation response, suggesting that BMI may influence the efficacy of letrozole-based OI. Our findings are consistent with previous research by Legro *et al*. and Imani *et al*., which indicated that obesity adversely affects ovulation and pregnancy rates with clomiphene citrate.[17,18] Moreover, our study extends these findings to letrozole, highlighting the compounded effects of obesity on fertility treatments beyond clomiphene citrate. In our study, median testosterone levels were higher in non-responders compared to responders (0.395 [0.054–0.9] vs. 0.54 [0.377–1.07]), reaching statistical significance. This contrasts with previous studies, which reported that higher basal serum testosterone levels were associated with better ovarian response.[19,20] These inconsistencies highlight the complexity of hyperandrogenism and its variable impact on ovarian function in women with PCOS.

Interestingly, other clinical and hormonal markers, such as FSH, LH, the LH/FSH ratio, testosterone, FAI, age, duration of infertility and PCOS phenotype, did not show significant associations with ovarian response to letrozole.

While some previous studies have suggested a role for elevated LH/FSH ratios and FAI in predicting OI success, our findings did not support this.[7,21] The lack of association emphasises the specificity of AMH in predicting ovarian responsiveness and suggests that AMH could be a more reliable biomarker for guiding individualised treatment strategies. Similarly, although FAI and HOMA-IR levels were higher in non-responders, these differences did not reach statistical significance. Insulin resistance, indicated by elevated HOMA-IR levels, is widely recognised as a key factor in the pathophysiology of PCOS.[22] However, our results suggest that these markers play a less direct role in predicting ovarian response, or that their predictive capacity requires further exploration through larger, multicentre studies.

The findings of our study have significant clinical implications for managing women with PCOS undergoing OI therapy. Measuring AMH levels before treatment can help identify patients who may not respond well to letrozole and might benefit from alternative or adjunctive treatments, such as the addition of gonadotropins or laparoscopic ovarian drilling. Additionally, using AMH as a predictive marker can assist in patient counselling, helping to set realistic expectations regarding treatment outcomes and reducing the emotional and financial stress associated with infertility treatments.

Despite its valuable insights, our study is limited by its small sample size, which restricts the ability to accurately measure effect size and limits the generalisability of the findings. As the study population was drawn from a single hospital, broader applicability may be constrained. Nonetheless, our study provides important preliminary data that can guide future research. Expanding the study to include a larger, more diverse population would enhance statistical power, improve generalisability and strengthen confidence in the findings for clinical practice. Additionally, variations in AMH measurement techniques and population-specific factors may influence the applicability of our AMH cut-off values. Therefore, larger multicentre studies using standardised assays are essential to confirm and refine these results, ultimately enhancing treatment protocols.

Conclusions

AMH emerges as a pivotal biomarker for predicting ovarian response to letrozole in women with PCOS, surpassing other clinical and hormonal parameters in its predictive accuracy. This provides a more targeted approach to fertility treatment. Future studies should focus on elucidating the biological mechanisms underlying AMH's predictive role and integrating its assessment into routine clinical practice to enhance the precision and effectiveness of OI therapies.

Author contributions

AK and RM conceived the study. ST and AKJ compiled the data. MS assisted with the biochemical analysis, while HCS and MK conducted the statistical analysis. AK and ST prepared the manuscript. RM and NM critically revised the manuscript and provided significant intellectual input. All authors reviewed and approved the final manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

