Cell-free DNA as a Biomarker in Cancer and other Inflammatory diseases

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Abstract

In the arena of liquid biopsy cell-free DNA (cf DNA) is a promising candidate biomarker due to minimally invasive procedure. It helps to detect and monitor different pathophysiological processes like inflammation, infection, autoimmune disease, especially cancer. The amount of cfDNA gets increased in pathological conditions which can help to detect disease states. Though in both malignancy and inflammatory conditions cf DNA levels get increased it is higher among malignant ones. It is unclear whether the abundance of cfDNA into the serum/plasma of cancer patients due to solely malignancy or other chronic inflammatory conditions has an impact here. So, studies conducted to detect cfDNA amount in cancer and inflammatory diseases are discussed here to get a clear picture. The purpose of this review was to assess the potential clinical value of the plasma cfDNA concentrations and strand integrity index as a supplementary tool for diagnosis and management of cancer and to distinguish it from inflammatory diseases and also assess cfDNA level and integrity relationship with disease severity.

Keywords: *Cell-free DNA, Cancer, Inflammation, cf DNA integrity, Biomarker.*

Introduction

Cancer is going to rank as one of the major causes of mortality and the most powerful barrier to increase the expectancy of life of the people in this 21st century (New Statistics Show Cancer Burden Rising in the World, Lung Cancer Biggest Killer, 2018). Estimates predict that in 2030 the number of people worldwide dying from cancer would reach 11 million (P & B, 2008). Detection of cancer at an early stage significantly increases the life expectancy of patients. To detect cancer early several biomarkers are used. But most of the currently available biomarkers are not sufficiently sensitive or specific to be implemented in routine clinical approaches. In addition, invasive procedures are also required for certain cancer forms, such as lung or breast cancer, to collect information for pathological analyses. Much effort has been made in the search for biomarkers that enable non-invasive evaluation, screening, disease staging and monitoring to simplify cancer management (Kohler et al., 2011). Cell-free DNA (cfDNA) could be such a potential biomarker. The non-invasive property of cfDNA measurement is that it can easily be separated from human plasma/serum or from other bodily fluids (Utting et al., 2002). Degenerated fragments of DNA that are released into the blood plasma/serum are cell-free DNA (cfDNA). It has been shown that cfDNA is derived under normal conditions from the programmed apoptotic process rather than necrotic processes (STROUN et al., 2006; Suzuki et al., 2008) While in cancer patients, due to high cellular turnover, apoptotic and necrotic processes both play role in its genesis (Grivennikov et al., 2010). cfDNA can release due to both cancerous growth and inflammation. And the challenge is here to differentiate cancer from other inflammatory diseases by evaluating cfDNA level. Chronic inflammation can

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lead to cancer development (Grivennikov et al., 2010). Since inflammation also triggers the release of cfDNA into the blood (Kustanovich et al., 2019). The aim of this review was to evaluate the levels of cfDNA and stand integrity in cancer and various inflammatory diseases to differentiate cancer from inflammation and also assess its relationship with the severity of the disease.

Review Method

Initially, research papers were searched using keywords such as cfDNA or cell-free DNA or Circulating cell-free DNA, cancer, inflammation, inflammatory diseases, biomarker, etc. Subsequently, the papers that met those word requirements were thoroughly reviewed and their results were properly noted.

Cell-free DNA (cf DNA)

cf DNA is used to identify different types of DNA freely circulating in the bloodstream. A liquid biopsy allows physicians to obtain a variety of information about a tumor from a blood sample which is simple and non-invasive alternatives to traditional surgical biopsies. The existence of circulating, cell-free nucleic acids (cfNAs) in human blood was first identified by Mandel and Métais in 1948 (Mandel & Metais, 1948). In healthy individuals, cfDNA is present at low concentrations (O'driscoll, 2007; Boddy et al., 2005), and in higher concentrations in patients with graft rejection, stroke, injuries, and burns, etc. (O'driscoll, 2007; Chiu et al., 2006; Fowler et al., 1976). But in cancer patients, highest levels of cfDNA have been observed (Dorofeyeva, 1975 ; Europe PMC, 2019). Most studies have documented apoptosis or necrosis, or both as the primary source of serum and plasma free-circulating DNA. DNA degradation is a hallmark of apoptosis, where DNA in a chromosome is first break down to large fragments (50-300 kb) and then into multiples of nucleosomal units (180-200 bp) (Nagata et al., 2003). Skilled phagocytes or neighboring cells easily consume the material of apoptotic cells through processes that are not completely understood (Viorritto et al., 2007), and following this the DNA in lysosomes is fully digested by DNase II (Nagata et al., 2003). As apoptosis is programmed cell death, before appearing in the circulation, DNA fragments produced by this process are removed. If this apoptotic body engulfment is disrupted or cell death is increased, large quantities of circulating DNA are observed. High cfDNA has been observed in cancer due to high cellular turnover. It will certainly be a concern for inflammation but autoimmunity, cancer, etc. even entail increasing circulating DNA (Nagata et al., 2003; Viorritto et al., 2007). Other than apoptotic and necrotic cell death, phagocytosis, oncosis, active secretion, pyroptosis, neutrophil extracellular trap release (NETosis), and excision repair play important role in release of cfDNA (Suzuki et al., 2008; Thierry et al., 2016; Peters & Pretorius, 2011). Not only cfDNA level but also cfDNA integrity can give us important complementary information regarding the disease (Vizza et al., 2018). DNA fragments are formed more randomly in necrosis, having a size greater than 10,000 base pairs, but in case of apoptosis fragments with 180-200 base pairs or multiples of this unit in length are created (Wang et al., 2003). The cfDNA integrity is called the integrity index. It is the ratio between the fragments of long and short cfDNA. It has recently been suggested as a promising biomarker for oncology due to its high sensitivity and specificity (Cheng et al., 2017; Wan et al., 2017). In case of cancer, cell death occurs more randomly, that is mostly necrosis and other form of cell death occurs frequently. So, in cancer cfDNA with larger fragments will produce compared to a healthy individual. Hence cfDNA integrity index will be higher in cancer patients.

