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REVIEW

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A systematic review on the applicability of cell-free DNA level as an obesity biomarker

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Summary

Obesity has become a global health concern in recent decades. Utilizing biomarkers presents a promising approach to comprehensively monitor the progress of obesity and its associated health conditions. This review aims to synthesize the available evidence on the correlation between cfDNA level and obesity and to provide insights into the applicability of using cfDNA level as a tool for monitoring progression of obesity. Searches were performed in PubMed and Embase on April 1, 2022. Data and other relevant information were extracted and compiled into a structured table for further analysis. Among 1170 articles screened, 11 articles were included in this review and assessed qualitatively. The results demonstrated that existing evidence mainly focused on three populations, including healthy individuals, cancer patients and pregnant women. Majority of the studies on healthy individuals identified a significant association between cfDNA level and body weight status but not among cancer patients. Varying results were observed among pregnant women at different gestational trimesters. Our review summarized some preliminary evidence on the association between cfDNA level and obesity. More cohort studies in larger scale with comprehensive assessment have to be conducted to examine the applicability of cfDNA as a biomarker for severity and disease progression of obesity.

KEYWORDS
adiposity, cell-free DNA, circulating DNA, obesity

1 | INTRODUCTION

Obesity is defined as the excessive accumulation of adipose tissue that can present increased risks of fatality in individuals.[1,2] The prevalence of obesity has increased drastically due to the lifestyle and environmental changes in recent decades. Approximately 650 million of the world population is reported to be obese.[3] Obesity is commonly associated with predisposition to clinical problems such as cardiovascular diseases, diabetes, dyslipidemia, and neoplasms.[4–8] In view of this, more attention was given to monitor the progress of obesity-associated clinical symptoms. Although the body mass index (BMI) is commonly used to detect and screen out people with overweight and obesity, it fails to provide in-depth information on severity and duration of obesity and hence the pathogenesis of obesity-related health

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conditions.[9] Therefore, BMI should be used in conjunction with other measures to get a more comprehensive picture of obesity status and related health risks of an individual suffering from obesity. In particular, making use of biomarkers that consider the molecular pathways could be a more comprehensive approach in monitoring obesity status and assessing the progress of obesity-related health conditions.

Adiposity or obesity has been interpreted as a state of inflammation in molecular biology. The low-grade systemic inflammation in individuals with obesity as the result of excess macronutrient deposit in adipose tissues had been extensively studied.[10] Previous molecular studies had suggested obesity as a multifactorial disease with complications stemmed from induced changes in cellular immune response and inflammation state.[11,12] Apart from serving as fat storage organ, adipose tissue is also of great importance to body homeostasis with its endocrinologic role in body's metabolism and immune response.[13,14] Nutritional derangements among individuals with obesity and overweight were found to cause distorted immune function.[14] Adipose tissue macrophages are generally responsible for immune regulation by producing and releasing different pro-inflammatory and anti-inflammatory factors, such as leptin, adiponectin, resistin, cytokines, and chemokines.[15,16] It has been reported that obesity could lead to severe adipocyte disorders by altering both the amount and activity of resident immune cells. As adipocytes enlarge, blood supply to these cells may decrease, resulting in hypoxia.[17] Cell hypoxia and macrophage infiltration due to excessive fat storage could result in enhanced production of hypoxia-inducible factor 1α and pro-inflammatory adipokines.[12,18–20] This would create an imbalance in the immunological phenotypes that eventually cause the development of persistent local inflammation where several biologically active molecules are released.[21] This pro-inflammatory environment created by altered T-cell stoichiometry and cytokine secretion eventually contributes to the pathogenesis of metabolic disorders, cardiovascular diseases, and even cancer initiation.[5,7,21–23] For example, there has been a large body of evidence accumulated that links obesity to high-sensitivity C-reactive protein (CRP).[20] However, CRP has been proposed to not just function as a marker of inflammation, but it also has a direct heavy involvement in the pathogenesis of atherosclerosis as well as the subsequent non-communicable diseases such as cardiovascular diseases and type 2 diabetes.[24,25]

Circulating cell-free DNA (cfDNA) has been regarded as an emerging biomarker for clinical diagnosis and monitoring with its pathophysiological relevance in health conditions.[26,27] Circulating cfDNA in general is a mixture of single stranded and double stranded DNA found in plasma as a result of cellular mechanisms such as apoptosis and necrosis.[28] Among individuals with obesity, adipose tissue remodeling that can enhance the apoptosis and necrosis of adipocytes was observed, resulting in the increased release of cfDNA in their circulation.[29] In fact, the development of preclinical symptoms and pathogenesis of metabolic disorders in individuals with obesity were found to be in close relationship with host inflammatory pathway activation and the chronic systemic inflammation in white adipose tissue.[22,30] Adipocyte hypertrophy in association with increased cellular death can also lead to pro-inflammatory macrophage infiltration, with macrophages surrounding necrotic adipocytes giving crown-like structures.[21] As a result, enhanced cellular turnover and necrosis observed in adipocytes of individuals with obesity could be the potential linkage between the cfDNA level and body inflammation status.[21,31,32] Given its connection with various obesity-related conditions such as increased oxidative stress and heightened inflammatory status, an increasing number of studies have been conducted to investigate the relationship between the cfDNA level and obesity.

