STROBE Statement—checklist of items that should be included in reports of observational studies

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|  | **Item** |  |
|  | **No** | **Recommendation** |
| **Title and abstract** | 1 | Title: Case–control study of Diffusion-weighted Magnetic Resonance Imaging (MRI) and micro-RNA in diagnosis and staging of hepatic fibrosis in chronic hepatitis C virus infection. |
|  |  |  |
|  |  | **Objective:** To assess diffusion-weighted MR imaging (DWI) and micro-RNAs (miR) in diagnosis and staging of hepatic fibrosis in patients with chronic hepatitis C.**Methods:** This study was conducted upon 208 patients and 82 age and sex matched controls underwent DWI of the abdomen, miR and liver biopsy. The pathological score was classified according to METAVIR scoring system. The ADC and miR were calculated and correlated with pathological scoring.**Results:** The apparent diffusion coefficient (ADC) value was decreased significantly from controls (F0), patients with early fibrosis (F1 and F2) and those with late fibrosis (F3 and F4), (median 1.92, 1.53 and 1.25 x 10-3mm2/s) respectively (*P* = 0.001). The cutoff ADC value used to differentiate patients from controls was (1.83 x 10-3 mm2/s) with area under curve (AUC) of 0.992. Combined ADC and miR-200 revealed highest AUC (0.995) for differentiating patients from controls with accuracy (96.9%). The cutoff ADC used to differentiate early fibrosis from late fibrosis was 1.54 x 10-3 mm2/s with AUC of 0.866. Combined ADC and miR-200 revealed best AUC (0.925) for differentiating early fibrosis from late fibrosis with accuracy (80.2%). The ADC correlated withmiR-200 (*r* = -0.61, *P* = 0.001), miR-21 (*r* = -0.62, *P* = 0.001) and miR-29 (*r* = 0.52, *P* = 0.001). **Conclusion:** Combined ADC and miR offer an alternative surrogate noninvasive diagnostic tool for diagnosis and staging of hepatic fibrosis in patients with chronic hepatitis C. |
| **Introduction** |  |  |
| Background/rationale | 2 | The diagnosis of hepatic ﬁbrosis in patients with chronic hepatitis is essential for therapeutic and prognostic implications. Liver biopsy has been used as the gold standard for characterization of hepatic fibrosis; however, biopsy has several inherent problems, including sampling error, high cost, morbidity, and low patient acceptance. Biopsy is also too invasive for frequent monitoring to follow treatment response to expensive, and potentially toxic, antifibrotic therapy. An equally reliable, reproducible, and noninvasive alternative for the diagnosis and staging of fibrosis would potentially have greater clinical utility.Diffusion-weighted MR imaging and micro-RNA have shown promise in the detection and quantiﬁcation of hepatic ﬁbrosis |
| Objectives | 3 | The objective of this study was to assess diffusion-weighted MR imaging (DWI) and micro-RNAs (miR) in diagnosis and staging of hepatic fibrosis in patients with chronic hepatitis C |
| **Methods** |  |  |
| Study design | 4 | The study assessed 215 consecutive patients with biopsy-proven CHC. Patients were included if they had a histological diagnosis of CHC on a liver biopsy. The patients were defined by the presence of serum anti-HCV and HCV-RNA..  |
| Setting | 5 |  |
|  |  | This study was conducted in Mansoura University Hospital, Mansoura University; Mansoura Egypt assessed 215 consecutive patients with biopsy-proven CHC. Patients were included in the study if they had a histological diagnosis of CHC on a liver biopsy. The patients were defined by the presence of serum anti-HCV and HCV-RNA. Seven patients were excluded from our study due to presence of hepatocellular carcinoma (*n* = 3), cardiac cirrhosis (*n* = 2) and hepatic metastasis (*n* = 2). The final number of patients was 208, median age was 36.3±9.3, (129 male and 79 female). Age and sex matched 82 volunteers underwent MR imaging for reasons other than abdominal abnormalities; the median age was 38.3±10.2 years (47 male and 35 female). The patients and controls included in the study from October 2012 to December 2015 who underwent DWI for the abdomen, miR tests and liver biopsy. |
| Participants | 6 | Patients were included in the study if they had a histological diagnosis of CHC on a liver biopsy. The patients were defined by the presence of serum anti-HCV and HCV-RNA. Control: Age and sex matched 82 volunteers underwent MR imaging for reasons other than abdominal abnormalities; the median age was 38.3±10.2 years (47 male and 35 female).  |
| Variables | 7 | In the current study it was found that there was significant decrease in ADC values starting from controls, then patients with early hepatic fibrosis then those with late fibrosis. When the ADC results combined with the miRs (200b, 21 and 29b) this provides highly sensitive, specific and accurate tool to differentiate patients with hepatic fibrosis from normal control patients. When the results of ADC combined with miR-200b, this was the best in differentiating patients from controls with (96.9%) accuracy also it can differentiate early from late fibrosis with (80.2%) accuracy.  |
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| Data sources/ | 8\* | For each variable of interest, give sources of data and details of methods of |
| measurement |  | assessment (measurement). Describe comparability of assessment methods if there |
|  |  | is more than one group |
| Bias | 9 | Describe any efforts to address potential sources of bias |
| Study size | 10 | Explain how the study size was arrived at |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, |
|  |  | describe which groupings were chosen and why |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding |
|  |  | (*b*) Describe any methods used to examine subgroups and interactions |
|  |  | (*c*) Explain how missing data were addressed |

