

Homocystiene and C-Reactive Protein in Detection of Frailty

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Abstract

Background and Aim of the Work: Frailty is a state of reduced physiological reserve and is associated with increased susceptibility to disability. It is associated with a high morbidity and mortality. The aim of this work is to assess the association between the levels of homocystiene and C-reactive protein and frailty and to examine the ability of homocystiene as a new marker to detect frailty. **Subjects and Methods:** A total number of 104 elderly subjects (above 60 years old) were included in this study and they were subjected to history, examination, comprehensive geriatric assessment, and laboratory investigations including: C-reactive protein (CRP) and Homocystiene (Hcy). The patients were divided into frail and non-frail groups using Fried's criteria as applied by Avila-Funes *et al.*, 2008 and each group included 52 patients. **Results:** There was no significant difference between the two groups as regards age gender or smoking habits. But there were higher levels of CRP (>10 mg/dl) and homocystiene (>12 mmol/l) among frail cases and by comparing both homocystiene was more sensitive in detection of frailty. **Conclusion:** Frailty was associated with elevated homocystiene and CRP and homocystiene is more specific than CRP in detection of frailty.

Keywords

Frailty, CRP, Homocystiene

1. Introduction

Frailty is becoming increasingly common as the world's population ages. There is no single best definition of frailty but the most acceptable definition is postulated by Fried *et al.* defined frailty as: "A physiologic syn-

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drome characterized by decreased reserve and resistance to stressors, resulting from cumulative decline across multiple physiologic systems, and causing vulnerability to adverse outcomes” [1]. Different conceptual approaches have been applied to describe this phenomenon, including incorporation of physical characteristics and function [2], and utilizing a combination of clinical deficits and comorbidity domain [3].

Lang *et al.* [4] reported that clinical markers or indicators are insufficient to differentiate the frailty process from normal aging, and they gave rise to the necessity to detect frailty at a pre-clinical stage with the help of biomarkers. Evaluation of alterations in human biomarkers and their relationships to differing models of frailty may assist the determination of the initiation of the processes that eventually led to frailty [5].

Homocysteine is one possible candidate that may underlie the development of the frailty syndrome. Total plasma homocysteine has been shown to be inversely related to the intake and plasma levels of folate and B-vitamins. The deficiencies of B-vitamins may result in mitochondrial dysfunction with deleterious changes in cellular function. These could conceivably cause muscle weakness and atrophy, leading to sarcopenia with progressive physical decline. At the molecular level, B-vitamin deficiency may be mediated via hyperhomocysteinemia through mechanisms of oxidative stress, or by homocysteinylolation, which involves covalent binding of homocysteine to proteins. These modified proteins can trigger the inflammatory cascade, resulting in vascular endothelium damage and subsequently vascular events, further leading to functional decline and frailty [6].

Older frail patients exhibit evidence of increased inflammation, with higher levels of C-reactive protein [7]. A direct role of inflammation in the development of frailty is primarily based on the catabolic effects that proinflammatory cytokines have on muscles. TNF- α and other inflammatory signals increase IL-6 production which in turn stimulates production of CRP: all are markers of the same inflammatory process and each has detrimental effects on muscle [8]. Elevated CRP levels are also associated with many late-life chronic conditions, including Alzheimer’s disease, cardiovascular diseases, macular degeneration, and functional decline, disability, as well as all-cause mortality in older adults [9]-[11].

In this study, the researchers sought to determine if elevated homocysteine is associated with frailty in elderly, (as the role of homocysteine in frailty was not thoroughly investigated) and to assess whether it is better in detection of frailty than CRP (whose role in frailty has been investigated by many researchers but it is linked to many other chronic conditions) so that when homocysteine is used it can increase the sensitivity of the physical criteria in detection of frailty.