Cell-free DNA in Cancer

In 2000, researchers and scientists grew interested to identify the role of cfDNA as a marker for cancer diagnosis and prognosis (Cabral et al., 2010; Chang et al., 2003; Rainer et al., 2003). It can be a potential surrogate marker in this aspect (Bronkhorst et al., 2019). Fragments of cfDNA have the capacity to invade the neighboring or distal cells (Shapiro, 2013; Bendich et al., 1965; Szybalska & Szybalski, 1962; Borenfreund & Bendich, 1961; Gartler, 1959). It promotes recipient cell metastasis by inducing overexpression of many pro-metastatic genes via the independent pathway of TLR9/MYD88 (Niu et al., 2018; Fűri et al., 2015; Kostyuk et al., 2012). Thus it play role in the metastasis of cancer. Variation of cfDNA amount in the blood is high. Some studies have shown that cancer patient's cfDNA ranges from 0-5 ng/ml to >1000 ng/ml and from 0 to 100 ng/ml in healthy control (Thierry et al., 2016; Schwarzenbach et al., 2011; A. Szpechcinski et al., 2015). Others said it ranges from less than 10 ng/ml to more than 1500 ng/mL in normal subjects (Elshimali et al., 2013). There is also a marked difference in serum/plasma cfDNA levels among patients with various forms of tumor. This variation is responsible for various methodological approaches, including sample processing and storage, plasma DNA extraction and quantification methods, and the choice of target genes (A. Szpechcinski et al., 2015). A Research study of Spindler. K.L.G et al., showed significantly high level of cfDNA in metastatic colorectal cancer patients compared with the healthy group and all cancer patient groups (all p values were <0.0001) (Spindler et al., 2015). Concentration ranging between ~60 to 550 ng/ml in plasma/serum was observed in breast cancer patients, only slightly intersecting with safe controls range in which values encompass from 3 to 63 ng/ml (Canzoniero & Park, 2016; Fleischhacker & Schmidt, 2007). In several studies, cfDNA levels are suggested to increase in malignant patients and correlate with tumor size, metastasis of the lymph node, histopathological grade, and clinical stage (Sharon et al., 2017; Zanetti-Dällenbach et al., 2008; Kohler et al., 2009). It is suggested in many studies that cfDNA levels increase in patients with malignant lesions and correlated with tumor size, lymph node metastasis, histopathological grade, and clinical stage (Sharon et al., 2017; Zanetti-Dällenbach et al., 2008; Kohler et al., 2009). A Study on Renal cell carcinoma (RCC) patients and healthy individuals showed high cfDNA variation. Quantitative real-time PCR was done to check the DNA integrity level in both classes of subjects. It was checked by short and log fragment amplification (ACTB-384/ACTB-106 ratio) and compared to healthy individuals, the levels of both DNA fragments were elevated in RCC patients (ACTB-384: 1.77 vs. 0.61ng/ml, p=0.0003; ACTB-106: 1.31ng/ml vs. 0.77 ng/ml, p=0.003) (Hauser et al., 2010).