(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls wasaddressed

*Cross-sectional study*—If applicable, describe analytical methods taking account ofsampling strategy

(*e*) Describe any sensitivity analyses

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**Results**

|  |  |  |
| --- | --- | --- |
| Participants | 13\* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, |
|  |  | examined for eligibility, confirmed eligible, included in the study, completing follow-up, and |
|  |  | AnalysedPatients:The study assessed 215 consecutive patients .Seven patients were excluded from our study due to presence of hepatocellular carcinoma (*n* = 3), cardiac cirrhosis (*n* = 2) and hepatic metastasis (*n* = 2). The final number of patients was 208, median age was 36.3±9.3, (129 male and 79 female). Control: Age and sex matched 82 volunteers underwent MR imaging for reasons other than abdominal abnormalities; the median age was 38.3±10.2 years (47 male and 35 female). |
|  |  | (b) Give reasons for non-participation at each stage |
|  |  | (c) Consider use of a flow diagram |
| Descriptive | 14\* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information |
| data |  | on exposures and potential confounders**Table (1): Demographic and laboratory tests of patients and controls**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters**  | **Control** **(n=82)** | **Early Fibrosis (n= 112)** | **Late Fibrosis** **(n = 96)** | **P value** |
| Age | 38.3±10.2 | 34.1±8.9 | 41.4±7.8 | 0.001 |
| Gender  | 47:35 | 69:43 | 60:36 | 0.8 |
| ALT | 36.29±17.24 | 52±36.07 | 57.17±36.88 | 0.001 |
| AST | 35±15.71 | 49±25.12 | 58±35.12 | 0.001 |
| Albumin | 4.1±0.45 | 4.2±0.44 | 3.8±0.69 | 0.001 |
| Bilirubin | 0.88±0.48 | 0.81±0.26 | 1.02±0.46 | 0.001 |
| PCR | 95746±10111 | 391000±213876 | 254500±129314 | 0.001 |
| AFP | 7.5±1.57 | 5.007±2.95 | 10.47±6.78 | 0.001 |

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|  |  | (b) Indicate number of participants with missing data for each variable of interest |
|  |  | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) |
| Outcome data | 15\* | *Cohort study*—Report numbers of outcome events or summary measures over time |
|  |  | *Case-control study—*Report numbers in each exposure category, or summary measures of |
|  |  | Exposure**Table (2): The median ADC and micro-RNA of patients versus controls**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Fibrosis (n = 208)** | **Control (n = 82)** | ***P* value** |
| **ADC** **miR-200** **miR-21** **miR-29** | 1.43± 0.224.61± 1.212.70± 1.300.58± 0.26 | 1.92± 0.081.20± 0.811.29± 0.400.98± 0.16 | 0.0010.0010.0010.001 |