2. Patients and Methods

2.1. Study Design and Setting

The study is a Case-control study it included 104 Elderly participants (60 years old and above), both males and females were recruited from Ain Shams University hospital from inpatient wards and outpatient clinics from January 2011 till December 2012. One hundred and twenty participants were interviewed 16 were excluded 10 of them had renal impairment by labs (which lead to increased Homocysteine level) 8 were excluded due to technical difficulties. They were divided into two groups which are:

Cases Group: 52 frail elderly 60 years and older diagnosed by Fried’s criteria [12] as applied by Avila-Funes *et al.* [13]. The participants were considered to be “frail” if they had three or more frailty components among the five criteria.

Controls Group: 52 elderly 60 years and older matched with cases regarding age and gender. They are not frail or have 2 or less of frailty criteria.

2.2. Data Collection

Any patient who refused to participate in the study, patients who were suffering from acute infection, and any patients who were taking drugs that have antiinflammatory effects as steroids, statins and aspirin were excluded from this study.

Each patient then underwent comprehensive geriatric assessment in the form of detailed history and physical examination, cognitive function assessment by Minimental status examination (MMSE) [14] (The Arabic version used in this study was done by El-Okl *et al.* [15], functional assessment by Activities of daily living (ADL) [16], Arabic version [17], and Instrumental activities of daily living (IADL) [18] (An Arabic version of the test

was applied [19], and Geriatric depression scale 15 items (GDS-15) [20] was used to screen for depression the Arabic version of the test was applied by Shehta *et al.* [21] (Those items are done as a routine assessment of elder patients).

Frailty was defined according to the construct previously validated by Fried *et al.* in the Cardiovascular Health Study [12]. All five components from the original phenotype were retained; however, the metrics used to characterize the frailty criteria were slightly different and defined as follows [13]:

- Shrinking—Recent and unintentional weight loss of ≥ 3 kg in the prior year was identified and body mass index calculated. Participants who answered “yes” for weight loss or had a body mass index < 21 kg/m² were considered to be frail for this component.
- Poor endurance and energy—As indicated by self report of exhaustion, identified by two questions from the Center for Epidemiological Studies-Depression scale (CES-D) [22]: “I felt that everything I did was an effort” and “I could not get going.” Participants were asked: “How often, in the last week, did you feel this way?” 0 = rarely or none of the time; 1 = some or a little of the time; 2 = a moderate amount of the time; or 3 = most of the time. Participants answering “2” or “3” to either of these questions were considered as frail by exhaustion.
- Slowness—Meets criteria for frailty if time to walk 6 m was ≥ 8 seconds for height ≤ 173 cm or > 7 seconds for height > 173 cm in males, and ≥ 8 seconds for height ≤ 159 cm or > 7 seconds for height > 159 cm in females.
- Weakness—Participants answering “yes” to the following question were categorized as frail for this component: “Do you have difficulty rising from a chair?”
- Low physical activity—A single response was used to estimate physical activity. Individuals who denied doing daily leisure activities such as walking or gardening and/or denied doing some sport activity per week were categorized as physically inactive. Those who reported doing them were considered to be active.
- Patients who had 3 of the 5 items were diagnosed as frail.

2.3. Laboratory Investigations

Six mL whole blood was drawn from each participant after 12 hours fasting, and was divided into 2 tubes: 3 mL in EDTA-anticoagulated tube for homocystiene assay Plasma was separated from the blood samples within 1 hour of collection and stored at -80°C until assayed. The other 3 mL were allowed to clot in a plain tube and the resulting serum was used for quantitative c-reactive protein. Homocystiene was assayed by Enzyme immunoassay (EIA) kit: Axis[®] Homocysteine EIA (Axis-Shield Diagnostics Ltd, The Technology Park, Dundee DD2 1XA, United Kingdom) Hyperhomocysteinaemia was defined as levels greater than 12 mmol/l [23]. Quantitation of CRP was performed by immunoturbidimetric assay using Biosystems CRP-hs kit (Biosystems, SA, Barcelona, Spain) CRP level in normal healthy adults is usually low < 10 mg/dl [24].