To our best knowledge, there were no systematic reviews or meta-analyses reviewing the studies that focus on the association between cfDNA level and obesity. Therefore, this systematic review aims to summarize the existing evidence on the association between cfDNA level and body weight status. Furthermore, a comparison would be made to study whether the association between cfDNA level and body weight status would differ according to subject characteristics. This can consolidate existing evidence on the applicability of using cfDNA level as a biomarker in conjunction with BMI in monitoring the severity of obesity and the progression of obesity-linked complications among individuals suffering from obesity.

2 | METHODS

2.1 | Search strategy

The systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[33] An electronic literature search was performed in PubMed and Embase on April 1, 2022. Keywords used included ("cell-free DNA" OR "extracellular DNA" OR "circulating DNA" OR "free DNA" OR "plasma DNA" OR "cfDNA") AND ("obese" OR "overweight" OR "body weight" OR "BMI"). The search was restricted to human studies published in English in the period of 2000–2022. Additionally, reference lists of the assessed articles were also screened to identify potential relevant studies.

2.2 | Selection of articles

Figure 1 shows the flow chart of study search and selection process. Articles were included in this review should they fulfilled the following criteria: (a) original research examined the association between cfDNA level and body weight status; (b) assessed the cfDNA levels using validated instruments; (c) had a clear definition on classification of body weight status; and (d) publication as a full paper in a peer-reviewed scientific journal. When same study population was reported in multiple studies or reports, the publication that included the highest sample size would be chosen.

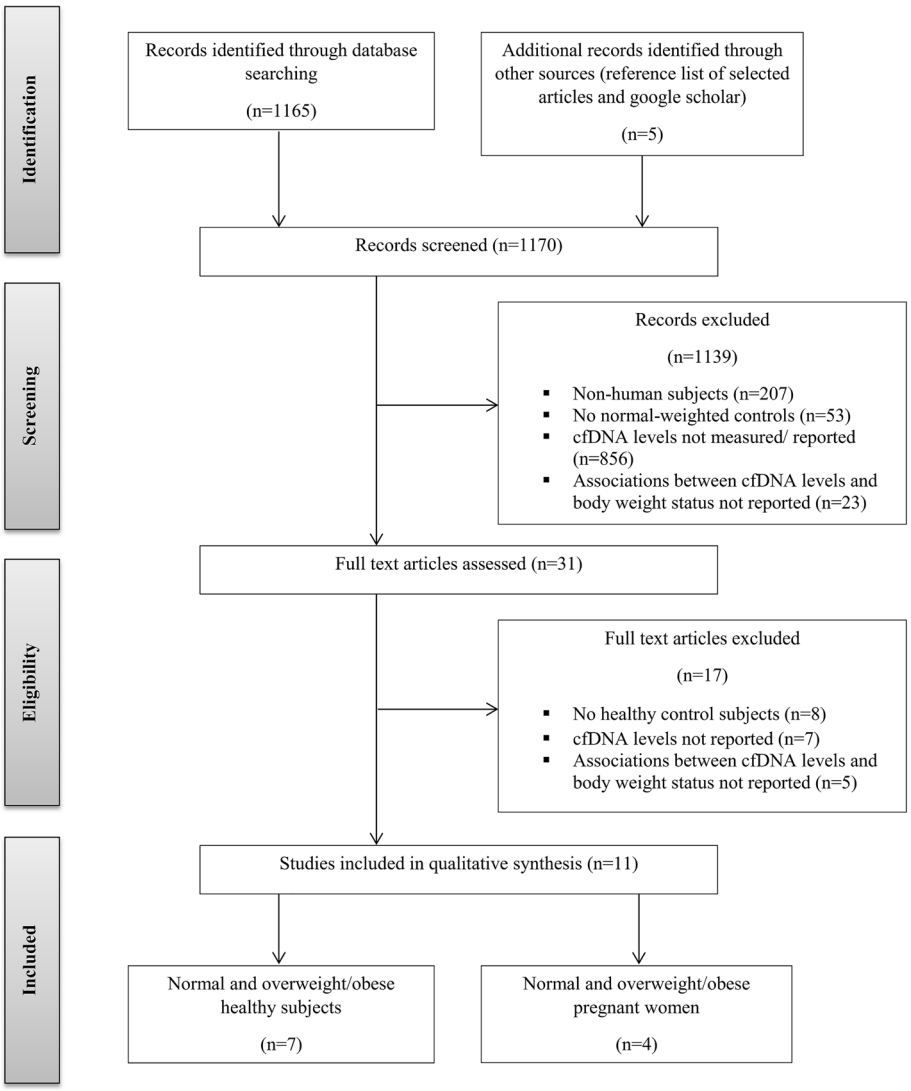

FIGURE 1 Flowchart showing the process of study selection.