References

1. Nader S. Ovulation induction in women with polycystic ovary syndrome. Nat Rev Endocrinol 2007;3:438-9.
2. Sunkara SK, La Marca A. The influence of AMH on IVF outcomes: A systematic review and meta-analysis. Hum Reprod Update 2011;17:46-60.
3. El-Halawaty S, Rizk A, Kamal M, Aboulhassan M, Al-Sawah H, Noah O, *et al*. Clinical significance of serum concentration of anti-Müllerian hormone in obese women with polycystic ovary syndrome. Reprod Biomed Online 2007;15:495-9.
4. Butt MS, Saleem J, Aiman S, Zakar R, Sadique I, Fischer F. Serum anti-Müllerian hormone as a predictor of polycystic ovarian syndrome among women of reproductive age. BMC Womens Health 2022;22:199.
5. van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, *et al*. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod 2010;25:221-7.
6. Jamil Z, Fatima SS, Cheema Z, Baig S, Choudhary RA. Assessment of ovarian reserve: Anti-Mullerian hormone versus follicle stimulating hormone. J Res Med Sci 2016;21:100.
7. Guo Z, Chen S, Chen Z, Hu P, Hao Y, Yu Q. Predictors of response to ovulation induction using letrozole in women with polycystic ovary syndrome. BMC Endocr Disord 2023;23:90.
8. Akgul OK, Guraslan H. Ovarian response can be predicted in women with PCOS who have ovulation induction with letrozole. J Coll Physicians Surg Pak 2023;33:217-21.
9. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-7.
10. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 6th ed. Geneva: World Health Organization; 2021.
11. Amer SA, Mahran A, Abdelmaged A, El-Adawy AR, Eissa MK, Shaw RW. The influence of circulating anti-Müllerian hormone on ovarian responsiveness to ovulation induction with gonadotrophins in women with polycystic ovarian syndrome: A pilot study. Reprod Biol Endocrinol 2013;11:115.

12. Rashid D, Bardan R. Anti-Mullerian hormone serum level as a predictor of responsiveness to clomiphene citrate therapy in Iraqi women with PCOS. Open Access Maced J Med Sci 2022;10:369-73.
13. Malhotra N, Mahey R, Cheluvaraju R, Rajasekaran K, Patkar D, Prabhakar P, *et al.* Serum Anti-Mullerian Hormone (AMH) Levels Among Different PCOS Phenotypes and Its Correlation with Clinical, Endocrine, and Metabolic Markers of PCOS. Reprod Sci 2023;30:2554-62. [doi: 10.1007/s43032-023-01195-y].
14. Dietz de Loos A, Hund M, Buck K, Meun C, Sillman J, Laven JS. Antimüllerian hormone to determine polycystic ovarian morphology. Fertil Steril 2021;116:1149-57.
15. Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, *et al*. Diagnosis of polycystic ovary syndrome (PCOS): Revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod 2011;26:3123-9.
16. Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:941-5.
17. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Alvero R, *et al.* The pregnancy in polycystic ovary syndrome II study: Baseline characteristics and effects of obesity from a multicenter randomized clinical trial. Fertil Steril 2014;101:258-69.e8.
18. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. J Clin Endocrinol Metab 1998;83:2361-5.
19. Wan RS, Ko JK, Yung SS, Ng EH, Li RH. Effect of basal serum testosterone level on the ovarian response and the cumulative live birth rate in infertile women undergoing *in vitro* fertilization. J Assist Reprod Genet 2023;40:883-90.
20. Xiao S, Li Y, Long L, Luo C, Mai Q. Basal serum testosterone levels correlate with ovarian reserve and ovarian response in cycling women undergoing *in vitro* fertilization. Gynecol Endocrinol 2016;32:51-4.
21. Tarlatzis BC, Grimbizis G, Pournaropoulos F, Bontis J, Lagos S, Spanos E, *et al*. The prognostic value of basal luteinizing hormone: Follicle-stimulating hormone ratio in the treatment of patients with polycystic ovarian syndrome by assisted reproduction techniques. Hum Reprod 1995;10:2545-9.
22. Lewandowski KC, Skowrońska-Jóźwiak E, Łukasiak K, Gałuszko K, Dukowicz A, Cedro M, *et al*. How much insulin resistance in polycystic ovary syndrome? Comparison of HOMA-IR and insulin resistance (Belfiore) index models. Arch Med Sci 2019;15:613-8.