Cell-free DNA in Inflammatory Diseases

The most important cause of death in the world is inflammatory diseases (chronic). Inflammation is a system of the body's defensive mechanism through which harmful foreign objects are detected and eradicate and healing starts. It can be either chronic or acute (Michels da Silva et al., 2019; Pahwa & Ishwarlal Jialal, 2019). It is marked as a major threat to human health by the World Health Organization (WHO). Globally, 3 out of 5 individuals die from chronic inflammatory diseases like stroke, chronic respiratory diseases, heart disease, diabetes, etc. (Barcelos et al., 2019; Tsai et al., 2019; Deepak et al., 2019). These inflammatory conditions cause cell damage. Following cell damage, cfDNA get released into the blood. The release of cfDNA into the bloodstream is often associated with systemic inflammation, the extent of which is proportional to the magnitude of systemic inflammation (van der Meer et al., 2019). The measurement of cfDNA levels has been recognized as a possible diagnostic method for treating patients with different inflammatory circumstances that represent cell necrosis and apoptosis, and can be used to determine the activity and severity of the disease (Siddiqi & Ridker, 2018). The highest levels of cfDNA were observed in patients with infec-

tions (3,469 and 1,659 μg/ml in survivors and non-survivors, respectively); acute inflammation patients were in the center, ranging from 175 to 645 μg/ml; the lowest, ranging from 50 to 79 μg/ml, was in patients with chronic inflammation (Frank, 2016).

For understanding psoriasis (chronic inflammatory disease) severity, the level of cfDNA may be used. Significantly higher levels of cfDNA (P=0.001) (170 ng/ul in mild to moderate vulgaris patients and in more severe, erythrodermic situations it was 405 ng/ul) compared with controls (72.65 ng/ul) was found in a study (Anani et al., 2020). Highly significant reductions in cfDNA (P= 0.042) level was caused by methotrexate treatment (Anani et al., 2020). Researchers have proposed through this research that circulating fragments of DNA have the potential to be a new biomarker to track and monitor Psoriatic patients and to investigate the efficacy of the medications used in treatment (Anani et al., 2020). First, Coimbra et al., showed insights into higher serum levels of cfDNA in patients with aggravated psoriasis vulgaris (Coimbra et al., 2010). Another research by Dielek et al., also indicated that the circulating nucleosome can be used as a potential biomarker to control the efficacy of the therapy in psoriatic patients (Dilek et al., 2013).

Critically ill patients die mostly due to septic shock and in its pathophysiology, apoptosis plays a significant role. In sepsis, this exceptional apoptosis releases multiple nucleosomes, which may overwhelm the mechanism of nucleosome clearance, ultimately resulting in elevated circulating levels of nucleosomes (Chen et al., 2012). This study showed that if we conduct the cfDNA level test, we can easily discern septic patients from non-septic critically ill patients during hospital admission, and these levels are associated with the degree of organ dysfunction and immunosuppressive response in sepsis. Zeerleder and his colleagues concluded in a study that patients suffering from extreme sepsis and septic shock had gradually increasing level of plasma nucleosomes compared to patients with fever and systemic inflammation (Zeerleder et al., 2003). Likewise, the detection of cfDNA in the bloodstream relates to the intensity and outcome of sepsis (Europe PMC, 2006; Dwivedi et al., 2012).

In hemodialysis patients, various pathological processes result in increased morbidity and mortality, impaired function, and tissue damage. In these patients, the summary impact of the various risk factors is cumulative, additive, interrelated, complex, and not completely unknown/ unpredictable. Therefore, a parameter that can assess these factors to predict post hemodialysis mortality will help to increase the patient’s quality of life. cfDNA works best in this case. The study's key finding was that the 850 ng/mL post-dialysis cfDNA level is the most reliable cutoff point for the 42-month mortality prediction (Tovbin et al., 2012). In an autoimmune disease like Systemic Lupus Erythematosus, rheumatoid arthritis, the majority of studies reported elevated levels of circulating cfDNA relative to controls (Barnett, 1968; Koffler et al., 1973; Hajizadeh et al., 2003).

In Chronic obstructive pulmonary disease (COPD), cfDNA level was higher (1,634 ng/mL) compared to stable COPD patients (781 ng/mL) and for healthy controls it was 352 ng/mL (P=0.0001, for both comparisons). This knowledge helps to classify patients with COPD who are at higher risk of poor outcomes.

Variation of level cf DNA in Cancer and other Inflammatory Diseases

According to Rudolf Virchow (1863), inflammation is one of the symbols of cancer since leukocytes are found within malignant tissues. From then, it has widely been agreed that if genetic anomalies are the 'match that lights the fire' of cancer, inflammation corresponds to the 'fuel that feeds the

flames' (Balkwill & Mantovani, 2001). Infectious agents are responsible for 15% of the overall global cancer burden, and inflammation is a vital constituent of these chronic infections. In addition, increase risk of malignancy is correlated with chronic inflammation caused by certain chemical and physical substances (Hanane, 1984), and with unclear etiology of autoimmune and inflammatory reactions (Ekbom et al., 1990). In pregnant women and in patients undergoing transplants, elevated levels of cfDNA were found (Sharon et al., 2017; Burnham et al., 2017; Tug et al., 2015; Breitbach et al., 2014). In non-malignant pathological processes (Schwarzenbach et al., 2011) , such as inflammation, diabetes, tissue damage, sepsis, and myocardial infarction (O'driscoll, 2007; Wilson et al., 2018; Volik et al., 2016; O'Connell et al., 2017), in conditions such as physiological (e.g., exercise) elevated cfDNA was also observed.