2.3 | Data extraction

Reviewer CC screened the title and abstract of the identified articles. Full text of all potentially relevant articles was then screened by two reviewers (CC and HWT) to extract relevant information into a structured table for further analysis. Any disagreements on the data extraction process were resolved by consensus or via discussion with a third reviewer (KT). The extracted data included the name of the authors, publication year, study period, location, study design, sample characteristics, methods used for cfDNA extraction and quantification and the reported associations between cfDNA levels and body weight status, and other information as required.

2.4 | Quality assessment

The methodological quality of all included studies was assessed using a modified version of the Joanna Briggs Institute's Critical Appraisal Checklist for Studies Reporting Prevalence Data.[34] A total of 9 items in 4 major domains (study design, sample selection, measurement method, and statistical analysis) was included in this modified checklist. Each of these articles was rated with a 9-point scale with the grading scheme as follow: 0–3 points indicating poor quality, 4–7 points as fair quality, and 8–9 points as good quality. The quality of each article was assessed independently by two researchers (CC and KT), in which any discrepancies on the rating were resolved by discussion with another researcher (HWT).

2.5 | Data synthesis and analysis

Narrative synthesis was used to integrate descriptions and findings of the included studies on the association between cfDNA levels and body weight status among individuals of different characteristics. Statistical synthesis was not conducted in this study because of the limited number of studies and the substantial differences on the parameters including exposures, target populations, and outcomes in these identified studies.

TABLE 1 Characteristics of studies included in the systematic review on the association between body weight status and cfDNA level.

ID	Studies	Country (sample size, *n*)	Study design	Study population	Mean age of participants	Body weight status	Specimen type	Measurement method of cfDNA level	Association between cfDNA levels and body weight status
1	Ferrandi et al.[35]	United States (14)	Randomized controlled trial	Individuals with normal body weight and obesity	Normal weight: 22.7 ± 1.6; obese: 26.0 ± 5.6	Mean body mass index of normal weight: 22.2 ± 2.0; Obese: 36.1 ± 3.2	Plasma	Extraction: QIAamp DNA Blood Mini-Kit (Qiagen) Quantification: RT-PCR, β-globin gene	No significant association was found between BMI and cfDNA levels in the baseline assessment of the randomized controlled trial
2	Ørntoft et al.[36]	Denmark (2817)	Cross-sectional	Colorectal cancer patients and healthy controls	66 (95% CI: 65.6; 66.2)	Mean body mass index: 26.3	Plasma	Extraction: QIAsymphony DSP Circulating DNA kit Quantification: droplet digital PCR	Significant association between BMI and cfDNA levels in healthy controls ($\beta = 0.03$, $P < 0.001$)
3	Shree et al.[37]	United States (1032)	Cohort	Pregnant women with singleton gestations at first trimester	Normal weight: 35.2 ± 4.0; overweight: 35.9 ± 4.1; obese: 36.5 ± 4.4	Mean body mass index of normal weight: 22.2 ± 1.8; Overweight: 27.0 ± 1.3; Obese: 35.8 ± 4.9	Plasma	Extraction: QIAsymphony Circulating DNA Kit Quantification: Qubit fluorometer	Total cfDNA level: no significant difference between women with normal body weight and with obesity ($P = 0.100$) Fetal cfDNA level: significantly lower in women with obesity, compared with women with normal body weight ($P < 0.001$)
4	Padilla-Sánchez et al.[38]	Ecuador (78)	Cross-sectional	Young and mature adults with normal body weight and with overweight or obesity	Normal weight young adults: male: 22.4 ± 2.4; female: 21.0 ± 1.5 Normal weight mature adults: male: 37.3 ± 5.7; female: 43.5 ± 7.4 Overweight/obese mature adults: male: 47.50 ± 12.80; female: 42.07 ± 8.46	Mean body mass index of normal weight young adults: male: 21.8 ± 2.2; female: 21.5 ± 1.4 Normal weight mature adults: male: 23.3 ± 1.4; female: 22.1 ± 1.8 Overweight/obese mature adults: male: 30.3 ± 5.2; female: 33.4 ± 6.2	Serum	Extraction: ChargeSwitch gDNA Serum Kit Quantification: RT-PCR	Significantly higher cf-mtDNA levels in subjects being overweight or compared with those with normal body weight ($P < 0.05$) Significantly higher cf-mtDNA levels in mature subjects compared with young subjects of normal body weight ($P = 0.013$) No difference detected in cf-mtDNA levels between mature adults with normal and high BMI Significant positive correlation between cf-mtDNA and BMI ($r = 0.257$, $P = 0.02$)

TABLE 1 (Continued)