3. Data Management

Analysis of data was performed by using the 12th version of Statistical Package for Social Science (SPSS). Description of all data in the form of mean (M) and standard deviation (SD) for all quantitative variables was done. Frequency and percentage was done for all qualitative variables. Comparison between quantitative variables was done using t-test to compare to compare two groups and ANOVA (analysis of variance) to compare more than two groups. Comparison of qualitative variables was done using the Chi-square test. Correlation coefficient also was used to find linear relation between different variables using r-test or Sperman correlation co-efficient. Significant level measured according to P value (probability), $P > 0.05$ is insignificant, $P < 0.05$ is significant and $p < 0.01$ is highly significant.

4. Results

As regards demographic criteria of the study population, there was no significant difference between cases and controls as regards: age, gender, living arrangement and smoking habits.

Regarding clinical characteristics frail patients had higher levels of ADL and IADL dependence in addition to higher grades of depression and cognitive impairment. There was a higher mean number of associated chronic disease among frail elderly. **Table 1** shows the distribution of chronic disease among frail and non frail partici-

Table 1. Comparison between the two studied groups as regards chronic diseases.

	Cases		Controls		T	P-value
	No.	%	No.	%		
Diabetes mellitus	24	46.2	14	26.9	4.1	0.04*
IHD	28	53.8	9	17.3	15.1	0.000**
Hypertension	26	50.0	14	26.9	5.8	0.01*
Stroke	10	19.2	2	3.8	6.0	0.01*
COPD	18	34.6	19	36.5	0.4	0.8
Arthritis	20	38.5	18	34.6	0.1	0.6
Visual impairment	40	76.9	18	34.6	18.8	0.000**
Hearing impairment	8	15.4	0	0	8.6	0.003**
Chronic liver disease	10	19.2	5	9.6	1.9	0.1
Thyroid disease	6	11.5	0	0	6.3	0.01*
Anemia	4	7.7	0	0	4.1	0.06

*P < 0.05 significant, **P < 0.01 highly significant.

pants there was higher percentage of diabetes mellitus (DM), Ischemic heart disease(IHD), hypertension, stroke, visual and hearing impairment among cases. The three most prevalent chronic illnesses among cases were visual impairment, DM, and IHD (the least common were hearing impairment, stroke and chronic liver disease). As for controls the three most prevalent chronic illnesses were chronic obstructive pulmonary disease (COPD), Visual impairment and arthritis.

Our study showed that there was higher mean CRP & homocystiene levels among frail cases compared to controls and the difference is significant statistically as shown in **Table 2**.

Finally by comparing the sensitivity and specificity of homocystiene and CRP in detection of frailty homocystiene was found to be more sensitive than CRP as it had a higher area under the curve than CRP as shown in **Figure 1** and **Figure 2**.

5. Discussion

Frailty has been recognized as a common clinical syndrome associated with a high rate of morbidity and mortality [25].

The current study assessed the association between the levels of homocystiene and C-reactive protein and frailty and to examine the ability of homocystiene as a new marker to detect frailty. Applying routine comprehensive geriatric assessments showed that frail patients had had higher levels of ADL and IADL dependence in addition to higher grades of depression and cognitive impairment and also a higher mean number of associated chronic diseases which was consistent with Espinoza *et al.* 2007 who stated that frailty is associated with multiple impairment and comorbidities [26].

The association between raised markers of inflammation and frailty has been searched in several studies as in our study we found that frail elderly had higher levels of CRP in comparison to non-frail controls Walston *et al.* 2002 found in results from Cardiovascular Health Study (CHS) that higher CRP levels are associated with higher frailty risk [7] & this was also approved by Hubbard *et al.* 2009 [8]. These data support the hypothesis of the role of inflammation in the development of frailty.