In the case of chronic inflammation, there is a lack of validation of cfDNA level. In subjects with chronic inflammation, the current diagnostic usefulness of plasma cfDNA measurement has not been validated properly. Thus the diagnosis of cancer and other inflammatory disorders is confusing. To determine the effect of chronic inflammation on plasma levels of cfDNA and to assess the possible therapeutic significance of this phenomenon to distinguish it from cancer diagnosis is vital (A. Szpechcinski et al., 2015).

There are a few studies that consider this fact and conduct their research accordingly. These researches showed us light through which we can easily distinguish cancer from inflammatory diseases. In these studies, researchers took three categories of the sample. One is healthy subjects; one is chronic inflammatory disease patients and another one is cancer patients group. cfDNA level was quantified in all three groups which help to set cut-off value of cfDNA in each condition.

In studies performed on patients with lung cancer (95.67 ng/ml), cfDNA concentrations were substantially higher than in healthy individuals (44.66 ng/ml) (T. Jiang et al., 2016; Gautschi et al., 2004; Kumar et al., 2010; Chiappetta et al., 2013). In addition, in patients with lung cancer, plasma cfDNA levels were substantially higher than in-patients with chronic respiratory inflammation (COPD, sarcoidosis, or asthma) (59.60 ng/ul) (A. Szpechcinski et al., 2015). In order to differentiate lung cancer from chronic respiratory inflammation, the difference of cfDNA level may be used as a biomarker. The integrity of patients' plasma/serum cfDNA was another essential finding in the research. Non-small cell lung cancer (NSCLC) (5.91) patients have had significantly higher cfDNA integrity than tuberculosis (3.85, $P = 0.000$) and stable control patients.

These results are in line with the hypothesis that high disintegration is a distinctive characteristic of cfDNA derived from the tumor (P. Jiang et al., 2015; Mouliere et al., 2011) and indicate that the integrity of cfDNA could also be used to differentiate cancer from other infectious/inflammatory diseases. Moreover, in this analysis, the impact of integrity of cfDNA (AUC = 0.722) to differentiate NSCLC from tuberculosis was higher than that of conventional tumor markers CA125 (AUC = 0.626), NSE (AUC = 0.716) and CEA (AUC = 0.589), suggesting that integrity of cfDNA showed marginally better parameters (Leng et al., 2018). In addition, the impact of cfDNA integrity on the separation of NSCLC patients from tuberculosis ones was higher than that of typical CA125, NSE and CEA tumor markers. Szpechcinski et al., in a related analysis found substantially higher levels of cfDNA in patients with non-small cell lung cancer relative to those with benign tumors ($p=0.0009$) and safe controls ($p<0.0001$) (Adam Szpechcinski et al., 2016). In healthy people, the plasma cfDNA integrity was also substantially lower than that found in patients with NSCLC or benign lung tumors ($p<0.0003$).

Thus we can see cell-free DNA is highest among cancer patients, mostly in the metastatic cases compared to other chronic inflammatory diseases and infections. We have to conduct more studies like these. Along with that if we able to measure the integrity index of cfDNA we can able to differentiate easily cancer from other disease since integrity index is always higher in cancer patients. For further specification of cfDNA as a biomarker of cancer, we can study the gene-specific mutation.

Conclusion

The deficit of an optimized and reliable cfDNA concentration and integrity assessment protocol will adversely affect the diagnostic usefulness of this candidate biomarker. Several studies, conducted observed that the levels of cfDNA increase from chronic to acute inflammation to serious infection and cancer to the highest form. If further research establishes a direct connection between the level of cfDNA and clinical inflammatory states, it would be an essential clinical tool. Measuring cfDNA is a simple and easy and cost-effective process, and can be done on a large scale. Thus it will be easy to measure cfDNA routinely in clinical practice anywhere plasma or serum peripheral blood can be drawn. Most notably, if it can be confirmed that cfDNA is present in pre-disease states at the lower end of the spectrum, such as in pre-diabetic patients or in retrospective blood samples from patients taken before the development of cancer it will help to diagnose the disease at an early stage. Since the integrity index is higher in cancer patients along with high cfDNA, we can easily distinguish cancer from other chronic inflammatory diseases and gene specific mutation of cancer will further increase its specification. In a nutshell, not only cfDNA amount measurement but also cfDNA integrity index measurement along with gene-specific mutation study will help to clearly differentiate cancer from other inflammatory diseases.

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