ID	Studies	Country (sample size, *n*)	Study design	Study population	Mean age of participants	Body weight status	Specimen type	Measurement method of cfDNA level	Association between cfDNA levels and body weight status
5	Haghiac et al.[31]	United States (30)	Cohort	Pregnant women with male fetuses at 38–40 weeks of gestation	Pregravid lean: 29.5 ± 7.1 Pregravid obese: 28.8 ± 5.5	Mean body mass index: lean: pregravid: 21.8 ± 2.8; at delivery: 27.5 ± 3.0 Obese: pregravid: 39.2 ± 6.7; at delivery: 45.7 ± 7.3	Plasma	Extraction: QIAamp DNA Blood Mini Kit (Qiagen) Quantification: RT-PCR, amplification of GAPDH and DYS14	Total cfDNA levels significantly higher in women with obesity when compared with those with lean body size ($P < 0.007$) No difference in fetal cfDNA levels between women with obesity and those with lean body size Significant positive correlation between total cfDNA and pregravid BMI ($r = 0.49$, $P = 0.006$) and BMI during pregnancy ($r = 0.57$, $P = 0.002$)
6	Nishimoto et al.[39]	Japan (131)	Cross-sectional	Patients who underwent medical examination	56 ± 12	Not reported	Plasma	Extraction: QIAamp DNA Mini Kit (Qiagen) Quantification: QuantiFluor ssDNA system (Promega)	Significantly higher cfDNA levels in those with visceral obesity (visceral fat area ≥ 100 cm^2) ($P < 0.05$) Significant positive correlation between cfDNA levels and visceral fat area ($P < 0.01$)
7	Khani et al.[40]	Iran (100)	Cross-sectional	Patients with prostate cancer (Pca), benign prostate hyperplasia (BPH), and healthy controls	PCa patients: 63.7 ± 8.0 BPH patients: 60.8 ± 8.1 Control: 60.6 ± 7.6	Not reported	Plasma	Extraction: NucleoSpin plasma XS (NS) Kit Quantification: Amplication of ALU115 and ALU247 using RT-PCR	No significant association was found between cfDNA level and BMI among PCa patients, BPH patients, and healthy controls Statistical association was found between total cfDNA level and weight among PCa patients (*P*

(Continues)

TABLE 1 (Continued)

ID	Studies	Country (sample size, *n*)	Study design	Study population	Mean age of participants	Body weight status	Specimen type	Measurement method of cfDNA level	Association between cfDNA levels and body weight status
									value = 0.009) but not in BPH patients and healthy controls
8	Lapaire et al.[41]	Germany (406)	Cohort	Pregnant women with singleton in second trimester (20–21 weeks of gestation)	26 ± 4.8	Mean body mass index of pre-pregnancy: 22.1 ± 4.1; at 20th and 21st gestational week: 24.1 ± 4.0; at delivery: 27.9 ± 4.1	Plasma	Extraction: High pure PCR template kit (Roche Diagnositcs) Quantification: RT-PCR, amplification of GADPH, SRY, and DYS14	Significant positive correlation between total cfDNA and maternal BMI at 20th and 21st gestational week and at delivery ($P = 0.034$ and $P = 0.014$) No significant association between fetal DNA and BMI at different time points of pregnancy No significant correlation between median gain of maternal BMI during pregnancy and total cfDNA levels
9	Kim et al.[42]	South Korea (64)	Cross-sectional	Gastric cancer patients and age-matched healthy controls	Gastric cancer patients: 66.7 ± 13.2 Healthy controls: 63.8 ± 6.8	Prevalence of overweight or obese: patients: 37%; healthy controls: 41%	Plasma	Extraction: QIAamp DNA Micro Kit (Qiagen) Quantification: RT-PCR	No associations reported between cfDNA and BMI in both gastric cancer patients and healthy controls
10	Jylhävä et al.[43]	Finland (1337)	Cohort	General Finnish population, aged 30 years and older	Men: 58.3 ± 7.9 women, no HRT: 60.5 ± 9.0 women, HRT user (estrogen): 58.6 ± 6.9 women, HRT user (estrogen + progestin): 57.2 ± 6.4	Mean body mass index of men: 27.5 ± 4.1; women, no HRT: 27.3 ± 5.2; women, HRT user (estrogen): 27.0 ± 5.0; women, HRT user (estrogen and progestin): 26.8 ± 4.3	Plasma	Extraction: Quant-iT DNA High Sensitivity Assay kit Quantification: Qubit fluorometer	Significant positive correlation between BMI and cfDNA level among women with no HRT ($P = 0.035$) but not in men, women HRT user (estrogen), and women HRT user (estrogen + progestin).
11	Atakul et al.[44]	Turkey (224)	Case control	Pregnant women underwent non-invasive prenatal testing	35.0 ± 8.0	Mean body mass index of women with normal cfDNA levels: 25.7 ± 6.2; women with high cfDNA levels: 26.4 ± 5.2	Plasma	Extraction: QIAamp Circulating Nucleic Acid Kit Quantification: Qubit fluorometer	No significant difference in body weight status and BMI between women with normal and high cfDNA levels

Abbreviations: BMI, body mass index; BPH, benign prostate hyperplasia; cfDNA, cell-free DNA; cf-mtDNA, cell-free mitochondrial DNA; HRT, hormone replacement therapy; PCa, patients with prostate cancer; RT-PCR, real-time polymerase chained reaction.

3 | RESULTS

3.1 | Study selection

A total of 1170 articles were initially identified from our search through PubMed and Embase. According to the pre-established criteria, 1139 articles were excluded as the criterion of assessing cfDNA in human subjects was not fulfilled. A total of 31 articles considered suitable for full text screening were further examined with criteria such as inclusion of healthy subjects for comparison and detailed description over the association between cfDNA level and body weight status. After the full-text screening, 11 articles reported the association of interest and were therefore included in this review. They comprised 10 observational studies (4 cohorts, 5 cross-sectional, and 1 case-controls) and 1 experimental study (randomized controlled trial). Figure 1 shows the flow chart of study search and selection process.