Homocystiene level was higher in cases than controls & the differences were statistically significant which agrees with results of Wong *et al.* 2013 that hyperhomocysteinemia is associated with the prevalence of frailty. It is also predictive of all-cause mortality independent of frailty. The study by Wong *et al.* included 4248 participants. One thousand one hundred-seventeen participants had high plasma total homocysteine levels of 15 micromoles per liter or more. Six hundred eighty-five subjects were categorized as frail. Among subjects who had high homocysteine levels, the adjusted risk of frailty as assessed during was 49% greater than those whose homocysteine was less than 15 micromoles per liter [6]. It also agrees to the results of Bates *et al.* 2010 that high homocystiene is also a robust and independent predictor of subsequent mortality in these older adults, both for

Table 2. Comparison between cases & controls as regards Homocystiene & CRP.

	Cases		Controls		P
	Mean	SD	Mean	SD	
CRP	31.3	38.9	14.4	13.7	0.004**
Homocystiene	15.4	10.3	6.8	6.8	0.000**

*P < 0.05 significant, **P < 0.01 highly significant.

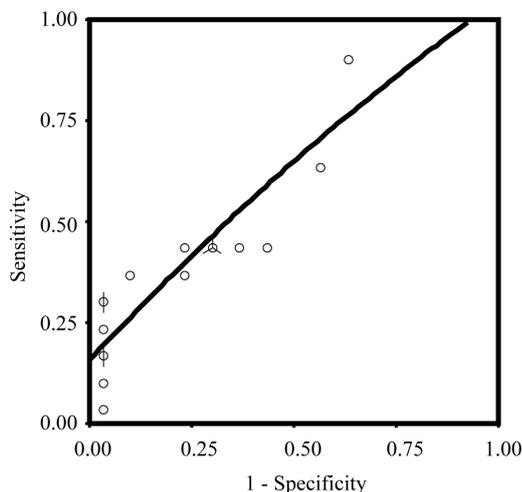


Figure 1. CRP area under the curve (0.65).

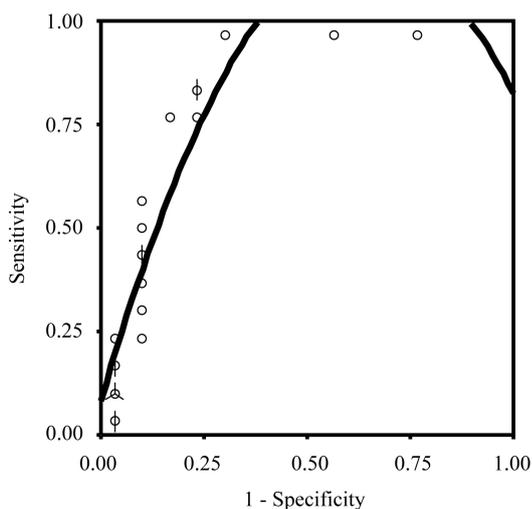


Figure 2. Homocystiene area under the curve (0.85).

all-cause mortality and especially for primary-cause vascular mortality [27]. These results support the hypothesized role of homocysteine in the development of frailty.

By comparing the sensitivity & specificity of CRP & homocystiene in the detection of frailty the latter was found to be better predictor of frailty, contrary to Wong *et al.* 2013 who stated that homocysteine & CRP are both associated with frailty but independent from each other. (The hypothesis of Hcy induced inflammation as a mechanism of physical decline was tested when high-sensitivity C-reactive protein was added to the fully adjusted model for frailty [6]. The effect estimates were altered minimally, implying that the relationship between Hcy and frailty in this cohort may be independent of the inflammatory pathway). Houwelingen *et al.* 2013 stated that CRP & homocystiene are equally related to mortality in elderly (85 years & older) [28].

6. Conclusion

Frailty is an important condition among the elderly but it needs further studies regarding risk factors and associations. The significant elevation of CRP (which is an inflammatory marker) in frail elderly, may give support to the theory of inflammaging. Also the increase in homocystiene level and its higher specificity for frailty than CRP suggest it might be used as a marker for frailty which can be used with Fried's clinical criteria to detect frailty. Lowering homocystiene level using vitamin B12 may affect the progression of this state. However this matter needs to be investigated in the future.

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