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|  |  | **Table (3): The ROC curve results with cut-off values of ADC and serum markers of patients and controls**

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| --- | --- | --- | --- | --- | --- |
| **Parameter** | **AUC** | **Cut-off point** | **Sensitivity** | **Specificity** | **Accuracy** |
| **ADC** | 0.992 | 1.825 | 98.6% | 97.0% | 97.1% |
| **miR-200** | 0.925 | 1.65 | 92.3% | 82.2% | 91.2% |
| **miR-21** | 0.865 | 1.35 | 82.2% | 76.0% | 84.2% |
| **miR-29** | 0.937 | 0.91 | 92.3% | 81.7% | 91.o% |
| **ADC & miR-200** | 0.995 | - | 100% | 96.0% | 96.9% |
| **ADC & miR-21** | 0.992 | - | 100% | 95.0% | 96.2% |
| **ADC & miR-29** | 0.992 | - | 100% | 95.9% | 95.9% |

**Table (4): The median, minimum and maximum of ADC and serum markers of patients with early and late fibrosis**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **Early Fibrosis (n=112)** | **Late Fibrosis****(n=96)** | ***P* value** |
| **ADC** | 1.5±0.2(1-1.9) | 1.25±0.17(0.9-1.5) | 0.001 |
| **miR-200** | 3.43±1.71(1.0-1.4) | 10.17±4.81 (1-28.4) | 0.001 |
| **miR-21** | 1.9±0.7 (1.0-4.2) | 3.6±1.17 (1.0-6.34) | 0.001 |
| **miR-29** | 0.7±0.18(0.12-1.00) | 0.4±0.2(0.07-1.0) | 0.001 |

**Table (5): The cut-off values of ADC, miR used to differentiate early from late fibrosis with area under the curve, sensitivity, specificity & accuracy**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **AUC** | **Cut-off point** | **Sensitivity** | **Specificity** | **Accuracy** |
| **ADC** | 0.866 | 1.53 | 99% | 67% | 81.7% |
| **miR-200** | 0.888 | 3.55 | 90.6% | 59.5% | 73.5% |
| **miR-21** | 0.877 | 2.38 | 91.7% | 70.3% | 80.2% |
| **miR-29** | 0.832 | 0.70 | 87.5% | 60.7% | 73% |
| **ADC & miR-200** | 0.925 | - | 71.7% | 97.2% | 80.2% |
| **ADC & miR-21** | 0.88 | - | 72.3% | 97.5% | 83.2% |
| **ADC & miR-29** | 0.879 | - | 74% | 96.5% | 85.1% |

*Cross-sectional study—*Report numbers of outcome events or summary measures |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their |
|  |  | precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and |
|  |  | why they were included |
|  |  | (*b*) Report category boundaries when continuous variables were categorized |
|  |  | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful |
|  |  | time period |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity |
|  |  | analyses |
| **Discussion** |  |  |
| Key results | 18 | Summarise key results with reference to study objectivesThis study aimed to evaluate diffusion-weighted MR imaging (DWI) and micro-RNAs (miR) in diagnosis and staging of hepatic fibrosis in patients with chronic hepatitis C.The ADC value was decreased significantly from controls, patients with early fibrosis and those with late fibrosis, combined ADC and miR-200 is the best predictor for differentiating patients from controls with accuracy (96.9%) and combined ADC and miR-200 is the best predictor for differentiating early fibrosis from late fibrosis with accuracy (80.2%).  |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. |
|  |  | Discuss both direction and magnitude of any potential biasFew limitations are reported in the current study. The first, there is small number of patients that limits the statistical power. Therefore, further studies are needed at a larger scale to confirm the results of this work. The second, this study applied diffusion weighted MR imaging. Further studies applied advanced diffusion modules such as diffusion kurtosis imaging and diffusion tensor imaging at 3-tesla will improve the results. Third, this study applied region of interest for localization. Further studies applied advanced post processing method such as machine learning and histogram analysis  |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity |
|  |  | of analyses, results from similar studies, and other relevant evidence |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results |
| **Other information** |  |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, |
|  |  | for the original study on which the present article is based |

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background andpublished examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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