3.2 | Study characteristics and quality

Table 1 provides an overview on the key characteristics of the 11 included articles.

Our review found that existing evidence on cfDNA mainly focused on three populations, including healthy individuals, cancer patients, and pregnant women. Of the 11 included studies, four of them evaluated and compared the cfDNA levels in individuals with normal body weight and obesity among non-disease group, with additional factors of concern included such as demographics, exercise intensity, and hormone replacement therapy.[35,38,39,43] Three of the studies assessed and compared the association between cfDNA level and body weight status among cancer patients and corresponding healthy controls.[36,40,42] Four focused on the cfDNA level variation in pregnant women with obesity and normal body weight with the aims to examine the effects of abnormal maternal weight on cfDNA levels of the mothers and fetus (Figure 2).[31,37,41,44]

Sample size of the included studies ranged from 14 to 2817 with 3 out of the 11 articles specified subjects' ethnicity (Finnish, Danish, and Iranian). Six articles had recruited gender-specific subjects (100% male or female) for the study, and all the articles with 100% female subjects focused on pregnant women. Mean BMI was specified in 8 out of the 11 articles. The other 3 of the articles reported the subjects' visceral fat area (VFA) or percentage of participants with obesity instead. Collection of cfDNA were performed using DNA extraction kits, with 7 of the studies using kit from QIAGEN (QIAamp DNA Blood Mini-Kit, QIAamp DNA Mini Kit, QIAamp DNA Micro Kit, QIAamp Circulating Nucleic Acid Kit, QIAsymphony DSP Circulating DNA kit and QIAsymphony Circulating DNA Kit). Other studies used extraction kits such as ChargeSwitch gDNA Serum Kit, NucleoSpin plasma XS (NS) Kit, Roche Diagnostic's high pure polymerase chain reaction (PCR) template kit, and Quant-iT DNA High Sensitivity Assay kit. As for nucleic acid quantification, 7 of the articles used PCR (RT-PCR and droplet digital PCR) and the rest used fluorometric systems (Qubit fluorometer and Promega QuantiFluor ssDNA system).

3.3 | Methodological quality

Details of the methodological assessment rating of the 11 included articles are shown in Table 2. Among the 11 articles, only seven provided clearly defined criteria for inclusion and exclusion of participants. Five of the articles did not clearly define the recruitment time frame. Eight studies clearly described the subjects and settings in detail. The method of sampling was considered appropriate in 7 articles, but most of the studies did not have an adequate sample size. All except one study provided a detailed description of how cfDNA level was measured, but only 7 had adequate description of body weight status assessment. The mean methodological quality score of the included articles was 6.4 (out of 9 points), with 1 article rated as poor quality, 5 articles rated as fair quality, and 4 rated as good quality.

3.4 | Association between body weight status and cfDNA level among healthy individuals

Seven studies had attempted to examine the association between body weight status and cfDNA level among healthy individuals,[35,36,38–40,42,43] but they adopted different parameters in reflecting the cfDNA level (five reported the total cfDNA level,[35,36,40,42,43] one reported the circulating cell-free mitochondrial DNA [cf-mtDNA],[38] and one reported plasma ssDNA levels[39]). Our review found that results varied according to the characteristics of participants. Ferrandi et al. identified comparable plasma cfDNA level between subjects with normal weight and obesity after underwent 30 min of high-intensity interval exercise (HIIE).[35] A study by Jylhävä et al.[43] tried to compare the cfDNA level among four distinctive study populations including men, women with no hormone replacement therapy (HRT), women with only estrogen HRT, and women with estrogen-progestin combination HRT and found that women with no HRT is the only group with significant correlation between BMI and cfDNA level.[43] Similar findings were also reported by Padilla-Sánchez et al. as they demonstrated a significant positive correlation between cf-mtDNA and BMI,[38] in which cf-mtDNA levels are significantly higher in subjects suffering from overweight and obesity when compared with individuals with normal body weight.[38] On the other hand, by using single-stranded DNA (ssDNA) to reflect cfDNA level, Nishimoto et al. reported that ssDNA levels are positively correlated with VFA, particularly among subjects with visceral obesity.[39] Similarly, in a study with 1930 healthy controls in Denmark, positive association between cfDNA levels and BMI was observed ($P < 0.001$).[36] However, such association was not observed among healthy subjects in the studies conducted in Iran[40] and South Korea.[42]

3.5 | Association between body weight status and cfDNA level among cancer patients

Our search identified three studies that have examined the cfDNA levels of cancer patients (colorectal cancer,[36] prostate cancer,[40] and

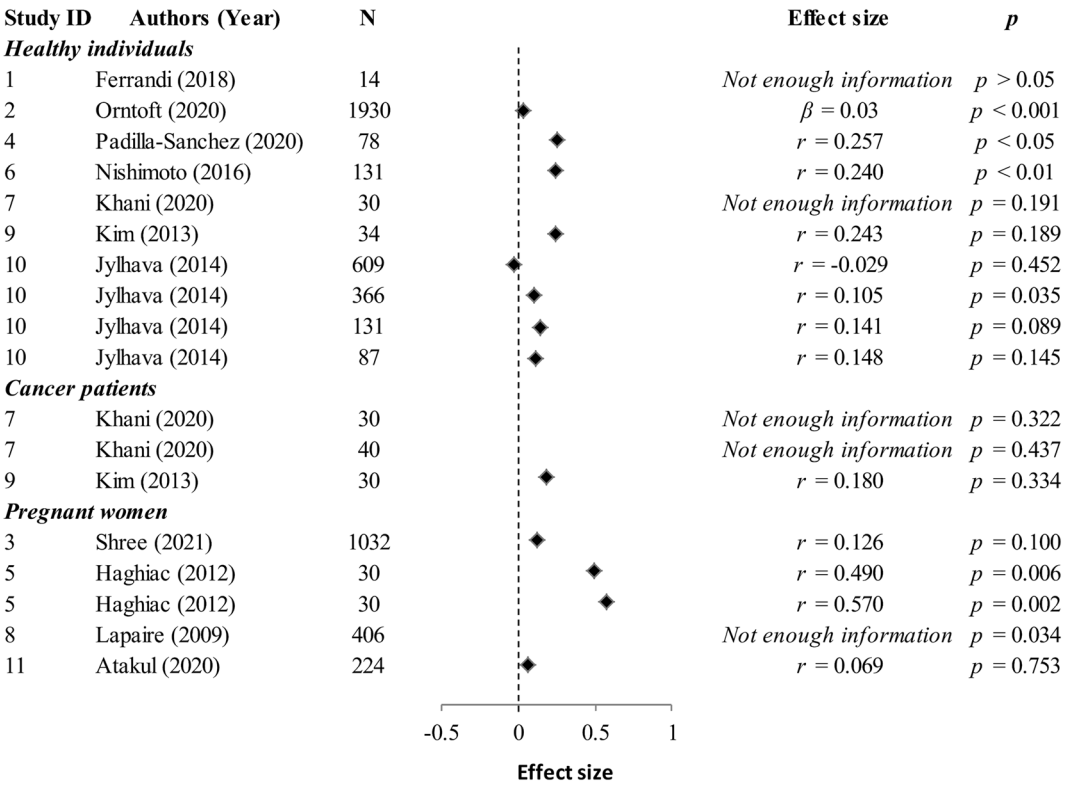

FIGURE 2 Forest plot of effect size on the association bewteen cell-free DNA (cfDNA) and body weight-related parameters.

gastric cancer[42]). However, no association was found between cfDNA levels and the body weight status among these cancer patients. In a study involving 887 colorectal cancer patients, although a significant association was observed between high cfDNA levels and decreased overall survival rate, cfDNA level and BMI were not significantly correlated.[36] Similarly, no significant association was found between cfDNA and body weight status (as assessed by BMI) in the other two studies involving prostate and gastric cancer patients.[40,42]

3.6 | Association between gestational obesity and maternal and fetal cfDNA levels

Of the 11 articles screened out from our search, four studies were found to focus on investigating the influence of maternal obesity on cfDNA levels in the pregnant women.[31,37,41,44] However, varying results were observed. While two cohorts (one from United States[31] and one from Germany[41]) both reported a significant positive correlation between total cfDNA level and maternal BMI during pregnancy, the other two studies had opposite observation.[37,44] A cohort study by Shree et al. reported no significant difference in total cfDNA level between pregnant women with normal body weight and obesity in their first trimester.[37] The same conclusion was also obtained in the case control study by Atakul et al., where no significant difference was detected in their BMI between pregnant women with normal and high cfDNA levels in first trimester.[44] Three of these articles also assessed the influence of gestational obesity on the fetal cfDNA level.[31,37,41] But only one had identified a significant correlation between the maternal body weight status and fetal cfDNA level.[37] It is pointed out by Shree et al. that fetal fraction (FF) of cfDNA in maternal blood is significantly lower in women with obesity as compared with pregnant women with normal body weight.[37] Yet, no association between maternal body weight status and fetal cfDNA was observed in the other two studies.[31,41]

4 | DISCUSSION

This is the first systematic review to assess and compare the association between cfDNA level and body weight status among different populations. Our review found that existing evidence mainly focused on three populations, including healthy individuals, cancer patients, and pregnant women. Distinctive patterns were observed across these three populations. In general, majority of the studies on healthy individuals identified a significant association between cfDNA level and body weight status, but such association was not observed among cancer patients including colorectal cancer,[36] prostate cancer,[40] and gastric cancer.[42] On the other hand, varying results were observed among pregnant women, suggesting that there might be other influencing factors on the cfDNA levels during pregnancy. Our review provided some preliminary evidence to support the further exploration over the applicability of using cfDNA level in monitoring the severity and progression of obesity.

TABLE 2 Quality assessment of studies included in the systematic review according to the Joanna Briggs Institute's Critical Appraisal Checklist for Studies Reporting Prevalence Data.

Article	Clearly defined criteria for inclusion and exclusion in the sample	Clearly defined subject recruitment time	Study subjects and setting described in detail	Study participants sampled in an appropriate way	Adequate sample size	Adequate description of body weight status assessment	Adequate description of cfDNA measurement	Adequate description of the data	Adequate description of statistical methods
Ferrandi et al.[35]	✓	×	✓	×	×	✓	✓	✓	✓
Ørntoft et al.[36]	✓	✓	✓	✓	✓	×	✓	✓	✓
Shree et al.[37]	✓	✓	✓	✓	✓	✓	✓	✓	✓
Padilla-Sánchez et al.[38]	×	×	×	×	×	✓	✓	✓	✓
Haghiac et al.[31]	✓	×	✓	✓	×	✓	✓	✓	✓
Nishimoto et al.[39]	✓	×	×	×	×	✓	✓	✓	✓
Khani et al.[40]	×	×	×	×	×	×	×	✓	✓
Lapaire et al.[41]	×	✓	✓	✓	✓	✓	✓	✓	✓
Kim et al.[42]	✓	✓	✓	✓	×	×	✓	✓	✓
Jylhävä et al. [43]	×	✓	✓	✓	✓	×	✓	×	✓
Atakul et al.[44]	✓	✓	✓	✓	✓	✓	✓	×	✓

Abbreviation: cfDNA, cell-free DNA.

Our review on the recent evidence identified that majority of the studies on healthy individuals reported a significant positive association between cfDNA level and body weight status. Apart from correlating with BMI, cfDNA level was also found to be positively correlated with VFA.[39] This suggests that cfDNA level could be used in screening obesity. While BMI has been criticized in failing to provide in-depth information on severity and duration of obesity and hence the pathogenesis of obesity-related health conditions,[9] the applicability of cfDNA as a biomarker is not just limited to screening obesity but also reflecting the severity and duration of obesity and even other obesity-associated pathological conditions. In fact, the main source of cfDNA is suggested to be apoptosis and necrosis, and these two cellular processes are likely to be altered among individuals with obesity due to adipose tissue remodeling. [39] Specifically, conditions associated with obesity, such as increased oxidative stress, reduced oxygen pressure, and heightened inflammation, can lead to cellular degeneration and accelerated turnover in adipose tissue. All of these can eventually lead to the pathogenesis of various metabolic disorders.[22,30] The high involvement of cfDNA in these cellular processes indicates the plausibility of using cfDNA as an unspecific biomarker for monitoring the severity and disease progression of obesity and its associated non-communicable diseases. While some existing evidence already identified the elevated cfDNA level among insulin resistant type 2 diabetes patients,[45] evidence proving the association between cfDNA and obesity-associated complications is still very preliminary. The applicability and utility of cfDNA as an unspecific marker can be further confirmed by comparing with other obesity-related biomarkers such as CRPs, interleukin-6, and adiponectin.[25,46]

In addition, the results seem to vary according to the characteristics of study participants. Although we found that the association between cfDNA and BMI has been confirmed in several large-scale observational studies with more than 1000 participants,[36,37,43] all three of these studies were conducted on Caucasians, and conflicting results were observed in studies on other populations such as African and Asian. While significant association was observed in studies conducted in Ecuador[38] and Japan,[39] this was not observed in the studies conducted in Iran[40] and South Korea.[42] Such discrepancies could be due to the relatively small sample size (ranging from 78 to 131). Studies in larger scale with comprehensive assessment have to be conducted in Asian and African population to confirm the applicability

of cfDNA as a biomarker for severity and disease progression of obesity in these populations. Apart from ethnicity, other characteristics such as exercise and hormone treatment status were also found to influence the association. For example, a large-scale cohort study conducted in Finland found that significant association was observed only in women with no HRT, but it was not observed in men with not HRT and women with HRT (both estrogen only and estrogen-progestin combined treatment).[43] Also, a small-scale randomized controlled trial in the United States found that performing 30 min of acute HIIE could significantly increase the cfDNA level in both individuals with normal bodyweight and obesity.[35] However, there was no significant difference in baseline cfDNA levels and the fold change after exercise between the two groups. More studies are needed to explore applicability of cfDNA as obesity-related biomarkers under different circumstances.

Liquid biopsy with cfDNA analysis is an emerging technique in cancer molecular diagnosis.

For example, in a Korean study on gastric cancer patients, a significant drop of cfDNA level was observed in post-operation patients ($P < 0.001$).[42] Furthermore, in a study involving 887 colorectal cancer patients, a significant association was observed between high cfDNA levels and decreased overall survival rate.[36] Such evidence suggests the use of cfDNA in the purpose of prognostic monitoring the medical conditions in patients. In fact, the notion of using cfDNA on estimating the obesity-related non-communicable disease risk was originated from the emerging role of circulating tumor DNA in cancer liquid biopsy and management.[47–49] However, we did not observe any significant correlation between cfDNA and BMI among cancer patients in the included studies. One possible reason is the potential dominating influences of the severe patient's conditions that interfered with the cfDNA released in the circulations. This suggests that the applicability of cfDNA as a tool for monitoring progression of obesity might be limited to mainly healthy individuals.

Utilization of cfDNA also came into practice in the obstetrics field. Four of the 11 reviewed studies were found to focus on investigating the influence of maternal obesity on cfDNA levels in the pregnant women,[31,37,41,44] in which three of them also assessed the influence on the fetal cfDNA level.[31,37,41] FF is one of the most important parameters to be checked in prenatal care. The analysis of fetal-originated cfDNA from maternal peripheral blood aided aneuploidy screening, explaining the principle of non-invasive prenatal testing (NIPT).[50,51] Our review found that the mean FF was only significantly lower in women with obesity at first trimester,[37] and such correlation was not found in pregnant women at their second and third trimesters.[31,41] However, the negative association between maternal BMI at first trimester and FF[37] cannot be explained by dilution of excess maternal cfDNA as the association between maternal BMI and total cfDNA was only significant at second and third trimesters,[31,41] which make this observation of phenomenon warrant for further investigation. Nevertheless, these provide some preliminary evidence that the body weight status of the mother could influence the cfDNA balance in the maternal blood. Given the health significance of cfDNA on the pregnancy outcome and neonatal health,[52] further investigation to confirm the influence of maternal body weight status during pregnancy on cfDNA level could be beneficial.

Despite the accumulating evidence, the usage of cfDNA as a molecular target in assessing body wellness and progress of obesity-related diseases is still in a barren stage. Barriers of using cfDNA into drawing clinically relevant decisions come in two directions.[42] First, pre-analytical steps of sample handling should be standardized. Given the relatively short half-life of cfDNA, concentration of cfDNA upon extraction can be influenced by storage conditions, time of processing, and extraction method.[53,54] Use of different DNA extraction kit also offers distinct size profiles and extraction efficiencies, making standardization of extraction method essential.[55] Furthermore, the sizing of cfDNA cellular destruction comes as a ladder form such that the fragmentation from different mechanisms could result in variable length of DNA. While the modal length for apoptotic-released DNA is around 166 bp, cfDNA is found to be of shorter length (~134 bp, sometimes as short as 90 bp)[56,57] in which cf-mtDNA can be even more fragmented (ranging from 40 to 300 bp) due to its nature as "naked DNA" (without histone-aided high order packing).[58,59] Establishment of standard operating protocol may help optimizing the utility of cfDNA level as a prognostic tool for people with obesity.[36] Second, there is still a lack of cut-off value or reference interval available for interpretation. Current evidence on cfDNA and obesity mainly assessed and compared the difference of cfDNA level between populations with normal body weight and obesity without the establishment of reference intervals. Our review identified that only one out of the 11 studies have tried to set cfDNA reference intervals in healthy individuals, in a large-scale case control study ($n = 2817$).[36] More comprehensive studies that can address the properties and dynamics of cfDNA are needed to establish the reference intervals of cfDNA.

To our best knowledge, this review is among the first that gathers and reviews evidence over the relationship between cfDNA and body weight status. While cfDNA has been suggested as a marker to tumor profiling functions, this review attempted to provide an answer towards the question of whether cfDNA can be qualified as an unspecific marker in reflecting the severity of obesity and the progression of its-associated complications. Our review provided some preliminary evidence over the applicability of using cfDNA level in conjunction with BMI in monitoring the severity and progression of obesity, especially among healthy individuals and pregnant women. However, it is important to consider the following limitations when interpreting the findings of this review. While we tried to conduct a comprehensive analysis on the association between cfDNA and body weight status across different populations, only limited studies were found in the cancer patients and pregnant women. More studies are needed to confirm the pattern of findings identified in this review. Expanding the range of populations considered in this review could potentially improve the applicability and generalizability of the findings. However, it is necessary to await further evidence regarding the use of cfDNA in diverse populations before drawing definitive conclusions. The accumulation of such evidence is crucial for ensuring that the benefits and limitations of cfDNA testing are well-understood across multiple

demographic groups, thereby facilitating its widespread adoption and utility. Another limitation is the high degree of heterogeneity among the included studies, particularly in DNA extraction tool, recruitment time frame, and sample size, which makes it difficult to draw a robust conclusion. More studies with standardized assessment method are needed to confirm the evidence on the association between cfDNA level and body weight status. Lastly, our literature search was restricted to manuscripts published in English. The omission of non-English studies may potentially bias the findings.

5 | CONCLUSION

This systematic review provided some preliminary evidence over the association between cfDNA level and body weight status and hence shed light on the potential applicability of using cfDNA level in conjunction with BMI in monitoring the severity and progression of obesity. Our review found that existing evidence mainly focused on three populations, healthy individuals, cancer patients, and pregnant women with distinctive patterns observed in each of these populations. Findings reveal high heterogeneity in methodology and limited number of large-size prospective cohort studies to substantiate the association between obesity and cfDNA level. The effects of obesity on cfDNA level were also found to vary by individuals' pre-existing medical conditions, physical activity level, and hormone treatment status. More cohort studies in larger scale with comprehensive assessment have to be conducted to confirm the applicability of cfDNA as an unspecific biomarker for severity and disease progression of obesity.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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